JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Storability and oil quality assessment of soybean (*Glycine max*) seeds under different storage conditions

Jaya Singh Bisen

Shri Rama Krishna College of Commerce and Science, Satna (MP) India

*Corresponding author

email: singhjaya685@gmail.com

Phone: +91 9098090901

Abstract

The present study investigates the effect of storability of soybean (*Glycine max*) seeds. The oilseeds, harvested during different years and stored under ambient or -20°C were investigated for their germination, viability, vigor, oil content and oil quality. The results revealed that during storage, the germination and seed vigor tend to reduce when the seeds are stored under ambient conditions, as compared to the seeds stored under -20°C as compared to control. The oil content is also reduced drastically, as was the fatty acid composition, in which proportion of major fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were reduced drastically for the seeds stored under ambient conditions, as compared to control seeds. The data were correlated using statistical tests.

Key Words: *Glycine max*, Soybean, Oilseeds, Seed quality, seed storability.

INTRODUCTION

India is the largest producer of oilseeds in the world and the oilseed sector occupies an important position in the agricultural economy of the country. Oilseeds are among the major crops that are grown in the country apart from cereals. Among the nine oilseed crops grown in the country, soybean stands apart in terms of acreage and production which goes up to an estimated production of 11.6 million tonnes per year[1].

Though India produces exceptionally good quantities of oilseeds annually, but still this level of production is not sufficient to meet the need of edible oil in the country. Hence, continuous efforts are on the boost the production of oilseeds utilizing the available resources. Storage of seeds is necessary not only to start a new crop during next season, but also to utilize the oils as and when required. However, viability and oil quality need to be maintained during storage[2]. In India, most of the farmers do not have proper storage facilities for seeds, and they heavily rely on storage at ambient conditions for the seeds to be used for next generation plantation. Even the warehouses, where the crops are stored for oil extraction, are not equipped with low temperature facilities.

During storage, seed quality may decline to the level that they neither can be used for germination purposes nor for the commercial exploitation of oils. Storage longevity may vary and is influenced by the initial quality of stored seed as well as storage conditions. The oil seeds are very sensitive to the harsh environmental conditions. It is well known fact that the oil content readily oxidize, which deteriorate the seed health during storage[3]. Seed storage conditions can determine germination characteristics and vigor potential of seeds[4]. In India, since other factors that may affect the seed quality during storage (mechanical damage, pests etc.) are easy to manage, the environmental conditions remain the sole factor responsible for the deterioration of seeds during storage[5]. Keeping the above facts in mind, the present study is aimed to identify the storability of soybean seeds for germination and to ascertain the oil quality during the storage period.

MATERIALS AND METHODS

The soybean seeds were harvested during 2009 to 2015 from the crops cultivated at the premises of Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur India. The seeds of soybean (*Glycine max*), JS-97-52 cultiver, from year 2006 to the year 2015 were used in the study. These seeds were stored under three different storage periods, *i.e.*, short term (3 years, including the seeds from the harvest year 2014, 2013 and 2012) mid-term (6 years, including the seeds from the harvest year 2011, 2010 and 2009) and long term (9 years, including the seeds from the harvest year 2008, 2007 and 2006 cold storage -20°C). The freshly harvested seeds from the year 2015 served as control.

Seed germination and vigor

Soybean seeds were germinated between the paper (BP) technique as per ISTA method[6]. The seeds under study were tested for the standard germination, which is considered successful once the embryonic axis is longer than 1 cm. Seeds (20 number) removed year wise from the stored lot were placed in the filter paper, wetted with distilled water in three replicates. Seeds were spread uniformly on a Petri dish with wet filter paper and were covered with another moist paper. The box was closed with a tight-fitting lid. This box was kept in germinator maintaining adequate humidity and temperature of 20°C. The number of seeds germinated for 5 days was recorded daily.

The vigor index was calculated using the formula[7]:

Vigor index = Root length + Shoot length x Seed germination %

In vitro seed viability tests

In vitro seed viability test was performed according to ISTA (1999). One hundred seeds from each seed lot of soybeans were used for this test in five replicates of 20 seeds each. The presoaked seeds were bisected longitudinally and stained with 1% solution (w/v) of triphenyl tetrazolium chloride (TTC) in water. After staining, seeds were rinsed with tap water. Seeds were than visualized for staining pattern using a magnified lens and viability was recorded as viable (completely colored embryos), potentially viable (partially colored) and not viable (completely colorless embryos).

Determination of oil content and fatty acid profile

The oil content was measured by extracting the soybean seeds by hexane using Soxhlet extraction method and was calculated as percent value per 100 g seed[8] (AOAC, 1999). To determine the percentage of fat, the dried sample of plant was extracted with hexane (petroleum sprit, bp 40-60°C) for 16 h. The oil weight and percentage of oil was calculated after evaporating the hexane from the extract.

Fatty acid profile was analyzed using gas chromatography method. The extracted oil (5g) from each lot was treated with methanolic KOH to convert the fatty acids into methyl esters. The sample (1 µl) was injected to gas chromatograph. The column TG5 (Thermo Fisher, USA) was set at a temperature of 185 °C and flame ionization detector temperature was at 200 °C. The reference standard mixture of known composition was also run in the same operating conditions. The peaks in the chromatogram were identified using the chromatogram of reference standard[8].

RESULTS

The soybean seeds stored for a duration of nine years were investigated for the germination properties as well as changes in oil quality during the storage time.

Seed germination and seed vigor

The soybean seeds were germinated using “between the paper (BP)” method as per ISTA guidelines. Fig 1 shows the germination of soybean seeds of control group after 7 days. The seedling length was mainly due to the shoot length, as roots have not emerged significantly even after seven days. A clear drop in germination and in seedling length is observed for the short term stored seeds, where on small shoot is visible in some seeds. Almost no sign of germination was observed for the mid-term stored seeds of soybean. When the germination of long term stored seeds were observed, the germination was visible in seeds from all three years, though the percent was in decreasing order. The most remarkable observation was that the seedling length was shorter as compared to the control seeds. Table 1 shows the results of direct germination tests under controlled environment for soybean seeds. Soybean seeds from control lot showed 100% germination (all seeds used were germinated at the end of the experiment).

Fig 1: Germination of soybean seeds stored for the short term (harvesting year 2012-2014 at ambient temperature, mid term (harvesting year 2009-2011 at ambient temperature and long term (harvesting year 2006-2008 stored at -20°C).

Table 1: Germination percentage of stored seeds of soybean. Seeds were germinated by selecting 20 random seeds from each lot.

Storage period	Harvesting Year	Germination %
Control	2015	100
Short term (Ambient temp.)	2014	70
	2013	55
	2012	40
Mid term (Ambient temp.)	2011	15
	2010	0
	2009	0
Long term (-20°C)	2008	95
	2007	75
	2006	35

The germination percentage reduced drastically as the age of seeds increased for ambient storage seeds. For short term stored seeds, the germination dropped to 40% while for mid-term stored seeds, germination was almost zero. For long term and cold stored seeds, the germination for seeds of 2008 were found to be 95% (close to control), but here also, decrease in germination was noted. Statistical tests using one way ANOVA

showed that except for the seeds of year 2008 (long term stored under cold storage conditions), all other seeds had significantly less germination as compared to control seeds ($F_{9,20}$= 117.7, $p > 0.05$).

In a different set of experiment, the germination of soybean seeds was monitored on day-wise pattern for five days. The germination studies of the seeds of different storage periods (Table 2) for the soybean showed that no seed started germinating after 24 h. The seeds of year 2015 and 2014, and from 2008 to 2006, started germination from second day. After 5th day, the seeds of the years 2009 and 2010 failed to show any germination. The seeds of the year 2011 showed only 15% germination after five days. The seeds of the year 2012, 2013 and 2014 showed 40%, 55% and 70% germination respectively. Only seeds of year 2015 and 2008 showed 100% germination after five days.

Table 2: A comparison of day wise germination of soybean seeds of different storage periods for five days. In total, 20 seeds were taken.

No. of seeds germinated after five days					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
2015	0	3	8	17	20
2014	0	1	4	12	14
2013	0	0	1	10	11
2012	0	0	1	8	8
2011	0	0	0	1	3
2010	0	0	0	0	0
2009	0	0	0	0	0
2008	0	0	1	11	19
2007	0	0	1	9	15
2006	0	0	0	2	7

The seed vigor was calculated as a function of germination rate and the seedling length (combined shoot and root length). Table 3 shows the seed vigor index of soybean seeds stored for varying length of time. The control seeds of soybean showed the vigor index as 890.00. The seed vigor decreased as a function of time for ambient stored seeds and dropped to 0.00 in the year 2011 onwards. The cold stored seeds from 2008 showed vigor index as 817.0, close to the control seeds. The seeds from 2007 and 2006, though germinated, had lesser seedling length and hence lower vigor index.

Table 3: Seed vigor index of soybean seeds stored for varying length of time. The shoot and root length were averaged for 20 seeds. Short and mid-term seeds were stored at ambient temperature, while the long-term seeds were stored at cold storage conditions (-20°C).

Year of harvest	Mean Shoot Length (cm)	Mean Root Length (cm)	Seedling length (cm)	Germination (%)	Vigor index
2015	7.8	1.1	8.9	100	890.00
2014	4.3	0.8	5.1	70	357.00
2013	2.1	0.56	2.66	55	146.30
2012	1.1	0.32	1.42	40	56.80
2011	0	0	0	15	0.00
2010	0	0	0	0	0.00
2009	0	0	0	0	0.00
2008	7.4	1.2	8.6	95	817.00
2007	6.57	0.82	7.39	75	554.25
2006	6.23	0.61	6.84	35	239.40

In order to get more authenticity of the data obtained, a direct viability test using tetrazolium chloride was performed. The control seeds of soybean showed 6 completely colored, 13 partially colored and 1 colorless seed. The same results were obtained with cold storage seeds. This indicates that the soybean seed starts losing viability almost immediately after harvest. As the time progresses, the number of completely colored seeds of soybean decreases while number of partially colored and colorless seeds increases (Table 4).

Table 4: Seed viability of soybean and Niger seeds when stored for different time periods under the same conditions. Total 20 seeds were chosen for viability test.

Storage pattern	Year of harvest	Number of seeds		
		Completely viable seeds	Partially viable seeds	Non-viable seeds
Control	2015	6	13	1
Short term (ambient temp.)	2014	3	17	0
	2013	0	17	3
	2012	1	18	1
Mid term (ambient temp.)	2011	0	20	0
	2010	1	19	0
	2009	1	19	0
Long term (-20°C)	2008	6	12	2
	2007	6	13	1
	2006	2	16	2

The changes in fat profile is one of the important factor for the commercial utilization of the stored oilseeds. Fig 2 shows the oil percentage of soybean seeds stored for varying length of time as well as for

ambient and cold storage conditions. The control seeds had 18.83 ± 1.02 % of oil. The seed oil tends to decrease over time when stored under ambient conditions, and a steep fall was seen when seeds are stored for more than four years under ambient conditions. The cold stored seeds did not show much decrease in seed oil as compared to control. The statistical tests using one way ANOVA and Dunette's multiple comparison tests revealed that oil percentage was significantly lower for seeds stored under ambient conditions, but not significantly lower under cold storage conditions ($F_{9,20}$=131.9, p>0.05).

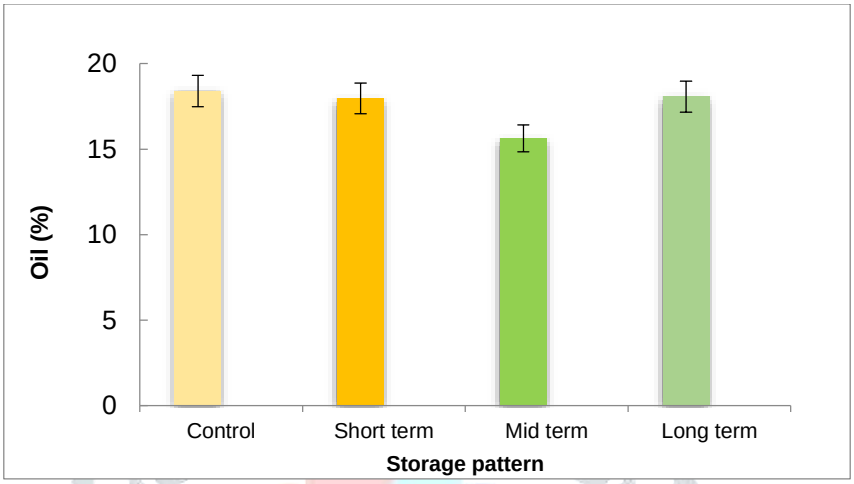

Fig 2: Effect of storage on oil percentage of soybean seeds stored under different length of time. Short and mid-term stored seeds were kept at ambient conditions, while long term seeds were stored at cold storage conditions (-20°C).

Table 5 shows the fatty acid profile summary for the soybean seeds stored for varying length of time and conditions. Control seeds showed presence of myristic acid (0.2%), palmitic acid (11.1%), stearic acid (4.2%), oleic acid (27.1%), linoleic acid (50.5%) and linolenic acid (6.2%). Other fatty acids comprised only 0.7%. A clear reduction in terms of important fatty acids was observed when seeds were stored under ambient temperatures, and the reduction was more as the time of storage increased. The seeds stored under -20°C could not show much decrease in important fatty acids as compared to control. The statistical tests using one way ANOVA and Dunette's multiple comparison tests revealed that oil percentage was significantly lower for seeds stored under ambient conditions, but not significantly lower under cold storage conditions ($F_{9,20}$=174.2, p>0.05).

Table 5: fatty acid composition of soybean oil extract from seeds stored under varying length of time and temperatures using gas chromatography.

Storage pattern	Year	Fatty acid percentage						
		C14:0 Myristic acid	**C16:0** Palmitic acid	**C18:0** Stearic acid	**C18:1** Oleic acid	**C18:2** Linoleic acid	**C18:3** Linolenic acid	Others
Control	2015	0.2	11.1	4.2	27.1	50.5	6.2	0.7
Short term (ambient temp.)	2014	0.2	10.9	4.2	26.8	49.8	6.1	2.0
	2013	0.18	10.7	4.1	25.0	49.1	5.8	5.12
	2012	0.17	10.5	3.9	25.1	48.2	5.4	6.73
Mid term (ambient temp.)	2011	0.11	9.9	3.7	24.7	47.5	5.3	8.79
	2010	0.11	9.4	3.4	23.1	47.1	5.3	11.59
	2009	0.09	8.8	3.1	20.3	47	5.1	15.61
Long term (-20°C)	2008	0.21	11.0	4.1	26.8	50.1	6.2	1.59
	2007	0.18	10.9	4.0	26.1	50.1	6.1	2.62
	2006	0.14	10.4	3.8	26.1	48.2	5.9	5.46

DISCUSSION

The present study focused on storage of seeds for longer periods of time at ambient conditions (uncontrolled temperature and relative humidity) as well as under cold storage environment. The aim was to figure out the best possible storage conditions for seed germination as well as for the possible exploitation of stored seeds for commercial purposes, *i.e.*, oil extraction and feeding and fodder uses. The study determined the changes in seed germination and vigor for the plantation purposes, which is of direct uses to the farmers, especially in India, where storing seeds in cold storage for long term can be financially impossible.

The purpose of storage is to maintain harvest quality of product, not to improve it[9]. The rate at which the seed aging process takes place depends on the ability of seed to resist degradation changes by protection mechanisms which are specific for each plant species[10].

The present study shows that the soybean seeds lost germination and seed vigor within a year of storage at ambient conditions. In a study, decline in seed germination after six and twelve months of storage was pronounced in the soybean genotypes. The authors discussed that the soybean seed was significantly more sensitive to the length of storage, as well as to storage conditions[11].

A declining trend was observed in total oil content and seed germination rates during the storage of soybean. The degree as well as speed of decline in seed vigor depend strongly upon plant species, storage conditions as well as quality of the initial seeds[12]. Soybean are known to be

susceptible to deterioration under environmental conditions as their embryonic axis is proximal to the funicular end, which renders seed in direct contact with both water and oxygen. Continuous exposures to moisture and oxygen make the oil oxidized compromising the seed vigor and quality[13].

The present study showed that fatty acid composition of soybean seeds stored under ambient conditions was decreased in terms of major unsaturated fatty acids, *i.e.* oleic acid, lineic acid, linolenic acid. In a study by Prabakaran *et al*.[14], the fatty acid composition of soybean flour was shown to decreases up to 90% during storage under high temperature. Changes occurring in seed during storage are valuable study tools to study seed quality and seed longevity. The high oil content seeds are known to show rapid changes in the oil quality under specific environmental and aging conditions[15].

REFERNCES

[1] Agrahari, Raj Kishan, Yuriko Kobayashi, Pankaj Borgohain, Sanjib Kumar Panda, and Hiroyuki Koyama. "Aluminum-specific upregulation of GmALS3 in the shoots of soybeans: A potential biomarker for managing soybean production in acidic soil regions." *Agronomy* 10, no. 9 (2020): 1228.

[2] Dupare, B. U., and Sharma Purushottam Verma. "Adoption of soybean production technology by the farmers in Malwa Plateau of Madhya Pradesh." (2019).

[3] Mahmood, Tariq, and Shahzad MA Basra. "Invigoration of low vigor sunflower hybrids by seed priming." (2009).

[4] McDonald, M. B. "Seed deterioration: physiology, repair and assessment." *Seed Science and technology* 27, no. 1 (1999): 177-237.

[5] Krishnan, P., S. Nagarajan, M. M. A. V. Dadlani, and A. V. Moharir. "Characterization of wheat (Triticum aestivum) and soybean (Glycine max) seeds under accelerated ageing conditions by proton nuclear magnetic spectroscopy." *Seed science and technology* 31, no. 3 (2003): 541-550.

[6] International Seed Testing Association. *International rules for seed testing. Rules 1999*. No. Suppl. 1999.

[7] Maisuria, K. M., and S. T. Patel. "Seed germinability, root and shoot length and vigour index of soybean as influenced by rhizosphere fungi." *Karnataka Journal of Agricultural Sciences* 22, no. 5 (2009): 1120-1122.

[8] FSSAI manual method for oils and fats. Food safety and standards authority of India (2021).

[9] (Sisman Can Burak. "Quality losses in temporary sunflower seed stores and influances of storage conditions on quality losses during storage." *Journal of Central European Agriculture* 6, no. 2 (2005): 143-150.)

[10] Balešević-Tubić, S., Đ. Malenčić, M. Tatić, and J. Miladinović. "Influence of aging process on biochemical changes in sunflower seed/influencia del proceso de envejecimiento en los cambios bioquímicos en la semilla

de girasol/effet du processus de vieillissement sur les changements biochimiques dans la graine de tournesol." (2014).

[11] Balešević-Tubić, Svetlana, Mladen Tatić, V. Đorđević, Zorica Nikolić, and V. Đukić. "Seed viability of oil crops depending on storage conditions." *Helia* 33, no. 52 (2010): 153-160.

[12] Ludwig, Vagner, Magno Roberto Pasquetti Berghetti, Fabiane Portella Rossato, Lucas Malmann Wendt, Erani Eliseu Schultz, Vanderlei Both, and Auri Brackmann. "Impact of controlled atmosphere storage on physiological quality of soybean seed." *Journal of Stored Products Research* 90 (2021): 101749.

[13] McDonald, Miller B. "Orthodox seed deterioration and its repair." *Handbook of Seed Physiology: Applications to Agriculture, Benech-Arnold, RL and RA Sanchez (Eds.). Food Products Press, New York* (2004): 273-304.

[14] Prabakaran, Mayakrishnan, Kyoung-Jin Lee, Yeonju An, Chang Kwon, Soyeon Kim, Yujin Yang, Ateeque Ahmad, Seung-Hyun Kim, and Ill-Min Chung. "Changes in soybean (Glycine max L.) flour fatty-acid content based on storage temperature and duration." *Molecules* 23, no. 10 (2018): 2713.

[15] Mbofung, Gladys CY, A. Susana Goggi, Leonor FS Leandro, and Richard E. Mullen. "Effects of storage temperature and relative humidity on viability and vigor of treated soybean seeds." *Crop Science* 53, no. 3 (2013): 1086.