International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue A 13, December 2019
ISSN:2320-7817(p) 2320-964X(0)

Original Article | Open Access

Effect of three different storage containers on the Protein Content and Reducing Sugar in four different varieties of Soybean seeds under tropical storage conditions

Dambhare Kirti [1], Gadewar Rajesh[2], Mahajan Ashish[3]

[1 &2] Sewadal Mahaila Mahavidyalaya, Nagpur (India), Email: kirtidambhare@gmail.com | 9422164019
[3] Kamla Nehru Mahavidyalaya, Nagpur (India)

Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Cite this article as:
Dambhare Kirti Gadewar Rajesh, Mahajan Ashish (2019) Effect of three different storage containers on the Protein Content and Reducing Sugar in four different varieties of Soybean seeds under tropical storage conditions, *Int. J. of. Life Sciences*, Special Issue, A13: 201-209.

ABSTRACT

The Biochemical changes such as Protein content and Reducing sugar are occurred in seeds during storage. Literature reported that the total uric acid, nitrogen and free fatty acid of grains increased considerably and non-reducing sugar, reducing sugars and total water soluble sugars decreased during storage. In the present study three different bags Polythene bag (C1), Cloth bag (C2) and Jute bag (C3) of dimensions 20 cm x 30 cm were used for the storage of soybean seed of four different varieties JS-335 (V1), AMS-99-33 (V2), TAMS-38 (V3) and TAMS-98-21 under ambient temperature and relative humidity for a period of 18 months. Portion of the seeds from each container were removed after 3 months (90 days) and examined for Protein content and Reducing sugar observations. In variety (V1), the protein content significantly decreased with increase in storage period. Among the containers Polyethylene bag (C1) showed significantly higher protein content (38.80 %) as compared to Cloth bag (C2) (38.70 %) and Jute bag (C3) (38.25 %) throughout the storage period. The seed protein content was decreased significantly in all four varieties JS-335, AMS-99-33, TAMS-38 and TAMS-98-21 (38.16%, 37.80%, 35.12% and 35%, respectively) after 540 days of storage. It might be due to aging or deterioration of seeds. Loss of germination or viability with increase in moisture content during storage has been found to be closely associated with decrease in protein content of soybean seed by increase in membrane permeability. The reducing sugar content was observed decreasing significantly in all four varieties JS-335 (V1), AMS-99-33 (V2), TAMS-38 (V3) and TAMS-98-21 (V4) during storage. The reducing sugar content was decreased significantly in JS-335, AMS-99-33, TAMS-38 and TAMS-98-21 (0.83 %, 0.80%, 0.78% and 0.76%, respectively) after 540 days of storage. Seeds stored in Polyethylene bag recorded maximum reducing sugar compared to Cloth and Jute bag at the end of storage. This may be due to higher amylase activity that further relates to the moisture content of the seeds.

Key words: Soybean, Stoarge containers, Biochemical studies, Protein content, Reducing Sugar.

INTRODUCTION

An important aspect in any agricultural improvement programme is the maintenance of quality in the storage of seeds. High temperature and high

humidity conditions which are the common ambient feature of subtropical and tropical areas, induced deterioration of seed quality. Although several reviews are available on the loss of seed viability during storage and its assessment has been standardized. Soybean; the raw materials for vegetable oils, occupy a significant place in India's national economy. India is the world's biggest oilseed growing country and, paradoxically, the world's biggest important of edible oils as well, the main reason for this can be traced to low productivity per hector.

In Vidarbha region of Maharashtra State, soybean crop are harvested in October-November. The seeds of soybean crops are stored for 7-8 months prior to sowing. Through sun drying after harvest, followed by storage, has been found to reduce the problem of loss of viability. Even keeping the seeds under ambient conditions in ordinary gunny bags, would result in significant loss of viability (Charjan and Tarar; 1992). However, seed is not dried to a relatively safe moisture content after harvest, its storability will be reduced (Gadewar *et al.*, 2009).

The demand for seed is fluctuating and very often there are large surplus stock of seed which need to be preserved till the time of next sowing. Such left-over seed experience in the hot and humid mansoon months, would significantly decline germinability. By the time of next sowing in June-July, the loss in vigour and viability of carry over seeds, may adversely affect field emergence and productivity (Basu, *et al.*; 1978, Charjan and Tarar; 1992, and Abdullah M. Alhamdan *et al.*; 2011). The oil seeds are poor storer and loose its viability very fast in adverse conditions of temperature and humidity.

Biochemical changes are occurred is seeds during storage. Charjan and Tarar, (1994) reported that the total uric acid, nitrogen and free fatty acid of grains increased considerably and non-reducing sugar, reducing sugars and total water soluble sugars decreased during storage. There are many biochemical changes occurred due to seed deterioration such as cellular, metabolic and chemical alterations including chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, changes in the enzymes and loss of membrane structure (Vieira *et al.;*2013).

Sharma *et al*., (2007) reported that the total soluble sugars, sucrose and reducing sugar content decreased up to 90 days of storage. Duranti and Gius, (1997) reported that the decrease in carbohydrates and protein content in deteriorated seeds. Protein and field emergence of groundnut seeds found decreased with advancement of storage period. Fabre and Planchon, (2000) revaluated the influence of nitrogen sources on yield and protein content and found correlation between the symbiotic N2 fixation in yield and seed protein content. Fante *et al*., (2011) observed the same pattern of banding relative to the total protein regardless of the treatment. Ávila *et al*., (2007) observed that polythene bag and metal tin were better storage containers than the bamboo bin and clay pot.

Kaviani and Kharabian, (2008) observed the highest amount of total protein content in seeds of plants grown in the soil treated with 30 g of KCl and 0.02 g of CaHPO4 per 100 kg of soil. Liu *et al*., (2008) studied properties of protein isolates from soybeans stored under various conditions and showed that properties of protein isolates prepared from the three conditions (mild, cold and ambient) does not affected significantly for 12 months of storage. Li *et al*., (2012) showed for every 10 mg/g increase in seed protein was accompanied by 4.3 mg/g decrease in sucrose in soybean seeds. Green *et al*., (1989) demonstrated advantages of the modified assay in Nelson-Somogyi method for reducing sugars estimation. Sharma *et al*., (2013) revealed that the content of starch, total soluble sugars and reducing sugars in soybean seeds decreased during storage for 180 days but it didn't show positional variations in their contents.

MATERIAL METHODS

Seeds of the following kinds and varieties i.e.JS-335, AMS-99-33, TAMS-38 and TAMS-98-21, (Denoted by V1, V2, V3 and V4 respectively) were obtained from "All India Co-ordinate Oil Seed project, College of Agriculture, Nagpur. The seed samples were packed in the respective containers Polyethylene bag 700 gauge (moisture vapour proof), Cloth bag (moisture pervious) and Jute bag (moisture pervious). Polyethylene bag, Cloth bag and Jute bag, are denotes by C1, C2 and C3 respectively.

All the three bags will be of 20 cm x 30 cm. The seeds were closed by stitching in fresh jute and cloth bags, whereas it was heat sealed in case of polyethylene bags. The respective containers were then stored in wire mesh almirah in mesonary building having cemented walls, roof and floor under ambient temperature and relative humidity for a period of 18 months. Portion of the seeds from each container were removed after 3

months (90 days) and examined for Physiological, Biochemical and Mycological observations.

0 Days, 90 Days, 180 Days, 270 Days, 360 Days, 450 Days, and 540 Days intervals are denoted by T1, T2, T3, T4, T5, T6 and T7 respectively.
Estimation of Protein was performed by Kjeldehl method whereas estimation of reducing sugar by Benedict's method.

Statistical analysis:
The data obtained from the experiments were statistically analyzed by using factorial CRD. (Complete Randomized Design), Using Web Portal of CCS Hariyana Agricultural University, Hisar: http://14.139.232.166/opstat/default.asp. The critical differences between the parameters like Soybean seed Varieties, containers and storage period were worked out at five per cent significance.

RESULTS & DISCUSSION

(a) Protein Content (%)
The effect of container and storage period on Protein content in all four varieties V1, V2, V3 and V4 is presented in Table 1.

In variety JS-335 (V1), the protein content significantly decreased with increase in storage period. However the rate of loss in protein content varied with the type of container used. Seeds stored in Polyethylene bag (C1) showed significantly higher protein content (38.16 %) as compared to those stored in Cloth bag (C2) (37.91 %) and Jute bag (C3) (36.75 %) up to 540 days (T7) days of the storage. Among the containers Polyethylene bag (C1) showed significantly higher protein content (38.80 %) as compared to Cloth bag (C2) (38.70 %) and Jute bag (C3) (38.25 %) throughout the storage period.

Table 1: Effect of Varieties (V), Storage Containers (C) and Storage Periods (T) and three factor interaction on Protein content (%) of soybean seeds during storage.

VxCxT	V1			V2			V3			V4		
	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
T1	39.41	39.41	39.41	39.22	39.22	39.22	38.56	38.56	38.56	38.41	38.41	38.41
T2	39.22	39.20	39.15	39.09	39.02	39.00	38.37	38.23	38.11	38.20	38.07	38.02
T3	39.01	38.95	38.81	39.00	38.81	38.69	38.25	38.01	37.74	37.91	37.66	37.49
T4	38.80	38.75	38.52	38.61	38.52	38.41	37.84	37.25	37.03	37.64	37.17	36.80
T5	38.59	38.45	38.19	38.42	38.31	38.00	37.42	36.42	36.24	36.86	36.34	36.14
T6	38.40	38.20	36.89	37.92	37.85	36.80	36.56	35.39	35.20	36.39	35.25	35.02
T7	38.16	37.91	36.75	37.80	36.92	36.40	35.12	34.91	34.53	35.00	34.72	34.22
Mean	**38.80**	**38.70**	**38.25**	**38.58**	**38.38**	**38.07**	**37.45**	**36.97**	**36.77**	**37.20**	**36.80**	**36.59**
SE (m)	**0.719**											
CD(P=5%)	**NS**											

*NS-Non Significant

Table 2: Effect of Varieties (V), Storage Containers (C) and Storage Periods (T) and three factor interaction on Reducing Sugar Content (%) of soybean seeds during storage.

VxCxT	V1			V2			V3			V4		
	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
T1	1.80	1.80	1.80	1.77	1.77	1.77	1.69	1.69	1.69	1.60	1.60	1.60
T2	1.75	1.65	1.62	1.66	1.60	1.59	1.59	1.54	1.50	1.55	1.50	1.44
T3	1.60	1.54	1.50	1.51	1.50	1.48	1.48	1.39	1.32	1.40	1.38	1.29
T4	1.48	1.41	1.39	1.43	1.40	1.37	1.31	1.09	1.00	1.29	1.01	1.19
T5	1.25	1.20	1.19	1.23	1.20	1.18	1.01	0.91	0.84	0.99	0.89	0.86
T6	1.12	1.01	1.00	0.96	0.92	0.89	0.92	0.88	0.77	0.90	0.86	0.75
T7	0.83	0.80	0.78	0.80	0.78	0.75	0.78	0.73	0.65	0.76	0.65	0.60
Mean	**1.40**	**1.34**	**1.33**	**1.34**	**1.31**	**1.29**	**1.25**	**1.18**	**1.11**	**1.21**	**1.13**	**1.10**
SE (m)	**0.015**											
CD(P=5%)	**0.043**											

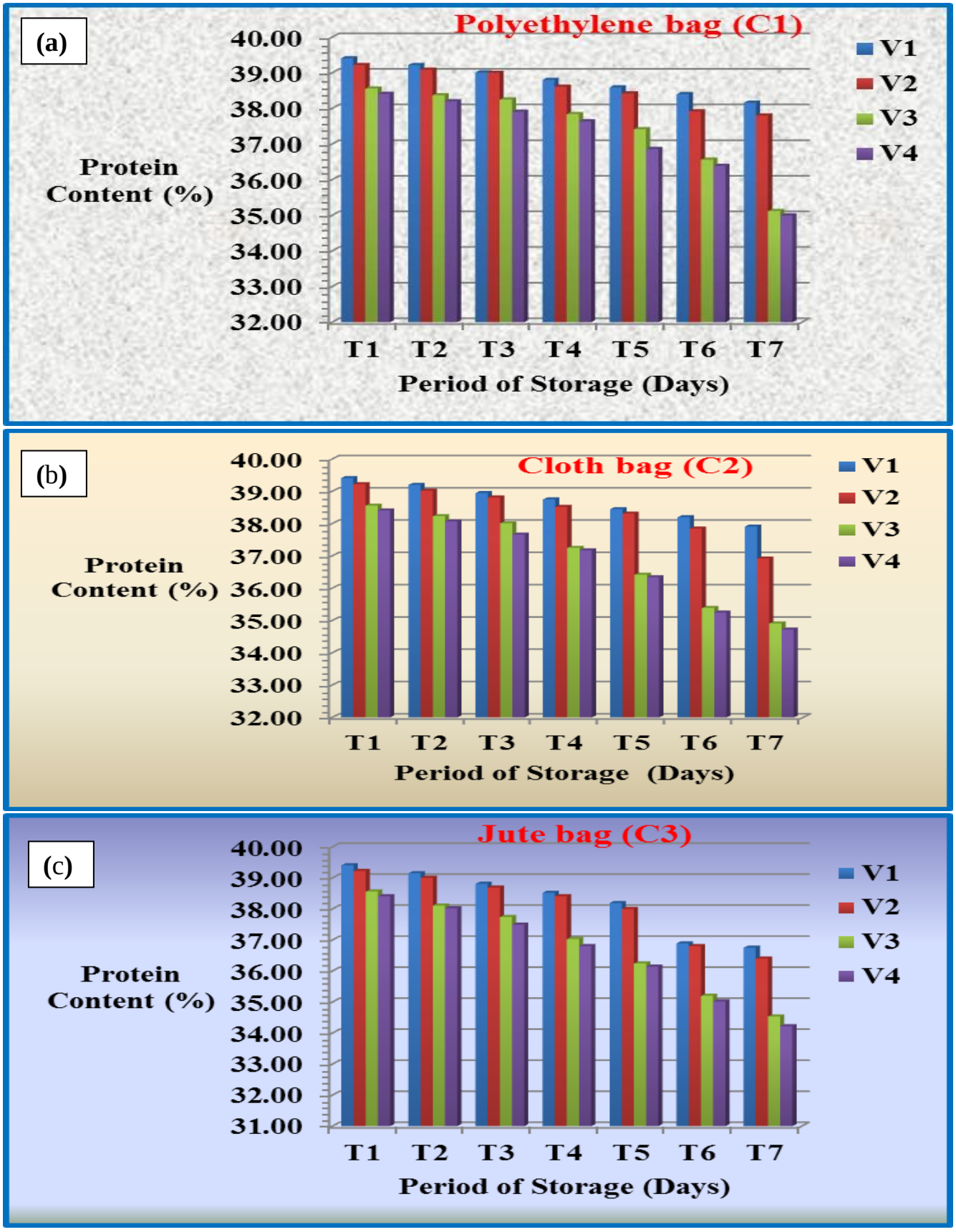

Figure 1: Effect of storage containers on Protein Content (%) in Soybean seed varieties.
(a) Polyethylene bag (C1), (b) Cloth bag (C2) and (c) Jute bag (C3).

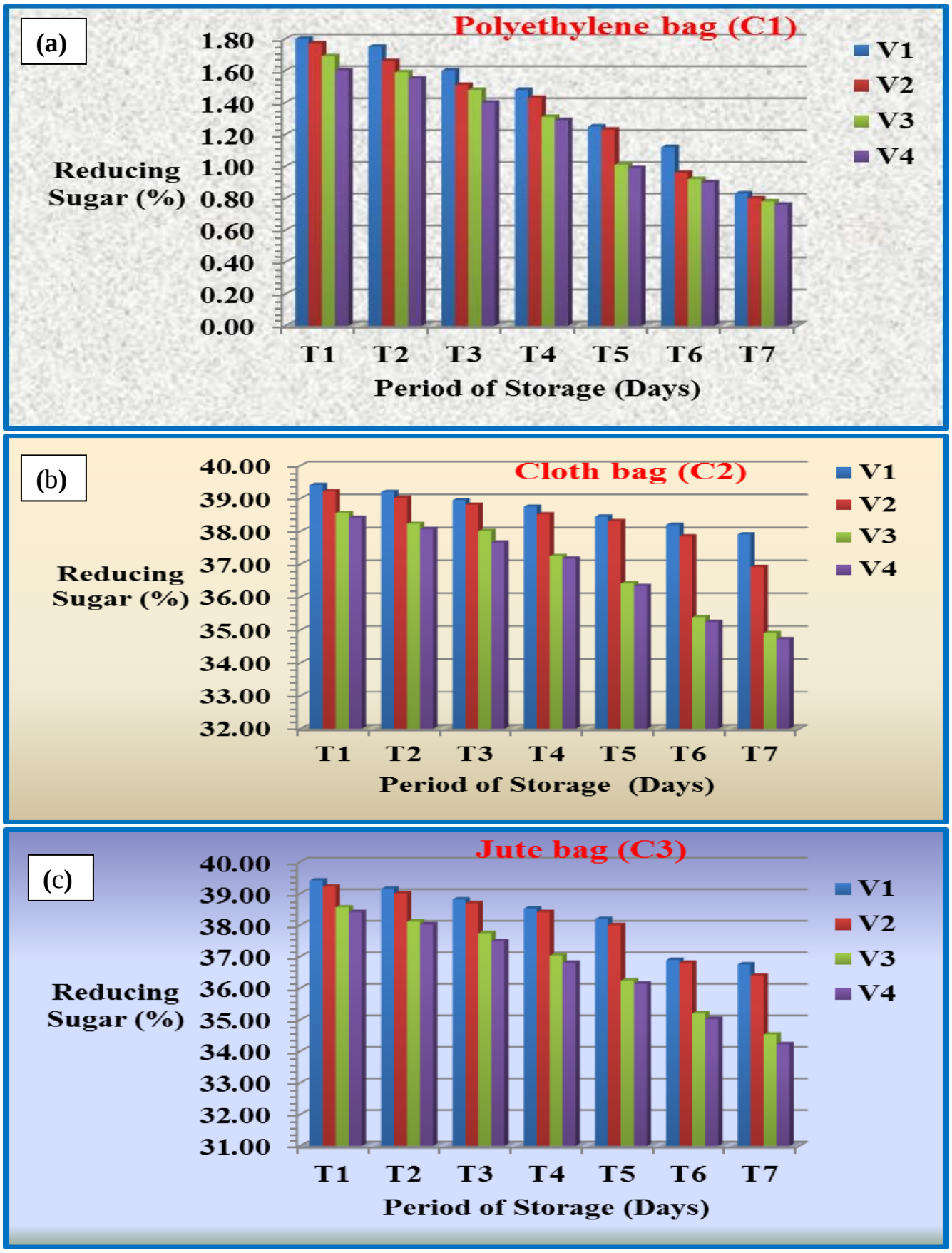

Figure 2: Effect of storage containers on Reducing Sugar (%) in Soybean seed varieties. (a) Polyethylene bag (C1), (b) Cloth bag (C2) and (c) Jute bag (C3).

In variety AMS-99-33 (V2), seed stored in Polyethylene bag (C1) showed significantly higher protein content (37.80 %) as compared to those stored in Cloth bag (C2) (36.92 %) and Jute bag (C3) (36.40 %) upto 540 days (T7) days of storage. Among the containers Polyethylene bag (C1) showed significantly higher protein content (38.58 %) as compared to Cloth bag (C2) (38.38 %) and Jute bag (C3) (38.07 %) throughout the storage period.

In variety TAMS-38 (V3), seed stored in Polyethylene bag (C1) showed significantly higher protein content (35.12 %) as compared to those stored in Cloth bag (C2) (34.91 %) and Jute bag (C3) (34.53 %) up to 540 days (T7) days of storage. Among the containers Polyethylene bag (C1) showed significantly higher protein content (37.45 %) as compared to Cloth bag (C2) (36.97 %) and Jute bag (C3) (36.77 %) throughout the storage period.

Similarly in variety TAMS-98-21 (V4), the seed stored in Polyethylene bag (C1) showed significantly higher protein content (35.00 %) as compared to those stored in Cloth bag (C2) (34.72 %) and Jute bag (C3) (34.22 %) up to 540 days (T7) days of storage. Among the containers Polyethylene bag (C1) showed significantly higher protein content (37.20 %) as compared to Cloth bag (C2) (36.80 %) and Jute bag (C3) (36.59 %) throughout the storage period.

Table 1 also shows that, among four varieties of soybean, seeds stored in Polyethylene bag (C1) exhibited significantly higher protein content percentage as compared to Cloth bag (C2) and Jute bag (C3). The variety JS-335 (V1) exhibited significantly higher protein content (38.58 %) as compared to AMS-99-33 (V2) (38.34 %), TAMS-38 (V3) (37.06 %) and TAMS-98-21 (V4) (36.86 %), irrespective of storage containers up to 540 days (T7) days.

(b) Reducing Sugar Content (%)

The effect of container and storage period on Reducing Sugar Content in all four varieties V1, V2, V3 and V4 is presented in **Table 2.**

In variety JS-335 (V1), the reducing sugar significantly decreased with increase in storage period. However the rate of loss in reducing sugar varied with the type of container used. Seeds stored in Polyethylene bag (C1) showed significantly higher reducing sugar (0.83 %) as compared to those stored in Cloth bag (C2) (0.80 %) and Jute bag (C3) (0.78 %) up to 540 days (T7) days of the storage. Among the containers Polyethylene bag (C1) showed significantly higher reducing sugar (1.40 %) as compared to Cloth bag (C2) (1.34 %) and Jute bag (C3) (1.33 %) throughout the storage period.

In variety AMS-99-33 (V2), seed stored in Polyethylene bag (C1) showed significantly higher reducing sugar (0.80 %) as compared to those stored in Cloth bag (C2) (0.78 %) and Jute bag (C3) (0.75 %) up to 540 days (T7) days of storage. Among the containers Polyethylene bag (C1) showed significantly higher reducing sugar (1.34 %) as compared to Cloth bag (C2) (1.31 %) and Jute bag (C3) (1.29 %) throughout the storage period.

In variety TAMS-38 (V3), seed stored in Polyethylene bag (C1) showed significantly higher reducing sugar (0.78 %) as compared to those stored in Cloth bag (C2) (0.73 %) and Jute bag (C3) (0.65 %) up to 540 days (T7) days of storage. Among the containers Polyethylene bag (C1) showed significantly higher reducing sugar (1.25 %) as compared to Cloth bag (C2) (1.18 %) and Jute bag (C3) (1.11 %) throughout the storage period.

Similarly in variety TAMS-98-21 (V4), the seed stored in Polyethylene bag (C1) showed significantly higher reducing sugar (0.76 %) as compared to those stored in Cloth bag (C2) (0.65 %) and Jute bag (C3) (0.60 %) up to 540 days (T7) days of storage. Among the containers Polyethylene bag (C1) showed significantly higher reducing sugar (1.21 %) as compared to Cloth bag (C2) (1.13 %) and Jute bag (C3) (1.10 %) throughout the storage period.

Table 2 also shows that, among the four varieties of soybean, seeds stored in Polyethylene bag (C1) exhibited significantly higher reducing sugar percentage as compared to Cloth bag (C2) and Jute bag (C3). The variety JS-335 (V1) exhibited significantly higher reducing sugar (1.36 %) as compared to AMS-99-33 (V2) (1.31 %), TAMS-38 (V3) (1.18 %) and TAMS-98-21 (V4) (1.15 %), irrespective of storage containers up to 540 days (T7) days.

DISCUSSION

(a) Protein Content (%)

Table 1 represent the effect of varieties, storage containers and two factor interactions on protein content of soybean seed during storage. The protein content of soybean seed is significantly influenced by different varieties stored in different containers during storage. The protein content decreased with increase in

storage period irrespective of varieties. The protein content was significantly higher in JS-335 followed by AMS-99-33 (V2), TAMS-38 (V3) and significantly lowers in TAMS-98-21 (V4) during all the periods of storage. The protein content of the soybean seed stored in Polyethylene bag (C1) was significantly higher than the seed stored in Cloth (C2) and Jute (C3) bags during all the periods of storage, irrespective of varieties.

The protein content of the soybean declined with slow rate with increase in period of storage. It might be due to aging or deterioration of seed. Loss of germination or viability with increase in moisture content during storage has been found to be closely associated with decrease in protein content of soybean seed by increase in membrane permeability (Hill and Breidenbach, 1974).

Meena *et al*., (2017) observed a decrease in protein content of soybean seeds during storage and concluded that, it is possible to extend the shelf life of soybean seeds up to 18 months without deterioration in biochemical parameters of the seeds viz., protein content under vacuum packaging. Similarly the decrease in protein content with increase in storage period was observed by Braccini *et al*., (2000) and Alencar *et al*., (2011) in soybean.

It has been reported in the literature that seed deterioration rate is strongly influenced by the type of container they are stored in (Singh *et al*., 2017; Orhevba and Atteh,, 2018; Saxena *et al*., 2015). In present study it is observed that decrease in protein content is at faster rate when seeds are stored in Cloth bag (C2) and Jute bag (C3) than Polyethylene bag (C1). It had been reported by Bellaloui *et al*., (2011) and Taski-Ajdukovic *et al*., (2010) that protein content can also be influenced by various genotypes present during storage. The genotype had found a strong effect on the protein percentage of the seed. Protein content was found to be related when a variation of glutamine concentration occurred (Ciabotti *et al*., 2016).
Khan *et al*., (2015) and Malek *et al*., (2012) reported that high yielding soybean genotypes should possess large dry matter weight, higher germination rate and viability at all growth stages. It has been observed that the variety JS-335 (V1) was better storer than the variety AMS-99-33 (V2) and TAMS-38 (V3), which is in agreement with our previous work (Dambhare and Gadewar, 2017). It was also observed that storage of seed in Polyethylene bag (C1) had significantly increased the storability of soybean seed over the seed stored in Jute bag (C3).

The results obtained from estimation of protein content have been illustrated graphically in **Figure 1.**

(b) Reducing Sugar (%)

In the present investigation, from **Table 2**, the reducing sugar content was observed decreasing significantly in all four varieties JS-335 (V1), AMS-99-33 (V2), TAMS-38 (V3) and TAMS-98-21 (V4) during storage. However, the reducing sugar was found more in JS-335 (V1) followed by AMS-99-33 (V2), TAMS-38 (V3) and TAMS-98-21 (V4). With the increase in storage period, reducing sugar in seed declined irrespective of variety, which leads to poor germination and vigour at the end of storage period. This may be due to higher protease activity that further relates to the moisture content of the seed (Shelar *et al*., 2008).

Decrease in reducing sugar over storage was also observed by Sharma *et al*., (2007); Filho et al., (2016) in soybean. The variety JS-335 with more carbohydrates maintained better seed quality as compared to other varieties AMS-99-33 (V2), TAMS-38 (V3) and TAMS-98-21 (V4), this is in agreement with the findings of Samaraha et al. (2009), who reported that sugar content have a positive correlation with seed germination and vigour.

Nitrogen as the main constituent of Proteins and carbohydrates is the major form of carbon, hydrogen and oxygen. During seed storage the proteins decreased and remained undegraded into free amino acids (Filho, 2015) and carbohydrates yield free sugar molecules. Thus, the hydrolysis of protein and carbohydrates could also be considered as one of the reason for loss of physiological vigour in the seeds at storage.

Many researchers reported that the reduction in the viability and vigour was strongly correlated with the decrease in reducing sugar. (Zhao *et al*., 2007; Shaban, 2013; Daniel and Edwin, 1985).

In the present investigation, the seeds stored in Polyethylene bag (C1) showed higher value of reducing sugar compared to Cloth bag (C2) and Jute bag (C3) after 540 days of storage. Decrease in reducing sugar in Cloth bag was also recorded by Saxena *et al*., (2015), Singh *et al*,. (2017), this result may be attributed to seed oxidation and respiration during storage that causes

biochemical change in seeds which ultimately results in decrease in reducing sugar. (Jyoti and Malik, 2013; Panobianco and Vieira, 2007 and Sharma *et al*., 2013).

The results obtained from estimation of reducing sugar content have been illustrated graphically in **Figure 2**.

CONCLUSION

The seed protein content was decreased significantly in all four varieties JS-335, AMS-99-33, TAMS-38 and TAMS-98-21 (38.16%, 37.80%, 35.12% and 35%, respectively) after 540 days of storage. Seeds stored in Polyethylene bag recorded maximum protein content as compared to Cloth and Jute bag. The reducing sugar content was decreased significantly in JS-335, AMS-99-33, TAMS-38 and TAMS-98-21 (0.83 %, 0.80%, 0.78% and 0.76%, respectively) after 540 days of storage. Seeds stored in Polyethylene bag recorded maximum reducing sugar compared to Cloth and Jute bag at the end of storage.

Conflicts of interest: The authors stated that no conflicts of interest.

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