International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 2386-2393

Received: 23-07-2017
Accepted: 27-08-2017

Bhaskaran M
Tamil Nadu Open University, Chennai, Tamil Nadu, India

R Jerlin
Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

T Venupriya
Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Bhaskaran M
Tamil Nadu Open University, Chennai, India

Understanding biochemical basis of seed deterioration in groundnut

Bhaskaran M, R Jerlin and T Venupriya

Abstract
Groundnut kernels deteriorate rapidly mainly due to chemical composition and moisture content. In this study, activity of hydrolytic enzymes and antioxidant enzymes was assessed in kernels of contrasting ground nut genotypes differing in their dormancy, pre-storage treatments and storage conditions. Kernels of ground nut genotypes stored under cold conditions registered significantly lower electrical conductivity then the ones stored under ambient conditions irrespective of pre-storage treatments. Kernels of dormant ground nut variety VRI 7 treated with halogen impregnated powder and stored under cold conditions showed lowest electrical conductivity than other treatments. Dormant genotype VRI 7 was found to possess significantly reduced activity of proteases than the non-dormant CO 7. Lipase activity and lipid perodixation were found to be less in dormant ground nut genotype VRI 7 (0.440 and 1.635) and halopolymer treatment was found to exhibit significant effect on this (0.041 and 1.650).

Keywords: Groundnut, kernel, dormancy, storage, biochemical, antioxidant

Introduction
Seed deterioration is an undesirable event associated with various cellular, metabolic and chemical alterations including chromosomal aberrations and damage to DNA, impairment of RNA and protein synthesis, changes in the enzymes and food reserves and loss of membrane integrity (Kibinza *et al.,* 2006) [15]. In groundnut, several biochemical and physiological changes occur during storage due to the presence of high fat and protein content, alternation in transcription and translation process (Shelar *et al.,* 2008; Walters *et al.,* 2010) [30, 32]. Accumulation of reactive oxygen species (ROS) in seed tissues plays an important role in the loss of seed viability during storage. Halogens are compounds that help to prevent seed deterioration and halogenations dry treatment was shown to exhibit beneficial effect of prolonging the shelf life of groundnut pods (Murugan, 1981 and Bindu Mathew, 1996) [20, 5]. Hence, this study is intended to gain information on the effect of pre-storage treatments and storage conditions on antioxidant enzymes involved in quenching of reactive oxygen species during the storage period.

Materials and Methods
Genetically pure seeds of groundnut varieties (CO 7, a non-dormant genotype and VRI (Gn) 7, a dormant cultivar) were obtained from Agricultural Research Station, Vaigai Dam, Tamil Nadu, and India and used. Graded seeds were subjected to seed treatments including halogenations with iodine and seed coating with iodine based halo polymer. Talc powder was used as a carrier for iodine gas talc powder was impregnated with iodine @ 3 gkg^{-1} and the mixture was incubated for a week for diffusion of iodine vapours into the carrier. Seeds were later treated with halogen powder and halo polymer @ 3 g kg^{-1} kernels.

Treatments
Seed treatments
T_1 - Control (Kernel)
T_2 - Seed dressing with halogen impregnated powder @ 3 g kg^{-1} of seeds
T_3 - Halo polymer treatment @ 3 g kg^{-1} of seeds
T_4 - Control (pods)

Varieties
V_1 - Dormant - VRI (Gn) 7

V_2 - Non dormant CO 7

Storage Condition

S_1 - Ambient (28 + 2^0C; 55 %)
S_2 - Cold (4 + 2^0C; 30 %)

Following biochemical assays were carried out to study the biochemical basis of seed deterioration in groundnut.
Electrical conductivity (Presley, 1958) [25]
Dehydrogenase activity (Kittock and Law, 1968) [16].
Oil content (Sadasivam and Manickam, 1995) [27].
Lipase activity (Jayaraman, 1981) [12]
Lipid Peroxidation (Bernheim *et al.,* (1948) [3]
Protein content (Alikhan and Young, 1973) [2]
Protease activity (Naithani, 1987) [21]
α-amylase activity (Paul *et al*., 1970) [23]

Results and Discussion

Increased production of free radicals, changes in the levels of free radical scavenging enzymes, degradation of proteins and DNA and increased accumulation of free amino acids were attributed to reduced germination and seedling vigour during ageing (McDonald, 1999) [18]. Decrease in enzymatic activity in stored seeds with increase in storage time led to reduction in germination and vigour (Khan *et al*., 2013) [2]. In this study, dormant variety (VRI (Gn) 7 maintained lesser electrical conductivity than non-dormant variety at the end of storage period under ambient storage conditions and the difference was 0.11. Dormant genotype VRI 7 would have started to deteriorate 45-60 days after harvest and seed coat of dormant variety was highly compact than non-dormant variety at the end of storage period leading to lesser accumulation of leachates. Lesser electrical conductivity was coincided with the seeds treated with halo-polymer than untreated control (Table 1). This was due to the weakening of cell membrane that has been ascribed to be the cause for leaching of metabolites and electrolytes through the semi-permeable membrane into the imbibing medium. The halogens by counteracting the free radical chain propagation reaction and consequent stabilization of lipo-protein moiety maintained the membrane integrity and as a result minimized the leakage of electrolytes from the cell.

Stabilization of membrane integrity and scavenging of free radicals by the halogens may be responsible for the relatively lower electrical conductivity of the halogen slurry treated seeds groundnut kernel (Sathiya Narayanan and Prakash, 2015) [28]. The increased electrical conductivity of other treatments may be due to faster deterioration of cell membrane which leads to auto-oxidation of poly unsaturated fatty acids in the membrane involving free radical chain reactions (Doijode, 1988) [7]. Irrespective of varieties and treatments, kernels stored under cold storage conditions registered lower electrical conductivity than the kernels stored under ambient conditions. This may be due to maintenance of biochemical activity in kernels stored under cold storage conditions whereas integrity of cell membranes would have weakened in kernels stored under ambient conditions.

Dehydrogenase enzyme is an indication of living tissue and is directly related with loss of viability. The dehydrogenase enzyme activity is a good stable metabolic marker to estimate the degree of vigour in seeds (Saxena *et al.,* 1987) [29]. The dehydrogenase enzyme activity was higher in dormant variety than non-dormant variety. The percentage increase was 9 %. This was due to the fact that deterioration was slower in dormant variety than non-dormant variety. The higher dehydrogenase enzyme activity was maintained in halo-polymer treated kernels (0.612 OD value) than untreated control (0.587 OD value) and the difference was 0.025 (Fig 1). Irrespective of varieties and treatments, the cold storage conditions had slightly higher dehydrogenase enzyme activity than ambient by 0.006. This indicated slower deterioration of living tissue under cold storage conditions. This was supported by Nithya (2012) [22] in sunflower, groundnut and soybean. The dehydrogenase enzyme was decreased from 0.620 to 0.580 with progressively advancement of storage period.

In oilseed crops, oil content is one of the important factors influencing deterioration of seed quality and viability. Seeds rich in oil have limited longevity due to their specific chemical composition. Oil content, composition and degradation pattern will be altered significantly due to storage conditions especially temperature and relative humidity (Koutroubas *et al*., 2000) [17]. In the present study, non-dormant genotype CO 7 had higher oil content than dormant variety irrespective of treatments and storage conditions (Fig 2). The halo-polymer treated kernels were found to maintain oil content better than control and other treatments (Fig 2). It may be due to stabilization of unsaturated fatty acid compound of lipoprotein membranes through halogen treatment and rendering them less susceptible to peroxidase change (Jenifer Sandhya, 2015) [13].

Lipases are key enzymes involved in mobilization of oil bodies during seed germination which catalyses the cleavage of carboxyl ester bonds of TAGs, releasing free fatty acids (FFAs) and glycerol (Feussner *et al*., 2001) [8]. The present study revealed that dormant variety had lesser lipase enzyme activity than non-dormant genotype (Fig 3). This might be due to the fact that deterioration rate was slower in dormant variety. Halo-polymer treated seeds recorded reduced lipase activity than untreated control by 0.008 (Fig 3). This might be because of the reduction in the process of lipid peroxidation by halogens so stability of poly unsaturated fatty acid had maintained. Lipid peroxidation and the generation of free radicals may contribute to seed deterioration. Peroxidation of unsaturated lipids affecting bio membrane permeability resulting in enhanced solute leakage are cited as the most probable sequence of deteriorative changes in seed (McDonald 1999) [18]. In the present study the lipid peroxidation was lower in dormant variety over non dormant variety. Lipid peroxidation was found to be reduced significantly during cold storage conditions when compared to ambient storage conditions. This might be due the slow cellular activity in cold storage. With the advancement of storage period, the lipid peroxidation activity was increased at the end of storage period (Fig 4).

Proteases are enzymes involved in hydrolysis of proteins into amino acids and responsible for degradation of proteins during storage. Reduction in protein content of seeds during ageing is coinciding with increase in protease activity. In the present investigation, protein content was higher in dormant variety than non-dormant variety (Table 2) where seed deterioration was faster. Irrespective of varieties and storage conditions, the halo-polymer treatment registered higher protein content and lesser protease activity (Table 3).

α-amylase is an important enzyme playing a vital role during germination of seeds which degrades the complex starch, maltose and release energy in the form of ATP, which is utilized by emerging seedlings (Bewley and Black, 1978) [4] and it is one of the enzymes which directly indicates the germination capability of the seeds. In the present

investigation the dormant variety maintained higher α-amylase activity (13.19) than non-dormant variety (12.54). This might be due to depletion of food reserve materials present in seeds was lesser in dormant variety. The halo-polymer treated kernels had higher α-amylase activity than untreated control (Table 4).

Groundnut kernels deteriorate faster under ambient storage conditions and such processes are slower in dormant varieties compared to non-dormant varieties. Further, the seed quality can be maintained by treating the kernels with halo-polymer that quenches the ROS which are generated during the seed deterioration.

Table 1: Effect of seed dormancy, halogenation and storage condition on electrical conductivity (dSm^{-1}) of groundnut seeds during storage

Varieties (V)	Treatments (T)	Ambient storage (S1)					Cold storage (S2)					Treatments Mean
		Period of storage (months)										
		P0	P2	P4	P6	Mean	P0	P2	P4	P6	Mean	
V1 (Dormant)	**T1**	**0.31**	**0.4**	**0.56**	**0.65**	**0.48**	**0.31**	**0.36**	**0.51**	**0.62**	**0.45**	**0.47**
	T2	0.31	0.36	0.45	0.61	0.43	0.31	0.34	0.41	0.56	0.41	0.42
	T3	0.31	0.33	0.39	0.52	0.38	0.31	0.31	0.35	0.47	0.36	0.37
	T4	0.31	0.35	0.42	0.57	0.41	0.31	0.33	0.39	0.52	0.39	0.40
	Mean	0.31	0.36	0.46	0.59	0.43	0.31	0.34	0.42	0.54	0.40	0.42
V2 (Non dormant)	T1	0.43	0.53	0.65	0.76	0.60	0.43	0.49	0.60	0.71	0.56	0.58
	T2	0.43	0.49	0.56	0.67	0.53	0.43	0.45	0.51	0.62	0.50	0.52
	T3	0.43	0.45	0.49	0.59	0.48	0.43	0.44	0.47	0.51	0.46	0.47
	T4	0.43	0.47	0.53	0.60	0.51	0.43	0.46	0.50	0.54	0.48	0.50
	Mean	0.43	0.49	0.56	0.65	0.53	0.43	0.46	0.52	0.60	0.50	0.52
Grand mean		0.37	0.42	0.51	0.62	0.48	0.37	0.40	0.47	0.57	0.45	0.47
T1		T 2	T3	T4	S1	S2	V1	V2	P0	P2	P4	P6
Mean	0.53	0.47	0.42	0.45	0.48	0.45	0.42	0.52	0.37	0.41	0.49	0.60

	V	T	S	P	VT	VS	VP	TS	TP	SP	VTS	VTP	VSP	TSP	VTSP
SEd	0.001	0.002	0.001	0.002	0.024	0.03	0.024	0.02	0.004	0.003	0.004	0.006	0.004	0.006	0.008
CD (P=0.05)	0.003	0.004	0.003	0.004	0.049	NS	0.049	NS	0.008	0.006	NS	0.012	NS	NS	NS

Legends

1 – Control; T2 – Halogen (Iodine) impregnated powder at 3 g kg^{-1}; T3- Halo polymer at 3 g kg^{-1}; T4- Pod without treatment
V1 (Dormant) – VRI (Gn) 7; V2 (Non dormant) – CO 7.

Table 2. Effect of seed dormancy, halogenation and storage condition on protein content (%) of groundnut seeds during storage

Varieties (V)	Treatments (T)	Ambient storage (S1)					Cold storage (S2)					Treatments Mean
		Period of storage (P months)										
		P0	P2	P4	P6	Mean	P0	P2	P4	P6	Mean	
V1 (Dormant)	**T1**	**26.8**	**26.2**	**25.1**	**24.2**	**25.6**	**26.8**	**26.4**	**25.3**	**24.6**	**25.8**	**25.7**
	T2	26.8	26.5	25.5	24.6	25.9	26.8	26.6	25.9	25.1	26.1	26.0
	T3	26.8	26.6	26.0	25.1	26.1	26.8	26.8	26.3	25.7	26.4	26.3
	T4	26.8	26.6	25.9	25	26.0	26.8	26.6	26.1	25.3	26.2	26.1
	Mean	26.8	26.5	25.6	24.7	25.9	26.8	26.6	25.9	25.1	26.1	26.0
V2 (Non dormant)	T1	26.0	25.4	24.1	23.3	24.7	26.0	25.6	24.5	23.8	24.9	24.8
	T2	26.0	25.7	24.8	23.9	25.1	26.0	25.9	25.2	24.5	25.4	25.2
	T3	26.0	25.8	25.1	24.4	25.3	26.0	26.0	25.6	24.9	25.6	25.5
	T4	26.0	25.7	25.0	24.2	25.2	26.0	26.0	25.4	24.6	25.5	25.3
	Mean	26.0	25.7	24.7	23.9	25.0	26.0	25.8	25.1	24.4	25.3	25.1
Grand mean		26.4	26.1	25.2	24.3	25.4	26.4	26.2	25.5	24.7	25.7	25.5
T1		T 2	T3	T4	S1	S2	V1	V2	P0	P2	P4	P6
Mean	25.2	25.6	25.9	25.7	25.4	25.7	26.0	25.1	26.4	26.1	25.3	24.5

	V	T	S	P	VT	VS	VP	TS	TP	SP	VTS	VTP	VSP	TSP	VTSP
SEd	0.095	0.134	0.095	0.134	1.523	2.153	1.523	1.523	0.269	0.190	0.269	0.380	0.269	0.380	0.538
CD (P=0.05)	0.188	0.266	0.188	0.266	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Legends

T1 – Control; T2 – Halogen (Iodine) impregnated powder at 3 g kg^{-1}; T3- Halopolymer at 3 g kg^{-1}; T4- Pod without treatment V1 (Dormant) – VRI (Gn) 7; V2 (Non dormant) – Co 7.

Table 3: Effect of seed dormancy, halogenation and storage condition on protease activity (OD value) of groundnut seeds during storage

Varieties (V)	Treatments (T)	Ambient storage (S1)					Cold storage (S2)					Treatments Mean
		Period of storage (P months)										
		P0	P2	P4	P6	Mean	P0	P2	P4	P6	Mean	
V1 (Dormant)	**T1**	**0.018**	**0.023**	**0.029**	**0.041**	**0.028**	**0.018**	**0.022**	**0.027**	**0.040**	**0.027**	**0.028**
	T2	0.018	0.020	0.025	0.035	0.025	0.018	0.019	0.023	0.030	0.023	0.024
	T3	0.018	0.019	0.022	0.029	0.022	0.018	0.018	0.020	0.026	0.021	0.021
	T4	0.018	0.020	0.023	0.033	0.024	0.018	0.019	0.021	0.030	0.022	0.023
	Mean	0.018	0.021	0.025	0.035	0.024	0.018	0.020	0.023	0.032	0.023	0.023
	T1	0.022	0.030	0.039	0.056	0.037	0.022	0.030	0.037	0.053	0.036	0.037

V2 (Non dormant)	T2	0.022	0.025	0.032	0.044	0.031	0.022	0.023	0.032	0.042	0.030	0.030
	T3	0.022	0.020	0.028	0.040	0.028	0.022	0.022	0.026	0.037	0.027	0.027
	T4	0.022	0.024	0.030	0.043	0.030	0.022	0.023	0.028	0.040	0.028	0.029
	Mean	0.022	0.025	0.032	0.046	0.031	0.022	0.025	0.027	0.028	0.030	0.031
Grand mean		0.020	0.023	0.028	0.041	0.028	0.020	0.023	0.025	0.030	0.027	0.027
T1		T 2	T3	T4	S1	S2	V1	V2	P0	P2	P4	P6
Mean	0.032	0.027	0.024	0.026	0.028	0.027	0.023	0.031	0.020	0.023	0.027	0.036

	V	T	S	P	VT	VS	VP	TS	TP	SP	VTS	VTP	VSP	TSP	VTSP
SEd	0.0001	0.0001	0.0001	0.0001	0.0017	0.0017	0.0017	0.0017	0.0003	0.0002	0.0003	0.0004	0.0003	0.0004	0.0006
CD (P=0.05)	0.0002	0.0003	0.0003	0.0003	0.0034	NS	0.0034	NS	0.0006	0.0004	NS	0.0008	NS	0.0008	0.0012

Legends

T1 – Control; T2 – Halogen (Iodine) impregnated powder at 3 g kg^{-1}; T3- Halopolymer at 3 g kg^{-1}; T4- Pod without treatment V1 (Dormant) – VRI (Gn) 7; V2 (Non dormant) – Co 7.

Table 4: Effect of seed dormancy, halogenation and storage condition on α-amylase activity (mg of maltose min^{-1}) of groundnut seeds during storage

Varieties (V)	**Treatments (T)**	**Ambient storage (S1)**					**Cold storage (S2)**					**Treatments Mean**
		Period of storage (P months)										
		P0	**P2**	**P4**	**P6**	**Mean**	**P0**	**P2**	**P4**	**P6**	**Mean**	
V1 (Dormant)	T1	13.42	13.20	12.94	12.53	13.02	13.42	13.31	13.02	12.64	13.10	13.06
	T2	13.42	13.25	13.10	12.86	13.16	13.42	13.33	13.16	12.93	13.21	13.18
	T3	13.42	13.39	13.26	13.01	13.27	13.42	13.41	13.33	13.10	13.32	13.29
	T4	13.42	13.36	13.18	12.92	13.22	13.42	13.39	13.23	13.05	13.27	13.24
	Mean	13.42	13.30	13.12	12.83	13.17	13.42	13.36	13.19	12.93	13.22	13.19
V2 (Non dormant)	T1	13.03	12.70	12.36	11.12	12.30	13.03	12.88	12.42	12.18	12.63	12.46
	T2	13.03	12.83	12.67	12.30	12.71	13.03	12.89	12.75	12.57	12.81	12.76
	T3	13.03	13.00	12.86	12.60	12.87	13.03	13.02	12.98	12.76	12.95	12.91
	T4	13.03	12.90	12.78	12.52	12.81	13.03	12.98	12.86	12.63	12.88	12.84
	Mean	13.03	12.86	12.67	12.14	12.67	13.03	12.94	12.75	12.54	12.82	12.74
Grand mean		13.22	13.08	12.89	12.48	12.92	13.22	13.15	12.97	12.73	13.02	12.96
T1		T 2	T3	T4	S1	S2	V1	V2	P0	P2	P4	P6
Mean	12.76	12.97	13.10	13.04	12.92	13.02	13.19	12.74	13.22	13.11	12.93	12.60

	V	T	S	P	VT	VS	VP	TS	TP	SP	VTS	VTP	VSP	TSP	VTSP
SEd	0.049	0.070	0.049	0.070	0.797	1.127	0.797	0.797	0.140	0.099	0.140	0.199	0.140	0.199	0.281

CD (P=0.05)	0.098	0.140	0.098	0.140	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Legends

T1 – Control; T2 – Halogen (Iodine) impregnated powder at 3 g kg^{-1}; T3- Halopolymer at 3 g kg^{-1}; T4- Pod without treatment V1 (Dormant) – VRI (Gn) 7; V2 (Non dormant) – Co 7.

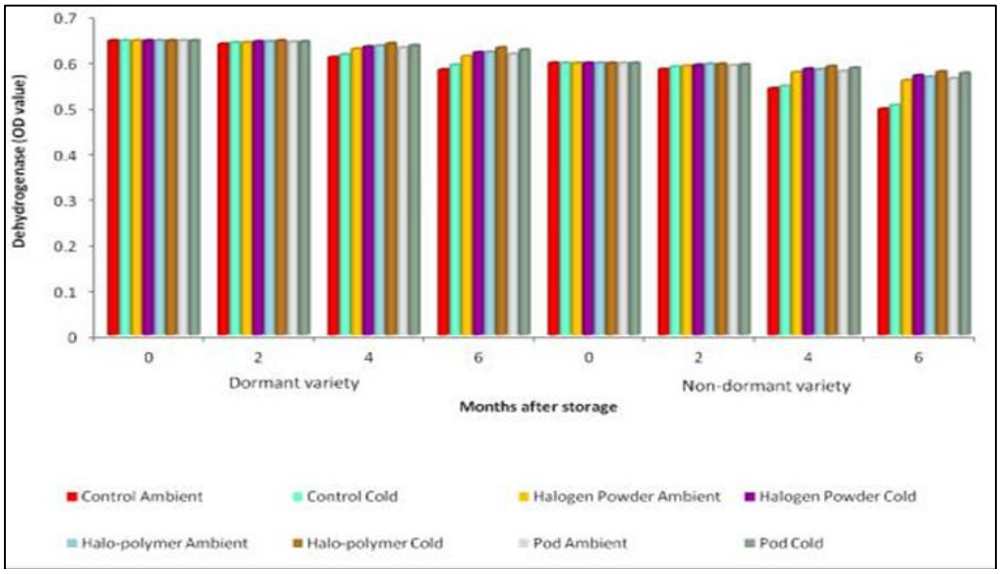

Fig 1: Effect of seed dormancy, halogenation and storage condition on dehydrogenase activity (OD value) of groundnut seeds during storage

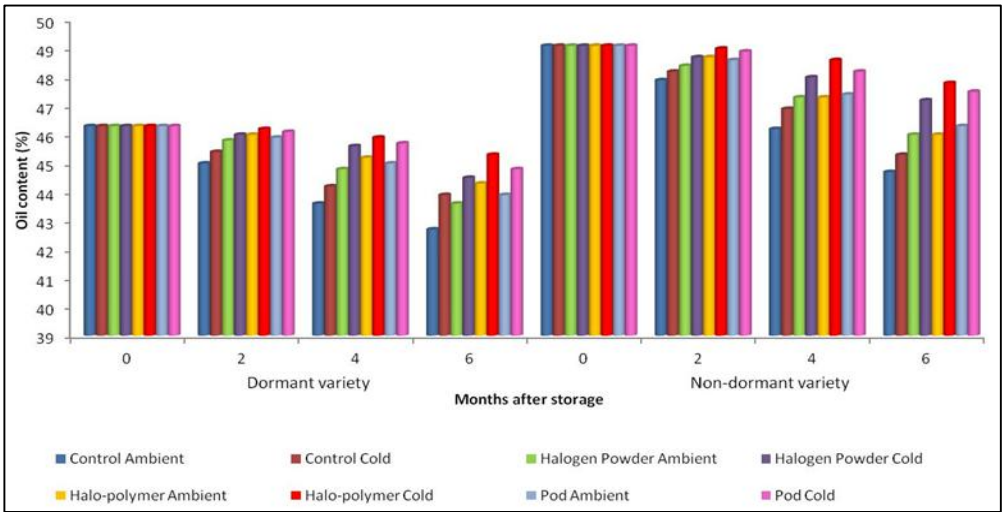

Fig 2: Effect of seed dormancy, halogenation and storage condition on oil content (%) of groundnut seeds during storage

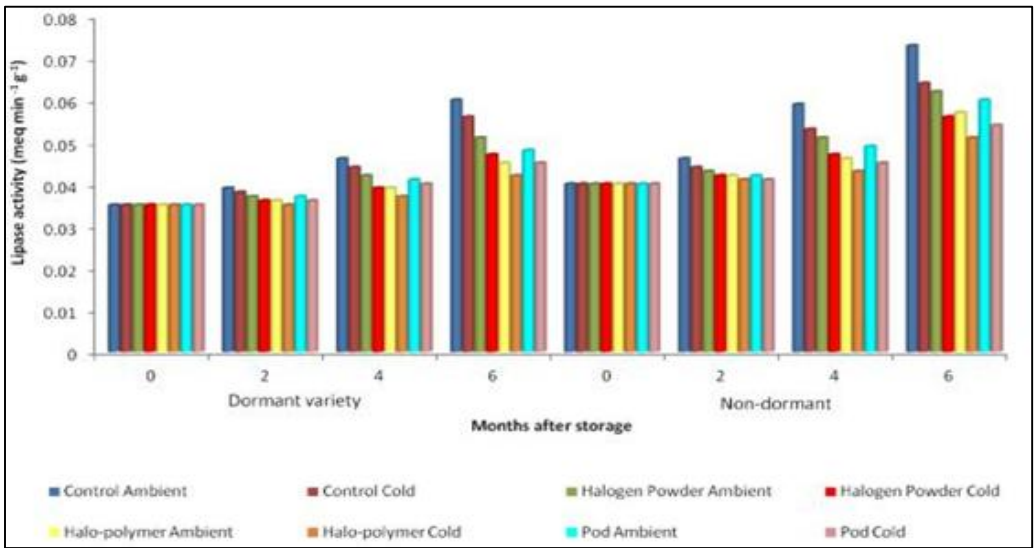

Fig 3: Effect of seed dormancy, halogenation and storage condition on lipase activity (meq min^{-1} g^{-1}) of groundnut seeds during storage

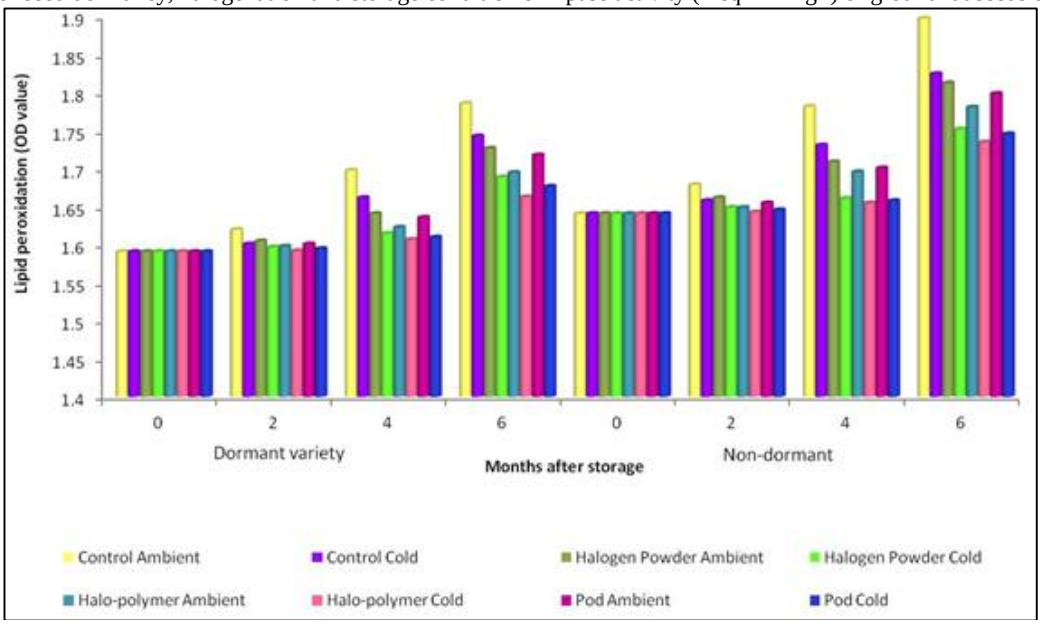

Fig 4: Effect of seed dormancy, halogenation and storage condition on lipid peroxidation (OD value) of groundnut seeds during storage

References

1. Aebi H. Catalase in vitro. Meth Enzymol. 1984; 105:121-126.
2. Alikhan ST, CG Young. Variation in protein content of field peas, Canadian J. Plant Sci. 1973; 53:37-41.
3. Bernheim F, ML Bernheim, KM Wilbur. The reaction between thiobarbituric acid and the oxidation products of certain lipides. J. Biol. Chem. 1948; 174(1):257-264.
4. Bewley JD, M Black. Physiology of development and germination. New York, 1994, 445-447.
5. Bindu Mathew. Evaluation of groundnut (*Arachis hypogaea* L.) genotypes for storability and vigour. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, 1996.
6. Demirkaya MK, J Dietz, HO Sivritepe. Change in antioxidant enzymes deterioration as related to moisture content during ageing, energy metabolism and deterioration: Standing the test of time. Plant Sci. 2010; 179:565-573.
7. Doijode SD. Effect of storage environment on brinjal (*Solanum melongena*) seed viability. Prog. Hortic. 1988; 20(3, 4):292-293.
8. Feussner I, H Kuhn, C Wasterneck. Lipoxygenase-dependant degradation of storage lipids. Trends Plant Sci. 2001; 6:268-73.
9. Gidrol X, A Noubhani, B Mocquot, A Fournier, A Pradet. Effect of accelerated aging on protein synthesis in two legume seeds. Plant Physiol. Biochem. 1998; 26:281-288.
10. Gidrol X, H Serghini, A Noubhani, B Mocoquot, P Mazliak. Biochemical changes induced by accelerated ageing in sunflower seeds. In: Lipid peroxidation and membrane damage. Physiologia Plantarum, 1989; 76:591-597.
11. Goel A, IS Sheoran. Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. J. Plant Physiol, 2003, 1093-1100.
12. Jayaraman J. In: Laboratory manual in Biochemistry. Willey Eastern Limited, New Delhi, 1981,133.
13. Jenifer Sandhya. Studies on mechanism of seed deterioration in groundnut kernel. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, 2015.
14. Khan AM, H Khan, R Khan, A Aziz. Decrease in enzymatic activity of stored seeds to estimate seed quality and predict field emergence in wheat. American J. Plant Physiol. 2013; 4:242-246.
15. Kibinza S, D Vinel, D Come, C Bailly, F Corbineau. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. Physiologia Plantarum. 2006; 128(3):496-506.
16. Kittock DL, AG Law. Relationship of seed vigour to respiration and tetrazolium reduction by germinating wheat seeds. Agronomy J. 1968; 60:286-288.
17. Koutroubas SD, DK Papakosta, A Doitsinis. Water requirements for castor oil crop (*Ricinus communis* L.) in a Mediterranean climate. Crop Sci. 2000; 40(1):33-41.
18. McDonald MB. Seed deterioration: physiology, repair and assessment. Seed Sci. & Technol. 1999; 27:177-237.
19. Murthy UMN, PP Kumar, WQ Sun. Mechanisms of seed ageing under different storage conditions for (*Vigna radiata* L.) Wilczek: Lipid peroxidation, sugar hydrolysis, maillard reactions and their relationship to glass state transition. J. Exp. Botany. 2003; 54:1057-1067.
20. Murugan V. Seed quality studies in groundnut (*Arachis hypogaea* L.) cv. TMV 7. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore. 1981.
21. Naithani SC. The rate of IAA oxidase, peroxidase and poly phenol oxidase in the fibre initiation on the cotton ovule. Beitr. Biologie Pflanzen. 1987; 62:79-90.
22. Nithya N. Studies on physiological and biochemical basis of cold storage of groundnut, sunflower and soybean seeds. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore. 2012.
23. Paul AK, S Mukherji, SM Sircar. Metabolic changes in rice seeds during storage. Int. J. Agric. Sci. 1970; 40(12):1031-1036.
24. Perur NG. Measurement of peroxidase activity in plant leaf tissue. Curr. Sci. 1962; 31(1):17.
25. Presley JT. Relationship of protoplast permeability of cotton seed viability and pre disposition of seedling disease. Plant Disease Reporter. 1958; 42:582.

26. Pukacka S, E Ratajczak. Age-related biochemical changes during storage of beech (*Fagus sylvatica* L.) seeds. Seed Sci. Res. 2007; 17:45-53.
27. Sadasivam S, A Manickam. Proteins and enzymes. In: Biochemical methods for agricultural sciences, Wiley Eastern Ltd., New Delhi, 1995, 33-143.
28. Sathiya Narayanan G, M Prakash. Influence of physical seed enhancement techniques on storability of sesame (*Sesamum indicum* L.) cv. VRI 1. Plant Archives. 2015; 15(1):459-463.
29. Saxena OP, G Singh, T Pakeeraiah, N Pandey. Seed deterioration studies in some vegetable seeds. Acta Horticulture, 1987; 215:39-44.
30. Shelar VR, RS Shaikhand, AS Nikam. Soybean seed quality during storage: a review. Agric. Reviews, 2008; 29(2):25-131.
31. Ushimaru T, S Kanematsu, M Katayama, H Tsuji. Antioxicidative enzymes in seedling of *Nelumbo nucifera* germinated under water. Physiologia Plantarum, 2001, 39-46.
32. Walters C, D Ballesteros, VA Vertucci. Structural mechanics of seed deterioration: Standing the test of time. Plant Sci. 2010; (179):565–573.