# International Journal of Chemical Studies

P-ISSN: 2349–8528 E-ISSN: 2321–4902 IJCS 2018; 6(6): 1125-1130 © 2018 IJCS Received: 01-09-2018 Accepted: 05-10-2018

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# Ageing induced biochemical and physiological changes during seed deterioration of groundnut (Arachis hypogaea L.)

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#### Abstract

Pods and kernels of two genotypes (GPBD-5 and DH-86) of summer groundnut were subjected to the accelerated ageing at 40° C and 90 % RH for various intervals (0, 2, 4, 6, 8 days). Accelerated ageing results in progressive loss of seed viability and vigour in both genotypes. A marked genotypic difference in storage of both genotypes in the form of pods and kernels on ageing had been recorded. Germination test and biochemical analysis were carried out for control and aged pod and kernels. The initial germination percentage was 97.86 % and after accelerated ageing germination decline to 49.32 %. During the ageing process decline in oil percentage and total soluble protein, whereas increase in total soluble sugars and phenols content was recorded. There was gradual decrease in the activity of  $\beta$ -amylase and increase in the activity of lipase was recorded during accelerated ageing. Hence the decrease in the changes in the biochemical content and the activity of enzymes involved in the degradation of seed reserves.

Keywords: groundnut, accelerated ageing, seed viability, amylases, lipase

#### Introduction

Groundnut (*Arachis hypogaea* L.) "King of oilseed crops" is believed to be native of Brazil (South America). It belongs to the family Leguminoceae. It is the 13<sup>th</sup> most important food crop, 4<sup>th</sup> most important source of edible oil and 3<sup>rd</sup> most important source of vegetable protein in the world. Groundnut possess high oil content (44-56%) and protein (22-30%) (Holaday and Pearson, 1974) <sup>[11]</sup> And is also a valuable source of vitamins E, K and B. It is a richest plant source of thiamine and niacin, which are low in cereals. Groundnut seed has short life and loses viability quickly under ambient condition. Groundnut seeds are more sensitive to storage conditions like high temperature, high seed moisture content and light exposure. Changes in the metabolic activities associated with seed deterioration are highly complex and poorly understood (Mc. Donald, 1999) <sup>[18]</sup>. Accelerated ageing technique has great potential for understanding the mechanism of ageing and associated deterioration process of seeds. The process of deterioration under accelerated ageing conditions are essential similar to those under normal condition, however, the major difference is that the rate of deterioration is much faster, this making it possible to study within reasonable time frame.

A change in enzyme activity in seeds due to ageing is a topic of scientific importance. Certain anabolic enzymes help in maintaining viability while some catabolic enzymes decrease viability. The free radical induced non-enzymatic peroxidation, which has the potential to damage membrane, is likely to be a primary cause of deterioration of stored seeds. Hence the present study was undertaken to elucidate some of the physiological and biochemical changes associated with ageing of seed of two groundnut genotypes.

#### Material and methods

The fresh pods of two genotypes of groundnut namely GPBD-5 and DH-86 harvested during summer of 2016 were obtained from NSP (National Seed Project) seed unit, University of Agriculture Sciences, Dharwad, Karnataka for present experiment. For accelerated ageing the pods and kernels were exposed to a temperature of 45 °C and 90 % RH (Byrd and Delouche, 1971)<sup>[7]</sup> for various time intervals (0, 2, 4, 6, 8 days). Seeds which were not exposed to the ageing treatment were referred as '0 days'. After each treatment, seeds were bench dried at room temperature, sealed in aluminium seed pouches and stored at 4° C till the experiment

analysis. The seeds were subjected to accelerated ageing treatments, from each lot four replications of 100 seeds were used for the experiment.

#### Seed germination (%)

The germinability of fresh seeds as well as those subjected to accelerated ageing was determined by incubating at  $25\pm1^{\circ}$ C; seeds sterilized with 0.1% HgCl<sub>2</sub> followed by repeated washings with sterile distilled water, in four replicates were kept in between germination papers saturated with distilled water. The evaluation of the normal seedlings was done on the 10<sup>th</sup> day as prescribed by ISTA (1985)<sup>[12]</sup>.

# **Total soluble protein**

One gram of seeds was crushed in pre-chilled pestle and mortar using 5 mL of water. The extract was centrifuged at 10000 rpm for 15 minute to collect the supernatant. The supernatant was used to quantify the protein content employing the method described by Lowry *et al.* (1951)<sup>[15]</sup>.

#### **Total soluble sugars**

#### Sample extraction and Acid Hydrolysis

One gram seed sample was crushed with 10 ml of 80 percent ethanol using mortar and pestle. The extract was filtered through whatman's No. 1 filter paper. One ml of filtrate was pipetted out in test tube and kept in hot water bath for evaporation till the alcoholic smell is lost. After evaporation, volume was made up to 5 ml with distilled water to get alcohol free extract. 1 ml of alcohol free extract was taken in test tube and mixed with 1 ml of 1 N HCl and boiled the mixture for 2-3 minutes. The hydrolysate was cooled and 1-2 drops of phenolphthalein indicator was added and neutralised with 1 N NaOH till pink colour was developed. 0.1 N HCl was used for reneutralisation till the discolouration of pink colour occurred. The hrdrolysate was made up to 5ml with distilled water. Further analysis of total soluble sugars was done by Nelson-Somogyi (1945) [21] using the hydrolysate. The total soluble sugars was expressed as mg per gram dry weight.

# Total oil content (%)

The analysis of oil content was done by Soxhlet extraction method. The oil collected after the extraction was transferred into a measuring cylinder which was placed over water bath for 30 min at 70 °C to ensure complete evaporation of solvent and volume and weight of the oil was recorded and expressed as oil content (%) (Warra *et al.*, 2011) <sup>[29]</sup>.

Oil content (%) = 
$$\frac{\text{Weight of the oil}}{\text{Weight of the sample}} \times 100$$

#### **Estimation of phenols**

A quantity of 5 g of grain powder was homogenized in 50 mL of 80% methanol, stirred continuously for 24 h at room temperature (Sadasivam and Manikam, 1992) <sup>[27]</sup>. The colorimetric method (Bray and Thorpe, 1954.) <sup>[7]</sup> was used for determination of total phenols using the Folin-Ciocalteu reagent. Total phenolic content was estimated based on the gallic acid standard curve (0, 20, 40, 60, 80, and 100  $\mu$ g/mL) and expressed as mg gallic acid equivalents (GAE) / g dry matter.

#### $\beta$ – amylase activity

β-amylase was extracted from germinating seeds and growing

seedlings groundnut seeds in 66 mM phosphate buffer (pH 7.0) containing 0.05M NaCl. The extract was centrifuged at 20,000 rpm for 15 min. The supernatant was used as a source of enzyme. All operations was done at 4°C.  $\beta$ - amylase activity of crude extracts was determined using the method of Clowick and Kaplan (1955)<sup>[8]</sup>.

### Lipase activity

Aged germinated whole seeds were collected and the seed coats were removed manually and 20 gram seed cotyledons were homogenized in chilled acetone at 4°C. The suspension was centrifuge at 3000 rpm and residue obtained was dissolved in 100 ml distilled water followed by centrifugation at 7500 rpm. The supernatant was used as a source of crude enzyme and was precipitated by ammonium sulphate (80% saturation) according to Michael *et al.* (2001) <sup>[19]</sup>. The titrimetric method of Maliks *et al.* (2000) <sup>[17]</sup> was used for determination of lipase activity.

#### **Results and Discussion**

#### Seed germination %

Germination percentage differed significantly among the genotypes in accelerated ageing test. The highest percentage of germination was obtained at 0 and 2<sup>nd</sup> day in both the genotypes and it decreased linearly as the ageing accelerates. In the pods, the highest percentage of germination was recorded at 0 days in GPBD-5 (98.75 %) and DH-86 (96.97 %). After 2 to 8 days of accelerated ageing, the seed germination percentage was decreased from 92.92 per cent to 50.45 per cent in GPBD-5 and 90.55 per cent to 48.18 per cent in DH-86. A mean decrease of 49.60 per cent in germination percentage was observed during the period (0-8 days) of ageing, in both the genotypes. A similar trend was observed in kernels storage form (Table 1A and 1B).

 Table 1A: Effect of accelerated ageing on seed germination (%) of different groundnut genotypes aged as pods

Construng		Days of accelerated ageing (AA)					
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	98.75	92.92	78.64	67.93	50.45	77.74	
DH-86	96.97	90.55	75.97	63.54	48.18	75.04	
Mean	97.86	91.73	77.31	65.73	49.32		
	Genotype		Days of AA		Intera	Interaction	
S. Em. ±	0.	14	0.22 0.1		0.3	31	
C. D. @ 0.01	0.	55	0.87		1.23		
C. V. (%)			0.6	59			

 Table 1B: Effect of accelerated ageing on seed germination (%) of different groundnut genotypes aged as kernels

Construng	Days of accelerated ageing (AA)						
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	98.75	86.41	74.48	63.21	46.89	74.00	
DH-86	96.97	87.98	71.08	60.16	44.60	72.16	
Mean	97.86	88.54	72.78	61.68	45.88		
	Geno	otype	Days of AA		Interaction		
S. Em. ±	0.11		0.18		0.25		
C. D. @ 0.01	0.45		0.71		1.00		
C. V. (%)			0.4	58			

The germination percentage of GPBD-5 pods was higher (5.05 %) than the kernels, similarly, in DH-86 it was (3.9 %) more in pods than the kernels. The germination percentage was observed to be highest during the early days in pods of GPBD-5 under accelerated ageing. Similar results of decrease in percentage of germination due to ageing in seeds storage was reported by Sarwad (1999)<sup>[28]</sup> in groundnut varieties. The

decrease in seed germination percentage was related to chromosomal aberrations that occur under long storage conditions (Kerter *et al.*, 1997)<sup>[14]</sup>. Decrease of germination percentage in aged seeds can be due to reduction of  $\alpha$ -amylase activity, carbohydrate contents (Bailly, 2004)<sup>[3]</sup> or denaturation of proteins (Nautiyal *et al.*, 1985)<sup>[20]</sup>. Maity *et al.*, (2000)<sup>[16]</sup> suggested that seeds deteriorate during storage and ageing is manifested as a reduction in percentage germination, resulting in less and weak seedlings. During the ageing process, seeds lose their vigour, ability to germinate and ultimately become less viable.

#### Total soluble sugars (TSS)

The results of total soluble sugar content (TSS) of two groundnut genotypes as influenced by accelerated ageing (AA) are presented in Table 2A and 2B. TSS differed significantly among the genotypes in accelerated ageing test. The lowest TSS was obtained at 0 and 2<sup>nd</sup> days of ageing of the seeds in both the genotypes and it increased linearly with the increasing days of accelerated ageing. In the pods, the lowest TSS was recorded at 0 days in GPBD-5 (105.53 mg/g) and DH-86 (109.17 mg/g). After 2 to 8 days of accelerated ageing, the TSS was increased from 125.00 mg/g to 184.00 mg/g in GPBD-5 and 131.20 mg/g to 194.40 mg/g in DH-86. A mean increase of 76.24 per cent in TSS was observed during the period (0-8 days) of ageing, in both the genotypes. A similar trend was observed in kernels storage form.

**Table 2A:** Effect of accelerated ageing on total soluble sugars(mg/g) in seeds of different groundnut genotypes aged as pods

Construng	Days of accelerated ageing (AA)					
Genotype	0 days	2 days	4 days	6 days	8 days	Mean
GPBD-5	105.53	125.00	138.63	152.63	184.00	141.16
DH-86	109.17	131.20	145.60	162.43	194.40	148.56
Mean	107.35	128.10	142.12	157.53	189.20	
	Genotype		Days of AA		Interaction	
S. Em. ±	0.:	50	0.	79	1.12	
C. D. @ 0.01	2.0	02	3.19		4.51	
C. V. (%)			1.	34		

**Table 2B:** Effect of accelerated ageing on total soluble sugars (mg/g) in seeds of different groundnut genotypes aged as kernels

Construng		Days of accelerated ageing (AA)					
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	105.53	119.07	127.77	147.53	165.73	133.13	
DH-86	109.17	124.40	131.77	151.73	172.40	137.89	
Mean	107.35	121.73	129.77	149.63	169.07		
	Genotype		Days of AA		Interaction		
S. Em. ±	0.1	21	0.1	32	2 0.46		
C. D. @ 0.01	0.83		1.31		1.85		
C. V. (%)			0.:	58			

The TSS of GPBD-5 pods were higher (6.03 %) than the kernels, similarly, in DH-86 it was (7.73 %) more in pods than the kernels. A similar observation was reported by Rao *et al.* (1970) <sup>[25]</sup> in groundnut. In groundnut the increase in TSS content under accelerated ageing is most likely due to impaired respiration. During seed storage it is documented that respiratory changes do occur which lead to various metabolic deficiency (Anderson and Baker, 1983) <sup>[1]</sup>. A decline in respiration rate due to ageing have been reported by Edje and Burris (1970) <sup>[9]</sup>; Woodstock *et al.* (1984) <sup>[30]</sup> in soybean.

### Total soluble protein (TSP)

The results of total soluble protein content (TSP) of two

groundnut genotypes as influenced by accelerated ageing (AA) are presented in Table 3A and 3B. In both genotypes there is a significant variation in total soluble protein content between genotypes. In the pods, the lowest TSP was recorded at 0 days in GPBD-5 (161.33 mg/g) and DH-86 (157.67 mg/g). After 2 to 8 days of accelerated ageing, the TSP was decreased from 153.67 mg/g to 142.00 mg/g in GPBD-5 and 150.33 mg/g to 141.69 mg/g in DH-86. A mean increase of 48.97 per cent in TSP was observed during the period (0-8 days) of ageing, in both the genotypes. A similar trend was observed in kernels storage form.

Construng	Days of accelerated ageing (AA)						
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	161.33	153.67	149.20	146.07	142.00	150.45	
DH-86	157.67	150.33	149.80	144.67	141.00	148.69	
Mean	159.50	152.00	149.50	145.37	141.50		
	Geno	otype	Days of AA		Interaction		
S. Em. ±	0.32		0.	0.71		0.50	
C. D. @ 0.01	1.27		2.85		2.01		
C. V. (%)			0.	81			

**Table 3A:** Effect of accelerated ageing on total soluble protein (mg/g) in seeds of different groundnut genotypes aged as pods

Table 3B: Effe	ect of accelerated	ageing on total	soluble protei	n
(mg/g) in seeds	of different groun	idnut genotypes	aged as kerne	els

Construns		Days of	of accelerated ageing (AA)				
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	161.33	152.67	144.67	144.97	141.63	149.05	
DH-86	157.67	148.67	147.67	143.93	139.67	147.52	
Mean	159.50	150.65	146.17	144.45	140.65		
	Geno	otype	Days of AA		Interaction		
S. Em. ±	0.	26	0.41		0.59		
C. D. @ 0.01	1.	1.05		1.67		2.36	
C. V. (%)			0.	68			

The TSP of GPBD-5 pods was 0.94 % higher than the kernels, similarly, in DH-86 it was 0.79 per cent more in pods than the kernels. Similar results of decline in protein content was also observed by Pallavi *et al.* (2003) <sup>[22]</sup> on their studies in fresh and aged seeds of sunflower. The decline in protein content in the genotype GPBD-5 might be due to the denaturation of protein or the decrease in the rate of photosynthesis during the process of ageing (Roberts, 1972) <sup>[26]</sup>. Gunstone (2001) <sup>[10]</sup> reported that in seeds of rapeseed the process of protein synthesis was affected by accelerated ageing and concluded that on ageing the rate of protein synthesis is decreased.

#### Total oil content (%)

When seeds were stored in accelerated condition for the period of 2 to 8 days it was seen that there is decrease in the oil content and a significant variation in seeds of both the genotypes. A progressive decrease in the seed oil content was observed with time and the decline was more conspicuous between the periods of 6 to 8 days of accelerated ageing. In the pods, the highest oil content was recorded at 0 days in GPBD-5 (46.27 %) and DH-86 (45.43 %). After 2 to 8 days of accelerated ageing, the per cent age oil content was increased from 45.77 per cent to 44.37 per cent in GPBD-5 and 45.17 per cent to 43.07 per cent in DH-86. A mean decrease of 4.64 per cent in oil content was observed during the period (0-8 days) of ageing, in both the genotypes. A similar trend was observed in kernels storage form. (Table 4A and 4B).

Table 4	4A: Effect of acce	elerated ageing	on total oil con	ntent (%) in
5	seeds of different	groundnut geno	otypes aged as	pods

Construe	Days of accelerated ageing (AA)						
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	46.27	45.77	45.30	45.11	44.37	45.36	
DH-86	45.43	45.17	44.77	44.23	43.07	44.53	
Mean	45.85	45.47	45.03	44.67	43.72		
	Geno	otype	Days	of AA	Interaction		
S. Em. ±	0.	10	0.16		0.23		
C. D. @ 0.01	0.42		0.66		0.93		
C. V. (%)			0.8	39			

 Table 4B: Effect of accelerated ageing on total oil content (%) in seeds of different groundnut genotypes aged as kernels

Construng	]	Days of accelerated ageing (AA)					
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	46.27	45.60	45.31	45.41	44.32	45.38	
DH-86	45.43	45.21	44.64	44.20	43.43	44.59	
Mean	45.85	45.41	44.98	44.81	43.88		
	Geno	otype	Days of AA		Interaction		
S. Em. ±	0.	04	0.07		0.10		
C. D. @ 0.01	0.	18	0.28		0.40		
C. V. (%)			0.3	38			

The total oil content of GPBD-5 pods was lowest (0.04 %) than the kernels, similarly, in DH-86 it was 0.13 per cent lower in pods than the kernels. With the increase in the days of ageing the decrease in oil content in seeds was also reported by Panayotov and Stoeva (2000)<sup>[23]</sup>. The decreased level of total oil content in the seeds during ageing may be due to lipid oxidation which is the major cause of oil and fat deterioration due to formation of hydro peroxides resulting from the reaction between oxygen and the unsaturated fatty acids (Araujo, 2004)<sup>[2]</sup>.

#### **Estimation of phenols**

The total phenol content of two groundnut genotypes were influenced by accelerating ageing (AA) and the results shows the increase in phenol content in both genotypes are presented in Table 5A and 5B. In the pods, the lowest phenol content (9.37 mg.GAE.g<sup>-1</sup>dry wt.) was recorded at 0 days in GPBD-5 while DH-86 recorded (10.30 mg.GAE.g<sup>-1</sup>dry wt.). After 2 to 8 days of accelerated ageing, the total phenol content was increased from 10.27 mg.GAE.g<sup>-1</sup>dry wt. to 13.63 mg.GAE.g<sup>-1</sup>dry wt. in GPBD-5 and 11.50 mg.GAE.g<sup>-1</sup>dry wt. to 14.83 mg.GAE.g<sup>-1</sup>dry wt. in DH-86. A mean increase of 0.44 per cent was observed during the period (0-8 days of ageing) respectively. A similar trend was observed in kernels storage form.

 Table 5A: Effect of accelerated ageing on total phenol content

 (mg.GAE.g<sup>-1</sup> dry weight) in seeds of different groundnut genotypes

 aged as pods

Construng	]	Days of accelerated ageing (AA)					
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	9.37	10.27	11.47	12.63	13.63	11.47	
DH-86	10.30	11.50	12.24	13.97	14.83	12.57	
Mean	9.83	10.88	11.85	13.30	14.23		
	Geno	Genotype		Days of AA		Interaction	
S. Em. ±	0.	15	0.24		0.33		
C. D. @ 0.01	0.60		0.95		1.34		
C. V. (%)			4.	8			

 

 Table 5B: Effect of accelerated ageing on total phenol content (mg.GAE.g<sup>-1</sup> dry weight) in seeds of different groundnut genotypes aged as kernels

Construng		Days of	ys of accelerated ageing (AA)				
Genotype	0 days	2 days	4 days	6 days	8 days	Mean	
GPBD-5	9.37	9.90	11.36	12.19	13.01	11.17	
DH-86	10.30	11.07	12.18	13.33	14.18	12.21	
Mean	9.83	10.48	11.77	12.76	13.59		
	Geno	otype	Days of AA		Interaction		
S. Em. ±	0.	16	0.25		0.36		
C. D. @ 0.01	0.65		1.02		1.45		
C. V. (%)		5.32					

The total phenol content of GPBD-5 pods was 2.68 % highest than the kernels, similarly, in DH-86 it was 2.94 per cent more in pods than the kernels. The total phenol content in the seeds of groundnut genotypes increased under accelerated ageing which may be attributed to cellular deterioration during ageing leading to its greater permeability for the cellular and subcellular content. Bewly and Black (1994) <sup>[5]</sup> reported that phenolic compounds may inhibit seed germination and cause dormancy by inhibiting cell elongation or they may deprive the embryo of oxygen because of consumption of oxygen for their oxidation.

#### β – amylase activity

β-amylase activity of two groundnut genotypes were influenced by accelerating ageing (AA) and the results shows the decrease in activity in both genotypes are presented in Table 6A and 6B. In the pods, the highest β-amylase activity (1.48 µmol/min/mg of protein) was recorded at 0 days in GPBD-5 while DH-86 recorded (1.44 µmol/min/mg of protein). At 2 to 8 days of accelerated ageing, the β-amylase activity was increased from 1.48 µmol/min/mg of protein to 1.50 µmol/min/mg of protein in GPBD-5 and 1.44 µmol/min/mg of protein to 1.49 µmol/min/mg of protein in DH-86, after that it decreased linearly. A mean increase of 2.73 per cent and then decrease of 38.66 per cent in β-amylase activity was observed during the earlier days of ageing and during the later period (4-8 days of ageing) respectively. A similar trend was observed in kernels storage form.

Table 6A: Effect of accelerated ageing on  $\beta$ -amylase activity (µmol/min/mg of protein) indifferent groundnut genotypes aged as pods

Construes	Days of accelerated ageing(AA)					
Genotype	0 days	2 days	4 days	6 days	8 days	Mean
GPBD-5	1.48	1.50	1.22	1.14	0.98	1.26
DH-86	1.44	1.49	1.19	0.97	0.87	1.19
Mean	1.46	1.50	1.21	1.06	0.92	
	Genotype		Days of AA		Interaction	
S.Em. ±	0.007		0.011		0.015	
C. D. @0.01	0.027		0.043		0.061	
C. V. (%)	2.13					

Table 6B: Effect of accelerated ageing on  $\beta$ -amylase activity ( $\mu$ mol/min/mg of protein) in different groundnut genotypes aged as kernels

Construe	Days of accelerated ageing(AA)					
Genotype	0 days	2 days	4 days	6 days	8 days	Mean
GPBD-5	1.48	1.48	1.19	1.07	0.92	1.23
DH-86	1.44	1.41	1.19	0.97	0.84	1.17
Mean	1.46	1.49	1.19	1.02	0.88	
	Genotype		Days of AA		Interaction	
S.Em. ±	0.003		0.009		0.012	
C. D. @0.01	0.022		0.033		0.049	
C. V. (%)	1.13					

Accelerated ageing didn't produce significant difference in the  $\beta$ -amylase activity between the pod and kernel storage. Similar results were observed by Rao and Wagle (1981)<sup>[24]</sup>. Bhanuprakash *et al.* (2010)<sup>[6]</sup> reported similar findings of decrease in the  $\alpha$ -amylase and dehydrogenase activity with ageing duration in onion seeds. The loss in activity of  $\beta$ amylase during ageing might be due to changes in phospholipid and membrane damage due to peroxidative changes associated with ageing (Beck and Ziegler, 1989)<sup>[4]</sup>.

#### Lipase activity

The lipase activity of two groundnut genotypes were influenced by accelerated ageing (AA) and the results shows the increase in activity in both genotypes are presented in Tables 7A and 7B. In the pods, the lowest lipase activity was recorded at 0 days in GPBD-5 (0.51  $\mu$  eq. of free fatty acid released/ min) and DH-86 (0.50  $\mu$  eq. of free fatty acid released/ min). After 2 to 8 days of accelerated ageing, the lipase activity was increased from 0.57  $\mu$  eq. of free fatty acid released/ min to 0.76  $\mu$  eq. of free fatty acid released/ min to 0.56  $\mu$  eq. of free fatty acid released/ min to 0.56  $\mu$  eq. of free fatty acid released/ min to 0.75  $\mu$  eq. of free fatty acid released/ min to 0.76  $\mu$  eq. of free fatty acid released/ min to 0.75  $\mu$  eq. of free fatty

**Table 7A:** Effect of accelerated ageing on lipase activity ( $\mu$  eq. offree fatty acid released/min assay) in different groundnut genotypesaged as pods

Genotype	Days of accelerated ageing(AA)					
	0 days	2 days	4 days	6 days	8 days	Mean
GPBD-5	0.51	0.57	0.63	0.65	0.76	0.62
DH-86	0.50	0.56	0.61	0.63	0.75	0.61
Mean	0.50	0.57	0.62	0.64	0.76	
	Genotype		Days of AA		Interaction	
S.Em. ±	0.002		0.003		0.005	
C. D. @0.01	0.009		0.014		0.020	
C. V. (%)	1.38					

**Table 7B:** Effect of accelerated ageing on lipase activity ( $\mu$  eq. offree fatty acid released/min assay) in different groundnut genotypesaged as kernels

Genotype	Days of accelerated ageing(AA)					
	0 days	2 days	4 days	6 days	8 days	Mean
GPBD-5	0.51	0.56	0.62	0.65	0.72	0.61
DH-86	0.50	0.52	0.63	0.63	0.74	0.60
Means	0.50	0.54	0.63	0.64	0.73	
	Genotype		Days of AA		Interaction	
S.Em. ±	0.002		0.004		0.004	
C. D. @0.01	0.008		0.018		0.017	
C. V. (%)	1.19					

The increase in activity of lipase during ageing might be due to the secretion of fungal lipase of the storage fungi which increased the rate of lipid degradation. Such type of increase in lipase enzyme activity was noticed in stored groundnut seeds by Kakde and Chavan (2011)<sup>[13]</sup>.

### Conclusions

The present experiment gives a clear picture of the effect of accelerated ageing on the mode of deterioration in the seeds of groundnut genotypes when stored in pod and kernel form. The comparison between pod and kernels storage of both groundnut genotypes revealed that there is superiority of pod over kernels storage for all seed quality attributes. Under accelerated ageing condition, the study revealed that among the two genotypes of groundnut GPBD-5 was superior over DH-86 in all the seed quality parameters and biochemical analysis throughout the storage period.

#### References

- 1. Anderson JD, Baker JE. Deterioration of seeds during aging. Phytopathology. 1983; 78(2):321-325.
- 2. Araujo JMA. Quimica de alimentos: Teoria e pratica. Viçosa. UFV, 2004, 416.
- 3. Bailly C. Active oxygen species and antioxidants in seed biology. Seed Sci Res. 2004; 14:93-107.
- 4. Beck E, Ziegler P. Biosynthesis and degradation of starch in higher plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1989; 40:95-117.
- Bewley JD, Black M. Cellular events during germination and seedling growth. Eds. Seed Physiology of Development and Germination. Plenum Press, New York, 1994, 147-197.
- Bhanuprakash K. Yogeesha HS, Arun MN. Physiological and biochemical changes in relation to seed quality in ageing Bell pepper. Ind. J Agric. Sci. 2010; 80(9):9-11.
- Byrd HW, Delouche TC. Deterioration of soybean seed in storage. Proceedings of the Association of Official Seed Analysis. 1971; 61:41-57.
- 8. Colowick SP, Kaplan NO. Methods of Enzymology. Academic Press. Inc. New York. 1955; 49(2):379.
- 9. Edje OT and Burris JA. Physiological and biochemical changes in deteriorating soybean seeds. Proc. Assoc. Off. Seed Anal. 1970; 68:158-166.
- Gunstone F. Production and consumption of rapeseed oil on a global scale. European J Lipid Sci. Technol. 2001; 103:447-449.
- 11. Holaday CE, Pearson JL. Effects of genotype and production area on the fatty acid composition, otal oil and total protein in peanuts. J Food Sci. 1974; 39:1206-1209.
- 12. International Seed Testing Association (ISTA). International rules for seed testing. Rules 1985. Seed Sci. Technol. 1985; 13:299-355.
- 13. Kakde RB, Chavan AM. Extracellular Lipase Enzyme Production by Seed-borne Fungi Under the Influenc of Physical factors. Intern. J Biol. 2011; 3(1):94-100.
- Kerter ST, Geneve RL, Houtz RL. Priming and accelerated aging affect L-isoaspartylmethyltransferase activity in tomato *Lycopersicones culentum* Mill. seed. J Experimental Bot. 1997; 48:943-949.
- 15. Lowry OH, Roserbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-phenol reagent. J Bio. Chem. 1951; 193:265-275.
- 16. Mait S, Banerjee G, Roy M, Pal C, Pal B, Chakrabarti D, *et al.* Chemical induced prolongation of seed viability and stress tolerance capacity of mung bean seedlings. Seed Sci. Technol. 2000; 28:155-162.
- Maliks SV, Kalia V and Pundir CS. Immobilization of porcine pancreas lipase on zirconia coated alkylamine glass using glutaraldehyde. Indian J Chem. Technol. 2000; 7:64-67.
- 18. Mc Donald MB. Seed deterioration: Physiology, repair and assessment. Seed sci. Technol. 1999; 27:177-237.
- Michael JH, Cichowicz DJ, Dierov JK. Lipolytic activity of California-Laurel (*Umbellularia californica*) seeds. J Am. Oil Chem. Soc. 2001; 78:1067-1071.
- 20. Nautiyal AR, Thapliyal AP, Purohit AN, Seed viability in Sal-IV. Protein changes accompanying loss of viability in *Shorea robusta*. Seed Sci. Technol. 1985; 13:83-86.
- 21. Nelson A, Somogyi M. Determination of reducing sugars by Nelson-Samogyi method in Sadasivam S. and

Manickam A. Biochemical Methods for Agricultural Sciences, Wiley Estern Ltd. New Delhi, 1945-1992.

- 22. Pallavi MS, Kumar SS, Singh KS, Reddy AV. Effect of seed ageing on physiological, biochemical and yield attributes in sunflower (*Helianthus annuus* L.) cv. Morden. Seed Res. 2003; 31(2):161-168.
- 23. Panayotov N, Stoeva N. Viability and some physiological indices of seeds of different ages of vegetable species of pepper. Capsicum and Egg plant Newslett. 2000; 19:111-114.
- Rao AS, Wagle DS. β-amylase activity in artificially aged soybean seeds. Bio logia Plantarum. 1981; 23(1):24-27.
- 25. Rao MRK, Sreeramulu N, Ran IM. Respiratory rate and food reserves in TMV-2 groundnut seeds (*Arachis hypogaea* L) in relation to loss of viability due to storage. Andhra Agric. J. 1970; 17(1):27-29.
- 26. Roberts EH. Storage environment and the control of viability. Viability of Seeds, London: Chapman and Hall Limited, 1972, 1-58.
- 27. Sadasivam S, Manikam A. Biochemical Methods for Agricultural Sciences, Wiley Eastern Limited, New Delhi, 1992, 150-151.
- Sarwad RK. Effect of accelerated ageing and seed treatment on storability of groundnut (*Arachis hypogeal* L.) varieties. M.Sc (Agri.) Thesis, Uni. Agric. Sci. Dharwad, 1999.
- 29. Warra AA, Wawata IG, Gunu SY, Aujaka KM. Extraction and physicochemical Analysis of some selected northern Nigeria, industrial oils, Archives Appl. Sci. Res. 2011; 3:536-541.
- 30. Woodstock LW. Furman K, Solomos T. Changes in respiratory metabolisrn during aging in seeds and isolated axes of soybean. Plant Cell Physiol. 1984; 25:15-26.