

Comparative study of protein content and reducing sugar of Soybean Seeds in the varieties JS-335 and AMS-99-33 under tropical storage conditions

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Abstract

Soybean is popular in Vidarbha and its consumption is increasing at a very rapid rate. Biochemical constituent such as Protein content and reducing sugar of soybean seeds is an important factor which influences the physiological soundness of seed. Among the different available varieties of soybean seed in Vidarbha region, most popular varieties are JS-335 and AMS-99-33. In the present work the comparative study of Protein Content and Reducing Sugar in the varieties JS-335 and AMS-99-33 have been presented. It has been observed that, during storage of Soybean seeds under tropical storage conditions in different containers for the period of 540 days, there is remarkable changes seen in Protein Content and Reducing Sugar at different rate in two different varieties JS-335 and AMS-99-33. The seed protein content was decreased significantly in both varieties JS-335 and AMS-99-33 (38.16% and 37.80% respectively) after 540 days of storage. Seeds stored in Polyethylene bag recorded maximum protein content as compared to Cloth and Jute bag. Whereas, the reducing sugar content was decreased significantly in JS-335 and AMS-99-33, (0.83 % and 0.80% 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.

Keywords: soybean, storage 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 importer of edible oils as well, the main reason for this can be traced to low productivity per hectare. 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)^[7]. However, seed is not dried to relatively safe moisture content after harvest; its storability will be reduced (Gadewar *et al.*, 2009)^[15].

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 monsoon 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^[4], Charjan and Tarar; 1992^[7], and Abdullah M. Alhamdan *et al.*; 2011). The oil seeds are poor storers and lose their viability very fast in adverse conditions of temperature and humidity. Many researchers reported that decay in food reserve in seeds may result into the loss

of vigour in seeds. Whereas, experiments on non-viable seeds proved that, there is sufficient food reserve remaining behind in the seed for further growth and development (Jyoti and Malik, 2013^[20]; Shelar *et al.*, 2008)^[36]. Some changes in chemical composition during deterioration were also observed (Vasileva, 2014)^[39].

Jyoti and Malik, (2013)^[20] reviewed that the soybean seeds stored at room temperature for one and half year at moisture contents varying from 8.0 to 11.8 % will result in decrease of both reducing and non-reducing sugars with a loss of vigour. Shelar, (2007)^[35] observed changes associated with seed deterioration are depletion in food reserve, increased enzyme activity, increased fat acidity and membrane permeability. As the catabolic changes continue with increasing age, the ability of the seed to germinate is reduced. Hou *et al.*, (2009)^[19] studied sugar variation in soybean seed and found that PI 243545 as a unique germplasm with the highest sucrose (105.48 mg/g) and total sugar (148.76 mg/g) content. Duranti and Gius, (1997)^[10] 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)^[11] reevaluated 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)^[12] observed the same pattern of banding relative to the total protein regardless of the treatment. Avila *et al.*, (2007)^[3] observed that polythene bag and metal tin were better storage containers than the bamboo bin and clay pot. Nikolova *et al.*, (2000)^[27] revealed that the degree of changes in protein spectra of seeds depends mainly on the type of mineral element under deficiency. 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. Wettlaufer and Leopold, (1991) ^[40] observed high sucrose containing seeds among domestic varieties of soybean which contain Chakaori in sucrose with very high percentage and that reducing sugar formation during seed boiling is a new approach for increasing sweet components. Green *et al.*, (1989) ^[16] demonstrated advantages of the modified assay in Nelson-Somogyi method for reducing sugars estimation. Sharma *et al.*, (2007) ^[33] studied changes in carbohydrate composition of germination soybean seeds under different storage conditions and observed that starch content in cotyledons declined from 90 to 180 days of storage in all the treatments whereas the total soluble sugars, sucrose and reducing sugar content decreased up to 90 days of storage. Sharma *et al.*, (2013) ^[34] 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. Gusakov *et al.*, (2011) ^[17] compared two methods for assaying Reducing Sugars in the determination of carbohydrate activities; the Nelson-Somogyi (NS) and 3,5-dinitrosalicylic acid (DNS) assays. The DNS assay gave activity values, which were typically 40–50% higher than those obtained with the NS assay. Sett, (2016) studied changes in levels of soluble sugar, reducing sugar and lipid during germination of seeds of *albizia procera*. He observed that with progression of germination, total lipid content decreased. Filho, (2016) ^[13] studied the effect of drying temperatures and storage of seeds on the growth of soybean seedlings and observed that the increase in the temperature of drying air affects the physiological quality of soybean seeds, and this effect is accentuated over time, especially on length of seedlings. From the literature survey, it has been observed that very less work is carried out for studying effect of storage material on Protein content and reducing sugar. Therefore it is very important to study the effect from the research prospective in the field of Soybean Seeds.

Material and Methods

Seeds of the two kinds and varieties i.e. JS-335 and AMS-99-33 (Denoted by V1, and V2 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 denoted by C1, C2 and C3 respectively. All the three bags will be of 20 cm x 30 cm. 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 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 carried out using Kjeldahl method, whereas estimation of reducing sugar was carried out using 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,

Isar: <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

a. Protein content

The effect of container and storage period on Protein content in all four varieties V1 and V2 is presented in Table 1.1.

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		
	C1	C2	C3	C1	C2	C3
T1	39.41	39.41	39.41	39.22	39.22	39.22
T2	39.22	39.20	39.15	39.09	39.02	39.00
T3	39.01	38.95	38.81	39.00	38.81	38.69
T4	38.80	38.75	38.52	38.61	38.52	38.41
T5	38.59	38.45	38.19	38.42	38.31	38.00
T6	38.40	38.20	36.89	37.92	37.85	36.80
T7	38.16	37.91	36.75	37.80	36.92	36.40
Mean	38.80	38.70	38.25	38.58	38.38	38.07
SE (m)	0.719					
CD (P=5%)	NS					

*NS-Non-Significant

■ Variety

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. 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.

■ Container

Table 1.1 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 %) irrespective of storage containers up to 540 days (T7) days.

Two factor interaction

■ Variety x Container (VxC)

The two-factor interaction between Varieties and Containers (VxC) on protein content is presented in Table 1.2. The effect for two factor interaction VxC was significant during all the periods of storage T1 to T7.

Table 2: Two factor interaction between Varieties and Containers (VxC) on protein content (%).

VxC	C1	C2	C3	Mean V
V1	38.80	38.70	38.25	38.58
V2	38.58	38.38	38.07	38.34
Mean C	38.69	38.54	38.16	
SE (m)	0.272			
CD (P=5%)	NS			

*NS-Non-Significant

The interaction between Variety x Container for protein content was not significant throughout the storage period. V1C1 had maintained higher protein content throughout the storage period. At the end of storage, higher protein content was found in V1C1 (38.69 %) and lowest protein content was found in V2C3 (36.16 %).

▪ **Variety x Storage Period (VxT)**

The two-factor interaction between Varieties and Storage Period (VxT) on protein content is presented in Table 1.3. The interaction between Varieties and Storage Period was significant for protein content for all three containers. During 90 days of storage, V1T2 recorded higher protein content (39.19 %) except for T1 which was found to gradually decrease from V1T3 to V1T7. Thus V1 maintained higher protein content throughout the storage period as compared to V2, V3 and V4. At the end of storage, V4T7 had recorded lowest protein content (34.65 %).

Table 3: Two factor interaction between Varieties and Storage Period (VxT) on protein content (%).

VxT	T1	T2	T3	T4	T5	T6	T7	Mean V
V1	39.41	39.19	38.92	38.69	38.41	37.83	37.61	38.58
V2	39.22	39.04	38.83	38.51	38.24	37.52	37.04	38.34
Mean T	38.90	38.64	38.36	37.95	37.45	36.66	36.04	
SE (m)	0.415							
CD (P=5%)	NS							

*NS-Non-Significant

▪ **Container x Storage Period (CxT)**

The two factor interaction between Container x Storage Period (CxT) on protein content is presented in Table 1.4. The interactions among Container x Storage period for protein content was significant for all the four varieties, and during storage period T2 of storage C1T2 recorded higher protein content (38.72 %) except T1, which further gradually declined for C1T3, C1T4, C1T5, C1T6 and C1T7. And lowest protein content was recorded in C3T7 (35.48 %).

Table 4: Two factor interaction between Container x Storage Period (CxT) on protein content (%).

VxT	T1	T2	T3	T4	T5	T6	T7	Mean C
C1	38.90	38.72	38.54	38.22	37.82	37.32	36.52	38.01
C2	38.90	38.63	38.36	37.92	37.38	36.67	36.12	37.71
C3	38.90	38.57	38.18	37.69	37.14	35.98	35.48	37.42
Mean T	38.90	38.64	38.36	37.95	37.45	36.66	36.04	
SE (m)	0.360							
CD (P=5%)	NS							

*NS-Non-Significant

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 1.5.

Table 5: 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		
	C1	C2	C3	C1	C2	C3
T1	1.80	1.80	1.80	1.77	1.77	1.77
T2	1.75	1.65	1.62	1.66	1.60	1.59
T3	1.60	1.54	1.50	1.51	1.50	1.48
T4	1.48	1.41	1.39	1.43	1.40	1.37
T5	1.25	1.20	1.19	1.23	1.20	1.18
T6	1.12	1.01	1.00	0.96	0.92	0.89
T7	0.83	0.80	0.78	0.80	0.78	0.75
Mean	1.40	1.34	1.33	1.34	1.31	1.29
SE (m)	0.015					
CD (P=5%)	0.043					

▪ **Variety**

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.

▪ **Container**

Table 4.47 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.

Two factor interaction

▪ **Variety x Container (VxC)**

The two-factor interaction between Varieties and Containers (VxC) on reducing sugar is presented in Table 1.6. The effect for two factor interaction VxC was significant during all the periods of storage T1 to T7.

Table 6: Two factor interaction between Varieties and Containers (VxC) on reducing sugar (%)

VxC	C1	C2	C3	Mean V
V1	1.40	1.34	1.33	1.36
V2	1.34	1.31	1.29	1.31
Mean C	1.30	1.24	1.21	
SE (m)	0.006			
CD (P=5%)	0.016			

The interaction between Variety x Container for emergence was significant throughout the storage period V1C1 had maintained highest germination throughout the storage

period. At the end of storage, highest germination was found in V1C1 (1.40 %) and lowest germination was found in V2C3 (1.10 %).

▪ Variety x Storage Period (VxT)

The two factor interaction between Varieties and Storage Period (VxT) on reducing sugar is presented in Table 1.7.

The interaction between Varieties and Storage Period was significant for emergence for all three containers. During 90 days of storage, V1T2 recorded higher emergence (1.67 %) except for T1, which was found to gradually decrease from V1T3 to V1T7. Thus V1 maintained higher emergence throughout the storage period as compared to V2. At the end of storage V2T7 had recorded lowest germination (0.67 %).

Table 7: Two factor interaction between Varieties and Storage Period (VxT) on reducing sugar (%).

VxT	T1	T2	T3	T4	T5	T6	T7	Mean V
V1	1.80	1.67	1.55	1.43	1.21	1.04	0.80	1.36
V2	1.77	1.62	1.50	1.40	1.20	0.92	0.78	1.31
Mean T	1.72	1.58	1.45	1.28	1.06	0.92	0.74	
SE (m)	0.009							
CD (P=5%)	0.012							

▪ Container x Storage Period (CxT)

The two-factor interaction between Containers x Storage Period (CxT) on reducing sugar is presented in Table 1.8.

The interactions among Container x Storage period for emergence was significant for all the four varieties, during storage period T2 of storage, C1T2 recorded highest germination (1.64 %) except T1, which further gradually declined for C1T3, C1T4, C1T5, C1T6 and T1C7. And lowest germination was recorded in C3T7 (0.70 %).

Table 8: Two factor interaction between Containers x Storage Period (CxT) on reducing sugar (%).

VxT	T1	T2	T3	T4	T5	T6	T7	Mean C
C1	1.72	1.64	1.50	1.38	1.12	0.98	0.79	1.30
C2	1.72	1.57	1.45	1.23	1.05	0.92	0.74	1.24
C3	1.72	1.54	1.40	1.24	1.02	0.85	0.70	1.21
Mean T	1.72	1.58	1.45	1.28	1.06	0.92	0.74	
SE (m)	0.008							
CD (P=5%)	0.021							

Discussion

a. Protein Content (%)

Table 1.1 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). 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) [18]. Meena *et al.*,

(2017) [26] 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) [26] and Alencar *et al.*, (2011) [2] 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 [37]; Orhevba and Atteh, 2018; Saxena *et al.*, 2015) [30]. 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) [5] and Taski-Ajdukovic *et al.*, (2010) [38] 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) [8].

Khan *et al.*, (2015) [21] and Malek *et al.*, (2012) [24] 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) which is in agreement with our previous work (Dambhare and Gadewar, 2017) [22]. 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).

b. Reducing Sugar (%)

In the present investigation, from Table 1.5, the reducing sugar content was observed decreasing significantly in both varieties JS-335 (V1) and AMS-99-33 (V2) during storage. However, the reducing sugar was found more in JS-335 (V1) followed by AMS-99-33 (V2). 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) [36].

Decrease in reducing sugar over storage was also observed by Sharma *et al.*, (2007) [33]; Filho *et al.*, (2016) [13] in soybean. The variety JS-335 with more carbohydrates maintained better seed quality as compared to variety AMS-99-33 (V2), this is in agreement with the findings of Samaraha *et al.* (2010), 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) [14] 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)^[30], Singh *et al.*, (2017)^[37], 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. (Mbofung, 2012^[25]; Jyoti and Malik, 2013^[20]; Panobianco and Vieira, 2007^[29] and Sharma *et al.*, 2013)^[34].

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