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Seed physiological and biochemical parameters of soybean (*Glycine max*) *as* influenced by different packaging materials and storage conditions

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Abstract
A lab experiment was carried out to study that seed physiological and biochemical parameters of soybean (*Glycine max*) *as* Influence by different packaging materials and storage conditions. Soybean seeds were stored in different packaging materials *viz*., gunny bags and high density polythene bags and vacuum packed bags stored at room temperature (25±2 ºC) and cold storage (4±1 ºC) for a period of 18 months. The treatments having six combinations and consisting of different containers *viz*., gunny bags, high density polythene bags and vacuum packed bags were replicated four times in both cold and ambient storage conditions in completely randomized design. The results of the study revealed that the least fluctuations in all seed biochemical parameters *viz.,* oil content and enzyme activity such as α-amylase, lipase and protease and seed physiological parameters such as mineral content (Cu, Mn, Zn and Mn), moisture content and electrical conductivity values were recorded in vacuum packed seeds than gunny, HDPE bags for soybean seeds stored under cold storage compared to room temperature throughout the storage period. Among the containers, the seeds stored in vacuum packed bags maintained the seed biochemical and seed physiological parameters with least deterioration compared to seeds stored in gunny bags and high density polythene bags.

Keywords: Moisture content, electrical conductivity, mineral content, biochemical parameters, oil content, vacuum packaging and cold storage

Introduction

Soybean [*Glycine max* (L.) Merill] is one of the most important protein and oil seed crops throughout the world. Its oil is the largest component of the world's edible oils. Soybean seed contains 20% oil and 40% protein. The world production of edible oils consists of 30% soybean. It is an ingredient of more than 50% of the world's high protein meal. The United States of America has the largest area under soybean cultivation with the highest yield and production (Anon., 1996) [56]. Soybean is globally grown over an area of 91.40 m ha, with a production of 204.00 m tons and a productivity of 2233 kg per ha (Anon., 2010) [3].

In agriculture, seed is a vehicle to deliver almost all agro-based technological innovations so that the farmers can exploit the genetic potential of new varieties. The availability, access and use of seeds of adaptable varieties are, therefore, the major determinants to attain the efficiency and productivity of other packages like irrigation, fertilizers and pesticides. This is one of the vital keys to increase crop production, enhance food security and alleviate rural poverty in the developing countries. Research on storability of seeds in India is of recent origin with the development of organized seed production and marketing. It is stipulated that 80 per cent of certified seeds produced in India require storage for one planting season and 20 per cent of seeds is carried over for subsequent sowing (Balasubramanyam *et al*., 1983) [6]. However, when the awareness and infrastructure is developed, substantial quantity of seeds can be stored for few planting seasons as a safeguard against monsoon failure and as a precaution against production of poor quality seeds. Seed deterioration has been ascribed to physical, physiological, bio-chemical and pathological detrimental changes occurring in seeds leading to death and has been characterized as inexorable, irreversible, inevitable, and minimal at the time of physiological maturity and variable among kinds of seeds, varieties and seed lots (Delouche *et al.,* 1973) [13]. The poor storability of the seeds of soybean is accounted for its high oil content, physiological fragility and thin seed coat, which leads to rapid loss of viability and vigour in storage, which inturn results in poor establishment of the crop in the field and low productivity. Research concerning to these aspects is very meagre. Keeping above these aspects in the view and considering their importance in maintaining viability for longer period the present investigation was carried out study that seed physiological and biochemical parameters of soybean (*Glycine max*) *as* Influence by different packaging materials and storage conditions.

Materials and Methods

A storage experiment was carried out under the laboratory for a period of 18 months at Department of Crop Physiology, University of Agricultural Sciences, Dharwad. In this research work, seeds of the soybean variety i.e. JS-335 collected from MARS, Seed Unit, were dried under sun and stored under different storage conditions and containers. The temperature maintained in the cold storage was around (4 °C±1 °C) and relative humidity was 65 to 70 per cent throughout the storage period while, for ambient storage, bags were stored in the laboratory at room temperature (25±2 °C). Soybean seeds were packed in 500 g vacuum packed bags (The machine used for vacuum packaging of different seeds was OLPACK 501/V manufactured by Interprise–Brussels S.A., Bruxtainer Division, Belgium) and 15 kg to gunny and high density polythene bags. After packaging of all the seeds in different containers, 50% bags were stored properly in the iron racks without stacking so that all the bags were uniformly exposed to the particular treatment condition; while 50% bags were stored under cold storage. The treatments having six combinations and consisting of different containers *viz*., gunny bags, high density polythene bags and vacuum packed bags were replicated four times in both cold and ambient storage conditions in completely randomised design. The observations on various seed physiological parameters *viz*., electrical conductivity (Presley, 1958) [39], and the per cent moisture were obtained by using MB 45 Halogen Moisture Analyzer from Ohaus, USA, while estimation of mineral content i.e. Copper (Cu), Zinc (Zn), Iron (Fe) and Manganese (Mn) contents in seed were estimated using Atomic Absorption Spectrophotometer (AAS-4141, Electronic Corporation of India Ltd.), oil content in seeds was estimated by the Soxhlet apparatus which was later on modified by Randall (1974) [44] and determination of all the biochemical parameters i.e. α-amylase activity, lipase enzyme activity and protease enzyme activity was estimated by methods devised by Bernfeld (1955) [55], Jayaraman (1981) [23] and Poulle and Jones, (1988) [40]**,** respectively at bimonthly interval upto18 months of storage. The analysis and interpretation of the experimental data was done as suggested by Panse and Sukhatme (1967) [38] with level of significance used as P=0.01.

Results and Discussion

Seed physiological parameters

Moisture content (%)

The moisture content of soybean seeds as influenced by different packaging materials shown in Table 1 was found to be differed significantly with storage period as well as storage conditions. At the initial period of storage (i.e., 2 months) the maximum moisture content (15.30%) was observed in seeds stored gunny bags at cold storage followed by high density polythene bags (12.97%), but it differed significantly with those stored at room temperature (10.98 and 10.87%), respectively; while minimum moisture content (9.39 and 9.37%) was recorded in vacuum packed bags kept at room temperature and cold storage and it did not differ significantly to each other. These results are in agreement with the findings of Davidson *et al.* (1982) [52] in shelled peanuts, Rajendraprasad *et al.* (1998) [42] in groundnut kernels, and Monira *et al.* (2012) [27] in soybean storability for longer period.

Electrical Conductivity (d Sm^{-1})

The electrical conductivity of vacuum packed seeds was less and a steady increase was seen with advancement in storage period. While, gunny bags as well as high density polythene packed seeds recorded higher EC values from early stage to 18 months of storage at room temperature (Table, 2). The end of storage period (18 months), the vacuum packed seeds recorded lower significantly lower values of electrical conductivity (0.534 and 0.537 dSm^{-1}) at cold storage and room temperature, significantly higher values of electrical conductivity (0.747 dSm^{-1}) were recorded in gunny bags at room temperature, and they were on par with high density polythene bags (0.733 dSm^{-1}). The values of electrical conductivity of seeds stored in vacuum packed seeds did not differ significantly in cold storage and at room temperature was not differed while the values of electrical conductivity was differed significantly in gunny bags as well as in high density polythene bags upto 18 months of storage. Similar results were obtained by Narayanaswamy *et al.* (1993) [34] in groundnut, Shanmugavel *et al.* (1995) [48] in soybean and Nataraj *et al.* (2011) [33] in sunflower.

Mineral’s Content

Copper Content (Cu, ppm)

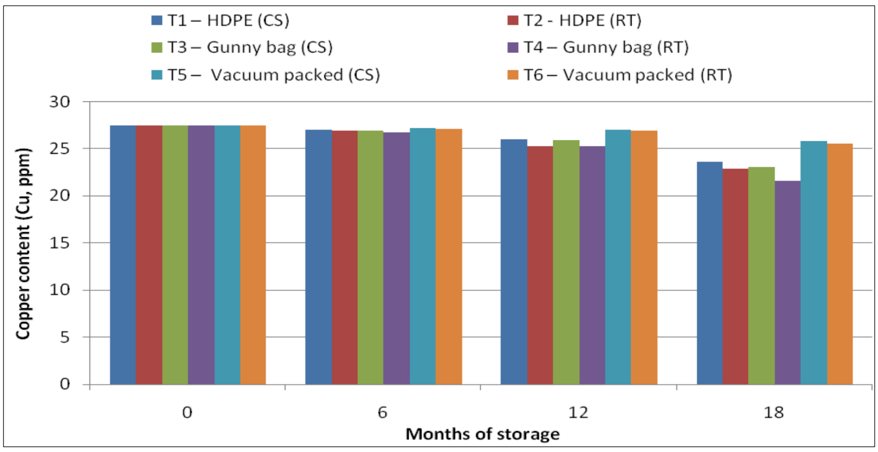

Fig 1: Influence of packaging and storage conditions copper content (Cu, ppm) at different time intervals of storage in soybean seeds

The observation on copper content (ppm) of soybean seeds as influenced by different packaging and storage conditions (Fig. 1) exhibited significant differences at all the stage period. There was a decline in copper content from 4th months and continued upto 18 months of storage in HDPE bag seeds stored under room temperature. At 18 months of storage period, the vacuum packed seeds recorded significantly higher copper content (21.33 and 21.25 ppm) when kept at cold storage and room temperature as compared to gunny bags (19.60 and 19.27 ppm) followed by HDPE bags (18.53 and 18.30 ppm), respectively.

Iron content (Fe, ppm)

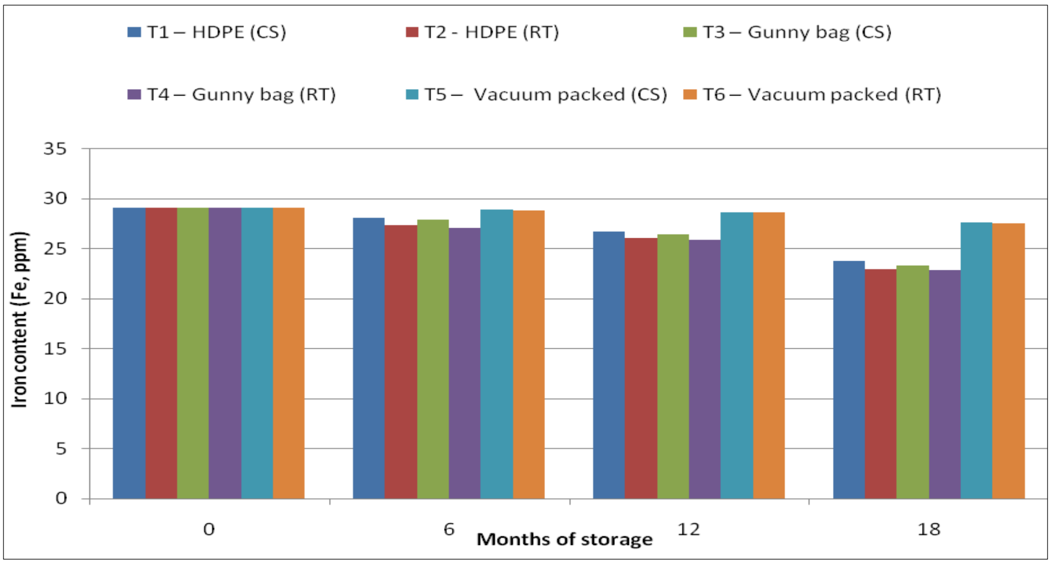

Fig 2: Influence of packaging and storage conditions iron content (Fe, ppm) at different time intervals of storage in soybean seeds

The iron content (ppm) of soybean seeds differed significantly between treatments and there was a gradual decline with progress in storage period (Fig. 2). At eight months of storage, significantly higher iron content (63.88 ppm) was recorded in vacuum packed seeds stored under cold storage and was on par with those stored under room temperature (63.44 ppm). Significantly lower iron content (59.87 ppm) was observed in gunny bag seeds under room temperature followed by high density polythene bags (61.10 ppm) stored at room temperature. The similar trend was observed at all the stages of storage even upto 18 months. Significantly higher iron content (62.95 ppm) was recorded in vacuum packed seeds stored under cold storage followed by stored under room temperature (62.91 ppm) at 18 months of storage. The lower iron content (53.80 ppm) was recorded in seeds stored in gunny bags followed by high density polythene bags (54.13 ppm).

Zinc content (Zn, ppm)

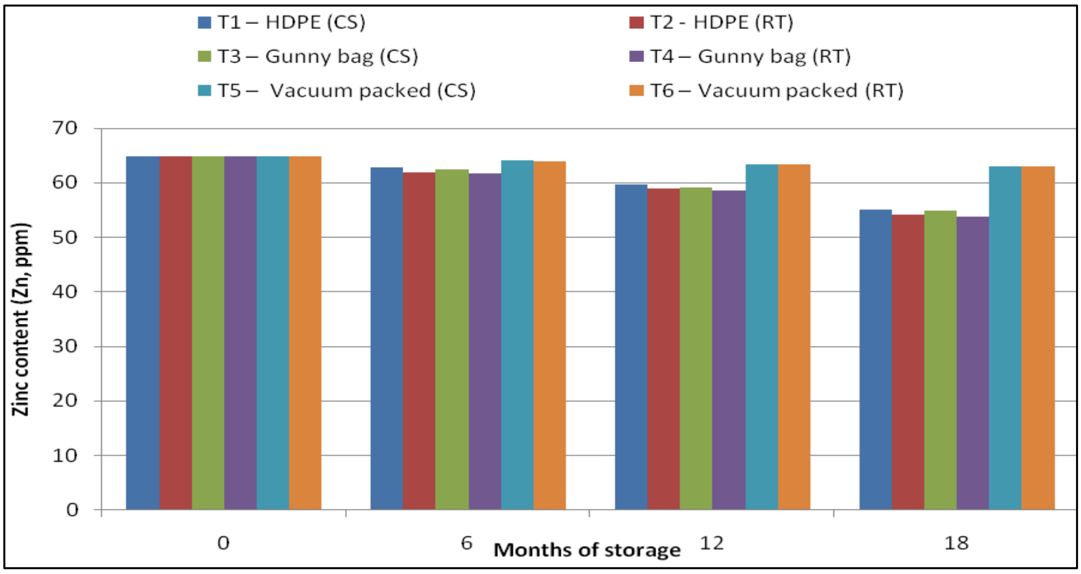

Fig 3: Influence of packaging and storage conditions zinc content (Zn, ppm) at different time intervals of storage in soybean seeds

The zinc content (ppm) of soybean seeds as influenced by different packaging and storage conditions indicated significant differences among the treatment during (Fig. 3). Significantly lower zinc content (31.87 and 32.20 ppm) was recorded in HDPE bag seeds kept under room temperature and cold storage at 10 months of storage period. While the significantly higher zinc content values (33.48 and 33.39 ppm) were recorded in vacuum packed seeds stored under cold storage and room temperature followed by gunny packed seeds (32.50 and 32.33 ppm), respectively. The similar trend was observed at all the stages of storage upto 18 months. The vacuum packed bags recorded significantly higher values of zinc content (32.78 and 32.70 ppm) kept under cold storage and room temperature compared to gunny bags (30.80 and 30.55 ppm) and HDPE bags (29.97 and 29.58 ppm), respectively.

Manganese content (Mn, ppm)

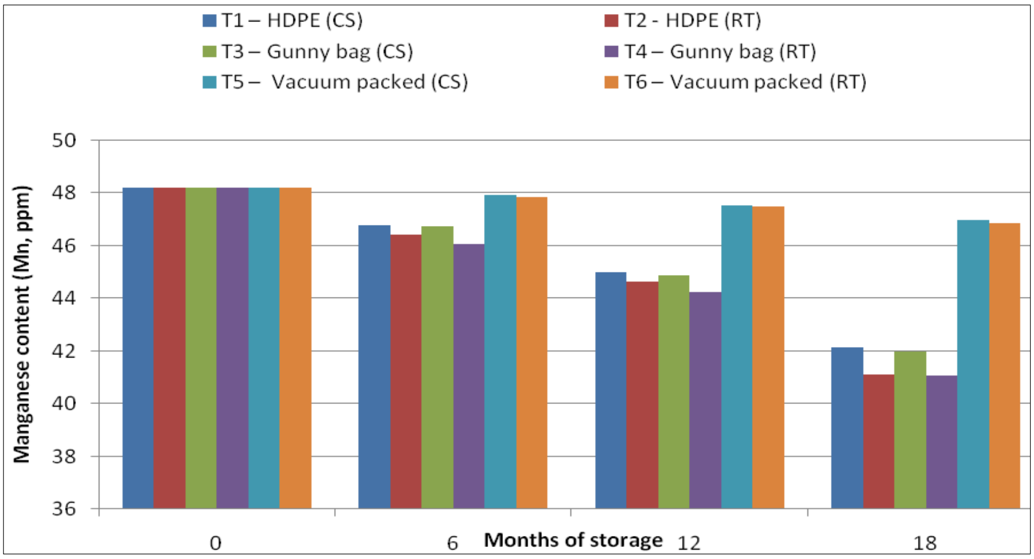

Fig 4: Influence of packaging and storage conditions on manganese content (Mn, ppm) at different time intervals of storage in soybean seeds

The manganese content (ppm) of soybean seeds as influenced by different packaging and storage condition differed significantly between the treatments during storage period (Fig. 4).

It is clear from the above results for all the mineral's content that the seeds stored in gunny bags under cold storage were on par with the high density polythene bags stored under cold storage similarly the gunny bags stored at room temperature was also on par with high density polythene bags stored at room temperature. The vacuum packed seeds could maintain higher mineral's content values throughout the storage period. The vacuum packed seeds could maintain higher manganese content values throughout the storage period. Mineral elements like zinc, copper, iron and manganese are very crucial for plant growth and human health, and play a key role in various physiological and biochemical processes. Results pertaining to mineral contents during study including copper, iron, zinc and manganese of soybean seeds showed gradual decrease with an advancement of storage period at all the stages of storage. Among the containers, the decrease in mineral content was very less in vacuum packed bags compared to HDPE, and gunny bags throughout the storage period under both ambient and cold storage. Variation in the mineral contents between the packaging materials could be attributed to redistribution of mineral elements in seeds and possible microbial contamination (Bognar *et al.*, 1990) [7]. In the HDPE bags mineral content values were higher than gunny bags, but it was lower than vacuum packed bags. Same results were obtained by Fagbohun and Faleye (2012) [17] in groundnut, Fagbohun *et al.* (2011) [19], in melon seeds, Echendu *et al.* (2009) [15] in cocoyam chips, Mata *et al.* (2003) [30], Perring and Pearson, (1987) [37], Deepa *et al.* (2011) [12] in chilli and Zagory and Kader (1989) [51] in green beans.

Higher reduction of iron content in HDPE and gunny bags may be attributed to its sensitivity to temperature. Kayisoglu and Demirci (2006) [24] reported that copper, iron, zinc and manganese contents in both conventional and vacuum packed bags were affected significantly and decreased with an increased storage period of 10 months. At 18 months of storage, vacuum packed bags recorded significantly higher values of copper, iron, zinc and manganese compared to HDPE, while significantly lower values were found in gunny bags stored under both ambient and cold storage. Similarly, Alinnor and Akalezi (2010) [4] also reported a decrease in Zn, Cu and Fe of cocoyam and white yam stored for six months. Higher mineral contents during cold storage compared to ambient storage could be attributed due to lower internal physiological and biochemical processes in the seed there by prolonging the shelf life of seeds during storage. Higher micronutrients in vacuum packed bags compared to gunny bags, may be due to decrease in ash content (Kayisoglu and Demirci, 2006) [24]. Similar findings were also reported in shelled melon seeds (Fagbohun *et al.*, 2011) [19], millet seeds (Fagbohun and Lawal, 2011) [19], cocoyam chips (Echendu *et al.*, 2009) [15], okra (Fagbohun *et al.*, 2011) [19], and greenbean (Perring and Pearson, 1987 and Zagory and Kader, 1989) [37, 51].

Seed biochemical parameters α-amylase activity (mg maltose released/min)

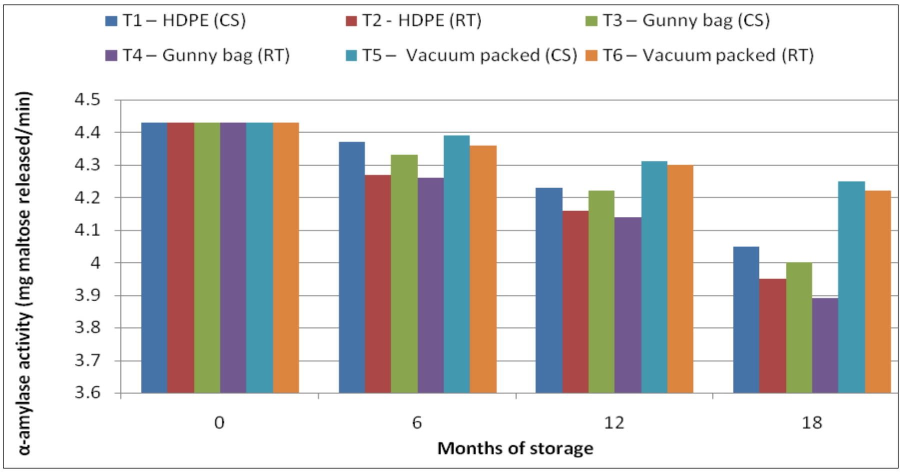

Fig 5: Influence of packaging and storage conditions on α-amylase activity (mg maltose released/min) in soybean seeds

The α-amylase activity (mg maltose released/min) of soybean seeds (Fig. 5) indicates significant differences among the treatments and it was considerably declined in gunny bag seeds and high density polythene bags from 10 months onwards and upto 18 months of storage. While, the α-amylase activity in vacuum packed seeds were found to be stable and slow decline was observed with progress in storage period. At the end of storage i.e., 18 months vacuum packed seeds stored under cold storage recorded significantly higher values of α-amylase activity (4.25 mg maltose released/min) over all other treatments, followed by vacuum packed seeds stored under room temperature (4.22 mg maltose released/min). Significantly lower values of α-amylase activity (3.89 mg maltose released/min) were recorded in gunny bag seeds under room temperature followed by high density polythene bags (3.95 mg maltose released/min). The vacuum packed seeds could maintain higher α-amylase activity throughout the storage period. In the present investigation, α-amylase enzyme decreased with an increased storage period. High density polythene and gunny stored seeds recorded higher α-amylase activity compared to vacuum packed seeds. Decreased α-amylase activity with an increase in storage period may be due to reduction of free sugars and amino acids. Similar observations were also reported in wheat seeds (Chauhan *et al.*, 2011) [9] and in naturally aged gram, chickpea and wheat seeds (Agarwal and Kharlukhi, 1987) [2], in pea (Davis, 1977) [53] and in mustard (Sana *et al*., 2009) [46].

Lipase activity (meq. free fatty acid/min/g)

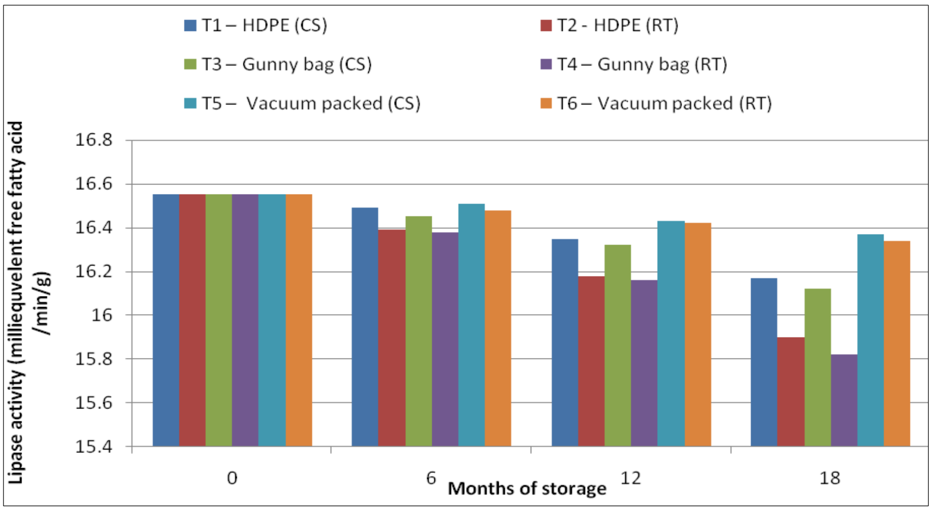

Fig 6: Influence of packaging and storage conditions on lipase activity (milliequvelent free fatty acid/min/g) at different time intervals of storage in soybean seeds

The data on lipase activity (meq. free fatty acid/min/g) of soybean seeds as influenced by different packaging and storage conditions differed significantly between the treatments during storage period (Fig., 6). The lipase activity in vacuum packed seeds was minimum and a gradual decline with progress in storage period. While lipase activity of gunny bag seeds as well as high density polythene packed seeds was found to reduce greatly from the initial stage to up to 18 months of storage stored under room temperature. At the end of storage i.e., 18 months vacuum packed seeds stored under cold storage recorded significantly higher lipase activity (16.37 meq. free fatty acid/min/g) over all other treatments, followed by vacuum packed seeds stored under room temperature (16.34 meq. free fatty acid/min/g). The lower lipase activity (15.82 meq. free fatty acid/min/g) was recorded in seeds stored in gunny bags under room temperature followed by high density polythene bags (15.90 meq. free fatty acid/min/g). Lipase activity were found to be slightly less in vacuum packed bags compared to HDPE and gunny bags. Our results are in good agreement with results of

Dhaliwal *et al.* (1991) [14] and Chaitanya *et al.* (2000) [10], Mohamed (2011) [26] in wheat and Eze and Chilaka (2010) [16] in cucurbitaceous seeds. Increase in protease activity during storage may be due to decline in protein content (Chaitanya *et al.*, 2000) [10]

Protease activity (mg amino acid released/min/ml)

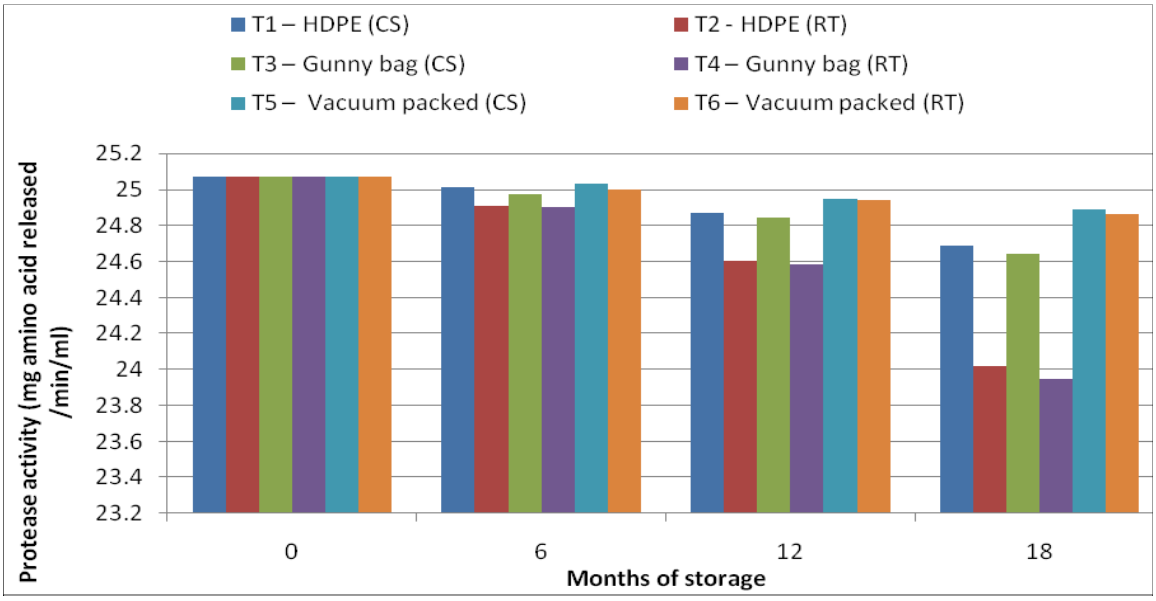

Fig 7: Influence of packaging and storage conditions on protease activity (mg amino acid released/min/ml) at different time intervals of storage in soybean seeds

The protease activity (mg amino acid released/min/ml) of soybean seeds differed significantly between treatments during storage period (Fig., 7). The protease activity in vacuum packed seeds was unchanged and gunny bags as well as high density polythene packed seeds from initial stage to 18 months of storage at room temperature. At the end of storage, vacuum packed seeds stored under cold storage recorded significantly higher protease activity (24.89 mg amino acid released/min/ml) over all other treatments, followed by vacuum packed seeds stored under room temperature (24.86 mg amino acid released/min/ml). The lower protease activity (23.95 mg amino acid released/min/ml) was recorded in gunny bag seeds stored at room temperature high density polythene bags (24.02 mg amino acid released/min/ml).. Similar results were observed by Chauhan *et al.* (2011) [9] in wheat, Scialabba *et al.* (2002) [47] in radish, Pallavi *et al.* (2003) [36] in sunflower. Same results of lipase activity were also observed in germinated seeds of castor bean (Ory *et al*., 1962) [35], *Jatropha curcas* (Abigor *et al*., 2002) [17] and the African bean (Enujiugha *et al*., 2004) [17]. Loss of viability of seeds has been correlated to enzymatic activity. Abdul-Baki (1972) [1] reported that the activity of respiratory and associated enzymes *viz.,* peroxidase, glutamic acid oxidase and catalase, activity of hydrolytic enzymes *viz., α-amylase, lipase,* proteases, phytases and phosphatases is associated with the degradation of organelles membranes, nucleoproteins, etc. In crop seeds, the development of amylase activity constitutes an important event in germination. During germination of seeds, a massive breakdown of the reserve substances begin with the help of amylolytic, proteolytic and lipolytic enzymes and the products are transported to the growing seedlings for their development. The remaining small amounts of protein represents enzymes concerned with metabolic processes during seed development and germination (Millerd and Thomson, 1975) [28]. Among the storage conditions, ambient storage recorded higher enzyme activities compared to cold storage. This may be due to higher temperature and higher metabolic activity under ambient storage. These findings are in agreement with those of Evans *et al.* (1997) and Sopanen and Lauriere (1989) [50], Huang *et al.* (1983) [22] in castor, Mostarin *et al.* (2012) in bush bean, Sana *et al.* (2009) [46] and Rehman and Shah (1999) [45] in wheat, Govind and Tumkur (1970) [54] in sorghum and Neg and Anderson (2005) [32] in quinoa, Liukkonen *et al.* (1992) [25] in oat seeds, Ghavidel and Davoodi (2011) [21] and Rahman *et al.* (2007) [41] in mungbean.

Oil content (%)

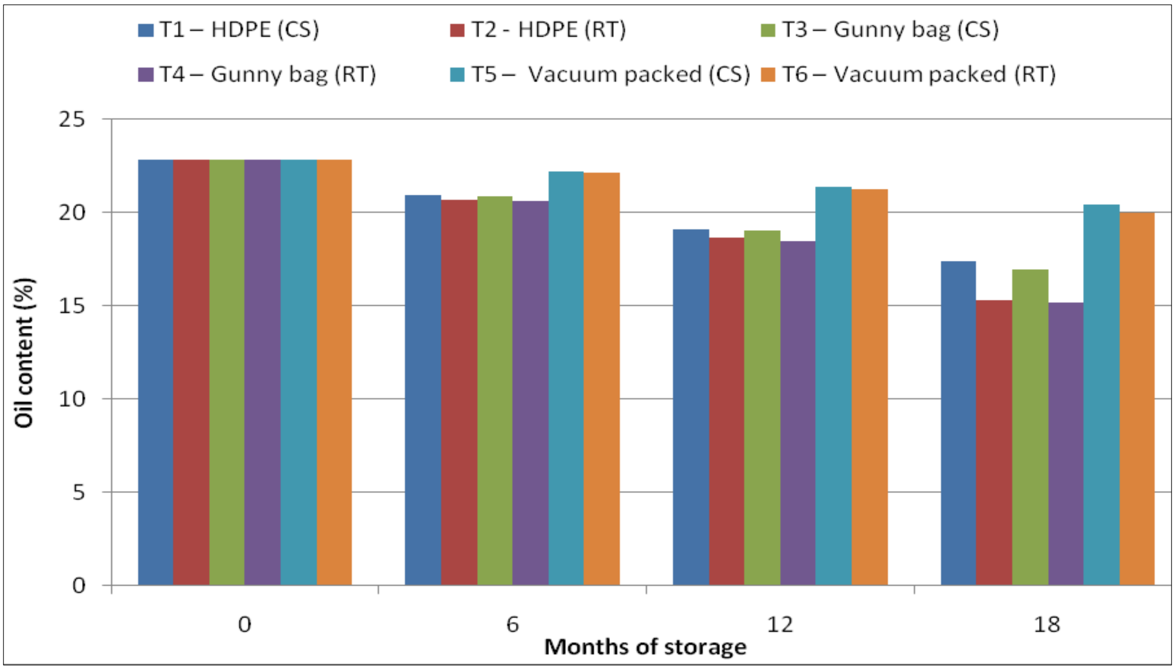

Fig 8: Influence of packaging and storage conditions on oil content (%) at different time intervals of storage in soybean seeds

The oil content (%) of soybean seeds differed significantly between treatments and there was a gradual decline was seen with progress in storage period (Fig., 8) reduction in gunny bags as well as high density polythene packed from the initial stage and until upto 18 months of storage, stored under room temperature. At the end of storage i.e., 18 months vacuum packed seeds stored under cold storage recorded significantly higher oil content (20.43%) over all other treatments, followed by vacuum packed seeds stored under room temperature (20.03%). The lower oil content (15.20%) was recorded in seeds stored in gunny bags samples, followed by high density polythene bags (15.33%). Martini *et al.* (2005) [31] reported that storage condition of oil seeds before industrial extraction might influence the quality of the crude oil. Similarly, Neg and Anderson (2005) [32] also showed that storage time and storage temperature had significant effect on free fatty acid content in Quinoa (*Chenopedium quinoa*) seed oil. Different longevity of seed storage as well as storage conditions exerts significant influence on oil content. It is clear from the results that the vacuum packed seeds could maintain higher oil content values compared to all other treatments. Similar results were also noticed by Ramamoorthy and Karivaratharaju (1986) [43] in groundnut, Branimir *et al.* (2007) [8], Kandil *et al.* (2013) in soybean, Akowuah *et al.* (2012) [5] and Canakci (2007) [11] in *Jatropha curcas* and Sisman (2005) [49] in sunflower.

Table 1: Influence of packaging and storage conditions on moisture content (%) at different time intervals of storage in soybean seeds

Treatments	Storage period (months)									
	0	2	4	6	8	10	12	14	16	18
T_1 – HDPE (CS)	9.30	12.97	12.03	12.11	12.10	12.13	12.09	11.90	11.85	11.20
T_2 – HDPE (RT)	9.30	10.87	10.50	10.33	11.13	11.09	11.07	10.90	10.09	9.95
T_3 – Gunny bag (CS)	9.30	15.30	15.41	15.20	14.88	14.60	14.57	14.40	14.23	14.10
T_4 – Gunny bag (RT)	9.30	10.98	10.78	10.73	12.45	12.38	12.30	11.25	10.90	10.30
T_5 – Vacuum packed (CS)	9.30	9.39	9.35	9.34	9.33	9.31	9.30	9.30	9.29	9.27
T_6 – Vacuum packed (RT)	9.30	9.37	9.33	9.31	9.30	9.30	9.29	9.28	9.27	9.25
S.Em (±)	0.19	0.14	0.12	0.07	0.08	0.12	0.23	0.09	0.13	0.11
C. D. (1%)	NS	0.41	0.34	0.22	0.24	0.35	0.68	0.26	0.38	0.32

HDPE = High density polythene CS = Cold storage
NS = Non significant RT = Room temperature

Table 2: Influence of packaging and storage conditions on electrical conductivity (d Sm^{-1}) at different time intervals of storage in soybean seeds

Treatments	Storage period (months)									
	0	2	4	6	8	10	12	14	16	18
T_1 – HDPE (CS)	0.499	0.507	0.519	0.533	0.550	0.572	0.590	0.628	0.645	0.659
T_2 – HDPE (RT)	0.499	0.510	0.528	0.541	0.566	0.586	0.634	0.688	0.701	0.733
T_3 – Gunny bag (CS)	0.499	0.508	0.522	0.538	0.560	0.579	0.598	0.638	0.650	0.677
T_4 – Gunny bag (RT)	0.499	0.513	0.531	0.549	0.573	0.592	0.648	0.699	0.724	0.747
T_5 – Vacuum packed (CS)	0.499	0.502	0.505	0.510	0.514	0.517	0.520	0.524	0.528	0.534
T_6 – Vacuum packed (RT)	0.499	0.503	0.506	0.513	0.516	0.519	0.522	0.527	0.531	0.537
S.Em(±)	0.018	0.039	0.079	0.005	0.008	0.007	0.007	0.008	0.001	0.001
C. D. (1%)	NS	NS	NS	0.014	0.024	0.021	0.020	0.023	0.003	0.003

HDPE = High density polythene CS = Cold storage NS = Non significant RT = Room temperature

Conclusion

Seed physiological and biochemical parameters deterioration is an inexorable and an irreversible process. The quality and viability of soybean seeds are subjected to variations during storage conditions and it has been found that the life span of seeds depends on moisture content of the seeds, relative humidity, temperature, light and oxygen content under which the seeds are stored. It has been found in the present study that it is possible to extend the shelf life of soybean seeds up to 18 months without deterioration in seed biochemical parameters *viz.*, oil content and enzyme activity such as α-amylase, lipase and protease and seed physiological parameters such as mineral content (Cu, Mn, Zn and Mn), moisture content and electrical conductivity by storing them under vacuum packaging. Since seed is an important input in agriculture which determines not only the production but also the productivity, it is essential to maintain the seed quality as well as seed vigor.

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