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Effect of Storage Periods on Seed Quality Characteristics of Three Soybean (*Glycine max* (L) Merrill) Varieties

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ABSTRACT

Seed storage experiment was carried out to investigate the most appropriate period of storing and preserving soybean seed quality under ambient conditions. Germination percentage, seed vigour, moisture content, 1000 seed weight, protein and oil contents were assessed before storage (control), three months (90 days) and six months (180 days) after storage. The results indicated that soybean seeds of the control recorded a high germination percentage and vigour than those stored for three and six months. On an average, germinability reduced by 17 % and 38 % at 3 and 6 months of storage respectively than seeds of the control. Seed vigour also reduced by 23 % and 71 % at the 3rd and 6th months of storage than those of the control. Temperature and relative humidity readings were high and fluctuating under ambient storage conditions, and these conditions contributed to increase in moisture and 1000 seed weight. The percentage oil content of the seeds reduced in storage by 0.37 % and 0.44 % at three and six months of storage. However, protein content of the seeds increased at three and six months in storage by 0.23 % and 1.77 %. Data collected were subjected to analysis of variance using Statistix Student Version 9.0 and treatment means separated using Tukey's HSD (Honest Significant Difference) at probability level of 0.01.

Keywords: Soybean, seed, storage period, seed quality.

I. INTRODUCTION

Seeds are considered to be in storage from the moment they reach physiological maturity until germination [1]. The purpose of storage is to maintain quality of harvested produce but not necessarily to improve it [2]. Seeds need to have good storage quality to ensure that it maintains conditions until it is used for sowing. During seed storage, quality can remain at the initial level or it may decline to a degree that would cause the seed to be unacceptable for planting [3]. Some seeds are naturally short-lived (e.g. soybean, onion, peanuts etc.) [1]. Storability of seeds is mainly a genetic character and is influenced by pre-storage history of seed, seed maturation and environmental factors during pre-and post-harvest stages [4].

Soybean seed decline in quality faster than seeds of other crops [5]; [6]. It has been reported that the short life span of soybean in storage could be due to certain factors including the high oil content and perhaps high moisture content [7]. One of the major constraints to the production of soybean in the tropics is the rapid loss of seed viability and vigour during storage under ambient conditions [8]. The loss of germination is much more acute under tropical conditions [9].

Seed deterioration is also associated with storage duration [10]. 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 [10]. Shrinking and breaking of seeds during

storage are some of the physical changes that occurred in soybean seed in storage [11]. Seed deterioration leads to reduction in quality, performance and stand establishment [10]. As seed quality deteriorates during storage, vigour declines before loss in standard germination [3].

Moreover, [12] reported that farmers in the developing world still store their produce including seed under the ambient environment. [13] added that storage under ambient conditions has been observed to affect seed quality in general and germination in particular. Storage is improved under ambient conditions if seeds are well-packaged [14]. Irrespective of initial seed quality, unfavourable storage conditions, particularly temperature and relative humidity, contribute to accelerating seed deterioration in storage [5]. Germination and seedling vigour are severely affected if seed is stored at high relative humidity and deterioration is much faster if the storage temperature is also high [15]. The study was therefore designed to determine the effect of period of storage on seed quality characteristics particularly, germinability, vigour, 1000 seed weight, moisture content, protein and fat contents.

II. METHODS AND MATERIAL

The experiment was carried out from January to June, 2013. The soybean seeds were stored at the Department of Horticulture under ambient conditions. Germination test and 1000 seed weight determination were carried out at Department of Horticulture, Kwame Nkrumah University of Science and Technology (KNUST). The moisture content, protein and fat content were determined at the Department of Biochemistry, KNUST, and seed vigour (Seed conductivity test) determined at the Department of Crop and Soil Sciences (KNUST).

Source of Seeds

Seeds of three varieties of soybean (Nangbaar, Anidaso and Jenguma) were procured from CSIR - Crops Research Institute (CRI) and CSIR - Savanna Agricultural Research Institute (SARI). The maturity classes of Nangbaar, Anidaso and Jenguma are early (≤100 days), medium (101-110 days) and late maturing (110-115 days), respectively [16].

Experiment 1: Seed Production Experiment

The seeds were produced at research fields at Crops Research Institute using three varieties in Randomized Complete Block Design (RCBD) with three replications. The land was manually prepared using the zero tillage technology. Seeds were planted in ten rows in each plot of 5 m long at spacing of 60 cm between rows and 10 cm within rows. The distance between replicates was 1 m. Three seeds were planted per hill and thinned to two plants per hill at two weeks after planting. No soil amendment or fertilizer was applied. Weeds were effectively controlled during the growing period. Spraying was carried out at four and six weeks after planting with Lambda Super 2 SEC to control insect pests. All the good agronomic practices were observed. Depending on the maturity class, each variety was harvested at physiological maturity. Physiological maturity harvesting was carried out when 90% of the pods on the plant turned brown [17]. Pods harvested at physiological maturity were further dried for one week before threshing manually.

Experiment 2: Seed Storage Experiment

The seed storage trial was set up in a 3 x 3 factorial arrangement in Completely Randomized Design (CRD) with four replications. The first factor was variety at three levels (Nangbaar, Anidaso and Jenguma); the second factor was storage duration at three levels (no storage, three and six months-storage). Seeds were not treated with any chemicals or botanicals before and during storage. Seeds were stored in brown paper envelopes.

Harvested seeds obtained were then used for further laboratory analyses to determine germination percentage, seed vigour (seed conductivity test), moisture content, protein and oil content. Temperature and relative humidity readings of the storage room were also recorded.

***Determination of Germination Percentage*:** Germination test was carried out according to [18]. For each treatment, 400 seeds from the pure seed fraction of a purity test were used to conduct the germination test. The seeds were arranged in four replicates of 100 each on a counting board and planted in a levelled layer of moist sand in a perforated container. On day eight, each replicate was examined

and evaluated separately. Seedlings and seeds were counted and grouped into normal and abnormal seedlings, fresh ungerminated seeds, hard and dead seeds. Germination percentage was determined using Equ 1, (ISTA, 2007).

$$\text{Germination \%} = \left[\frac{\text{Number of germinated seeds}}{\text{Number of total seeds planted}}\right] \text{x } 100 \text{ .....Equ 1}$$

***Determination of Seed Vigour*:** Conductivity test was used in determining the vigour of the seeds [8]. Four replicates of 50 seeds of each treatment were drawn at random and tested for electrical conductivity. Seeds were placed in Erlenmeyer flasks containing 75 ml ultra-pure deionized water equilibrated to 25 °C, then maintained at 25 °C for 24 h. After 24 h of soaking, the flasks was swirled for 10-15 sec and seeds then taken out of water with a clean forceps. An electrical conductivity dip cell was inserted into the seep water until a stabilized reading was achieved and recorded. The mean of the two control flasks (sterilized distilled water) when measured served as background reading. Conductivity was calculated using Equ 2, [18].

$$\text{Conductivity, } (\mu S\ cm^{-1}g^{-1}) = \left[\frac{\text{Conductivity reading} - \text{background reading}}{\text{Weight (g) of replicate}}\right] \text{.....Equ 2}$$

Determination of Moisture Content: The low constant temperature oven method [19] was used to determine the moisture content of the seeds. Empty glass crucible was thoroughly washed, cleaned and dried for one hour at 130 °C and placed in desiccator to cool. The empty crucible and its cover were then weighed before and after filling. About 5 g milled soybean seed from each sample was weighed and transferred into a previously weighed empty glass crucible and placed in an oven maintained at a temperature of 105 °C and dry for 5 h. Four replicates were taken. At the end of the prescribed period, the container was covered and removed from the oven and allowed to cool in desiccator to room temperature. After cooling, the container with its cover and content was reweighed and figures recorded. Loss in weight was calculated as percentage moisture content using Equ 3, [19].

$$\text{\% Moisture (wt)} = \left[\frac{\text{weight of wet sample} - \text{weight of dry sample}}{\text{weight of wet sample}}\right] \text{x } 100 \text{....Equ 3}$$

***Determination of Crude Fat Content*:** The sample used for the moisture content determination was transferred into a paper thimble, labeled and put in a thimble holder for the crude fat determination [19]. 150 mL of petroleum spirit was poured into a pre-weighed 500 mL round bottom flask and assembled on a semi-continuous Soxhlet extractor and refluxed for 16 h. The hexane was recovered after removing the paper thimble from the thimble holder and the flask containing the fat heated for 30 min in an oven at 103^0 C to get rid of the residual hexane. The flask containing the fat was re-weighed after being cooled in a desiccator [19]. The increase in weight was calculated as percentage crude fat as shown in Equ 4.

$$\text{\% Fat} = \left(\frac{\text{weight of fat}}{\text{weight of sample}}\right) \text{x } 100 \text{ .....Equ 4}$$

Determination of Protein Content

The protein content was determined using the Kjeldahl method in three steps: digestion, neutralization and distillation, and titration [19].

Digestion: About 2 g of the sample was weighed into a digestion flask and mixed with 25 mL of concentrated H_2SO_4, selenium catalyst and few anti-bumping agents. The content of the flask was digested by heating in a fume chamber till the colour of the solution turned clear.

Neutralization and Distillation: after the digestion has been completed, the digestion flask was allowed to cool, the solution transferred into a 100 mL volumetric flask, and the volume made up to the 100 mL mark with distilled water. The distillation apparatus was flashed out with water and 10 mL of digested sample transferred into the distillation apparatus. The solution was neutralized with 18 mL NaOH and boiled under distillation water in a steam generator. Circulation was allowed for about 10 min. A conical flask was filled with 25 mL of 2% boric acid and 3 drops of mixed indicator (methylene blue and methylene red) added. The conical flask and its content were placed under the condenser in a position where the tip of the condenser was completely immersed in solution for 10 min and end of condenser washed with distilled water.

Titration: The nitrogen content was then estimated by titrating the ammonium borate formed in the conical

flask with 0.1M HCl solution. Titre values of the replicate samples were recorded and percentage nitrogen calculated using Equ 5. A blank sample was run at the same time as the sample is being analyzed [19].

$$\%\text{Nitrogen} = \left[\frac{(St - Sb)x\ NA\ x\ 100\ x\ 0.1\ x\ 0.014\ x\ 100}{\text{Sample weight x } 10}\right] \quad \ldots\text{Equ 5}$$

Temperature and Relative Humidity of Storage Room

The ambient storage room temperature and relative humidity readings were taken at specified times of 9:00 am, 12:00 pm and 6.00 pm. Acurite manufactured indoor digital humidity and temperature monitor (00325) was used in taking the readings.

Data analysis: Data collected were subjected to analysis of variance using Statistix Student Version 9.0 and treatment means separated using Tukey's HSD (Honest Significant Difference) at probability level of 0.01.

III. RESULTS AND DISCUSSIONS

Ambient conditions of storage environment

Relative humidity ranged from 61.7% to 86.6% whereas temperature ranged between 22.6 ºC and 28.8 ºC. The minimum relative humidity was recorded in January, 2013 and the maximum in June, 2013. The minimum temperature was observed in January, 2013 and the maximum in April, 2013 (Table 1).

Table 1: Average relative humidity and temperature in the storage environment

Month	Relative Humidity (%)	Temperature (ºC)	Maximum Relative Humidity (%)	Minimum Temperature (ºC)
January	61.68	27.86	66.65	22.61
February	63.37	28.32	80.68	24.46
March	72.82	28.54	83.42	24.77
April	70.10	28.81	84.50	24.77
May	74.19	27.92	86.50	24.16
June	76.21	27.09	86.58	23.85

Effects of varieties and storage periods on germination of soybean seed

There was significant variety x storage period interaction (P≤0.01) for seed germination capacity (Table 2). Nangbaar seeds without any storage registered the highest germination percentage (92.17%) though it was not significantly different from other varieties at no storage. Jenguma at six months of storage recorded the lowest germination percentage (34.75%). Seeds without storage gave significantly the highest germination percentage whereas those stored for six months recorded the least (Table 2). Seeds stored for three and six months reduced in germinability by 17% and 38% respectively than seeds not stored. These results indicated that germination percentage decreases with increase in storage period. However, the decline in seed germination at six months of storage was more pronounced under ambient conditions. The research findings also revealed that the soybean seed was significantly more sensitive to the length of storage, as well as the storage conditions. Similar results were obtained by [20] for soybean seed stored for six months under conventional conditions. They stated that in a group of tested varieties only one maintained germination above 80%. This also confirmed the findings of [21], who stated that irrespective of genotypes, the germination potential of soybean seeds decreased during storage. [9] also added that the germination potential (viability) is very short lived in soybean as compared to other oilseed crops and are often reduced prior to planting time. This loss of germination is much more acute under tropical conditions. The environmental conditions make it very difficult to maintain viability during storage.

Table 2: The effect of storage period on germination (%) of soybean seeds

Storage Periods	Soybean Varieties			Mean
	Nangbaar	Anidaso	Jenguma	
No storage	92.17	86.58	87.33	88.69
Three months	77.75	70.58	68.17	72.17
Six months	59.92	57.00	34.75	50.56
Mean	76.61	71.39	63.42	

Tukey HSD (0.01): Variety = 3.82; Storage Periods = 3.82; Variety x Storage Periods = 8.24.

Effects of harvesting stages and storage periods on vigour of soybean seed

There were no significant interactions for seed vigour. However, between varieties, Jenguma obtained the highest electrical conductivity value (37.87 µS cm-^{1}g-^{1}). Both Anidaso and Nangbaar recorded the lowest (35.20 µS cm-^{1}g-^{1} and 35.56 µS cm-^{1}g-^{1} respectively) though there were no significant differences between them (Table 3a). Seeds stored for six months recorded significantly the highest conductivity value (47.19 µS cm-^{1}g-^{1}) than other storage periods (Table 3b). However, [18] indicated that seed lots that have high electrolyte, that is, having high leachate conductivity, are considered as having low vigour, while those with low leakage (low conductivity) are considered high vigour seeds. The result implied that seed vigour decreased when the period of storage increased. At the beginning of seed storage, the vigour was high in all the varieties but reduced as time progressed, especially, at six months of storage. Seeds stored for three and six months recorded 23 % and 71 % reduction, respectively, in vigour as compared to seeds not stored. This support the statement made by [8] that one of the major constraints to the production of soybean in the tropics is the rapid loss of seed viability and vigour during storage under ambient conditions. [3] added that as seed quality deteriorates during storage, vigour declines before loss in standard germination.

Table 3a: Seed Conductivity (Vigour) of three soybean varieties

Soybean Varieties	Seed Conductivity (µS cm^{-1}g^{-1})
Nangbaar	35.56
Anidaso	35.20
Jenguma	37.87
Mean	36.21
Tukey HSD (0.01)	

Table 3b: Effect of storage periods on seed conductivity (Vigour) of soybean seed

Storage periods	Seed Conductivity (µS cm^{-1}g^{-1})
No storage	27.55
Three months	33.88
Six months	47.19
Mean	36.21
Tukey HSD (0.01)	

Effects of varieties and storage period on moisture content (%) of soybean seed

There was significant variety x storage period interaction (P≤0.01) for seed moisture content (Table 4). Anidaso seeds without any storage registered significantly the least (7.55%) moisture content. However, Nangbaar seeds stored for six months had the highest (9.27%) moisture content. Seeds without storage registered the lowest (7.80%) whereas seeds stored for six months recorded significantly the highest percentage moisture content (9.20%). The results indicated that the seed moisture content for the entire storage duration ranged between 7.55 and 9.27. These figures were within the safe moisture limit for long storage and implied that the seeds were dried properly before storage. [22] recommended that oilseeds storage for extended period is only possible if the seed moisture content is less than 10 % or preferably dried to 8 %. In addition, the experimental results showed that the moisture content increased in response to an increase in the storage duration under ambient conditions. The climatic information (Table 1) during the study period revealed that there was a rise in relative humidity from January to June. Therefore, the seeds absorbed moisture under ambient storage. Nevertheless, the rise in moisture content did not exceed the safe moisture limit.

Table 4: The effect of storage period on moisture content (%) of soybean seeds

Storage Periods	Soybean Varieties			Mean
	Nangbaar	Anidaso	Jenguma	
No storage	7.99	7.55	7.85	7.80
Three months	8.28	7.79	8.15	8.07
Six months	9.27	9.15	9.17	9.20
Mean	8.51	8.16	8.39	
Tukey HSD (0.01): Variety = 0.08; Storage Periods = 0.08; Variety x Storage Periods = 0.17.				

Effects of varieties and storage periods on protein content of soybean seed

There was significant variety x storage period interaction such that Nangbaar and Anidaso stored for six months had significantly the highest seed protein content (30.51% and 30.40% respectively). Nangbaar without storage (28.08%) had the least seed protein content (Table 5). Storing soybean seeds for six months recorded the highest mean percentage protein content (30.14%) while seeds without storage (28.37 %) obtained the least. Across the storage periods, Anidaso produced the highest protein content whereas Jenguma and Nangbaar registered the lowest (Table 5)

Nangbaar seeds without storage had an average protein content of 28.08 %, Anidaso had 28.68 % and Jenguma obtained 28.36%. [23] found the average protein content of Nangbaar to be 43.00±0.18% and 46.38±0.08% for Anidaso. This implied that the average percentage protein content obtained from this study was low as compared to the findings of [23]. The results also showed that the percentage protein content increased periodically in storage. However, according to [24], the total protein content of seed as calculated from its nitrogen content is generally assumed constant during storage. As fungal deterioration advances however and carbohydrate is used in the respiratory processes, protein increases when protein test is conducted and calculated.

Table 5: Effects of varieties and storage periods on protein content (%) of soybean seeds

Storage Periods	Soybean Varieties			Mean
	Nangbaar	Anidaso	Jenguma	
No storage	28.08	28.68	28.36	28.37
Three months	28.15	29.21	28.45	28.60
Six months	30.51	30.40	29.52	30.14
Mean	28.91	29.43	28.78	
Tukey HSD (0.01): Variety = 0.13; Storage Periods = 0.13; Variety x Storage Periods = 0.28.				

Effects of varieties and storage periods on oil content of soybean seed

There was significant variety x storage period interaction for seed oil content (Table 6). Anidaso seed without storage contained significantly the highest oil content (18.63%) whereas Nangbaar and Jenguma seeds stored for six months contained the lowest oil content (18% and 18.06% respectively). Soybean seeds without storage contained significantly high oil content (18.61%) as compared to seeds stored for six months, which recorded the least (18.17%). Anidaso had the highest oil content (18.53%). Nangbaar registered the least oil content (18.21%). [23] recorded an average oil content of 16.77± 0.23% for Nangbaar and 16.45±0.07% for Anidaso at physiological maturity. The implication was that the oil content obtained in this study was comparatively high to that of [23]. However, it confirmed the findings of [25] that at maturity, soybean contains 18% oil.

Furthermore, the results also revealed that as the storage duration increased, oil content reduced. According to [26], oil seeds are very sensitive to the harsh environmental conditions. It is hypothesized that their oil content readily oxidizes, which enhances deterioration of the seed in storage. Therefore, the reduction could be attributed to oxidation during storage. According to [7], the chemical composition of oilseeds causes specific processes to occur during storage. The seeds rich in lipids have limited longevity due to their specific chemical composition. For example, soybean seed storage demands special attention due to its oil content; otherwise, processes may occur that would lead to the loss of germination ability and seed viability [7]. Shrinking and breaking of seeds during storage are some of the physical changes that occurred in soybean seed in storage [11].

Table 6: The effect of storage periods on oil content (%) of soybean seeds

Storage Periods	Soybean Varieties			Mean
	Nangbaar	Anidaso	Jenguma	
No storage	18.59	18.63	18.61	18.61
Three months	18.04	18.53	18.14	18.24
Six months	18.00	18.44	18.06	18.17
Mean	18.21	18.53	18.27	
Tukey HSD (0.01): Variety = 0.05; Storage Periods = 0.05; Variety x Storage Periods = 0.11.				

Main effects of varieties and storage periods on 1000 seed weight of soybean seed

There were no significant interactions for seed weight. However, among varieties, Jenguma registered significantly the heaviest seed weight (126.30 g). Nangbaar and Anidaso (116.92 g and 116.97 g, respectively (Table 7a) obtained the least seed weight. [23], found the average 1000 seed weight for Nangbaar to be 115.5±7.2 g, 96.08±8.2 g for Anidaso. The result for Nangbaar was similar to that of [23]. However, that of Anidaso differed from what [23]

reported. Between storage periods, the 1000 seed weight was higher than that which was not stored (Table 7b) and could be attributed to the rise in relative humidity in every month in storage.

Table 7a: 1000 seed weight (g) of soybean as affected by varieties

Soybean Varieties	Seed Weight (g)
Nangbaar	116.92
Anidaso	116.97
Jenguma	126.30
Mean	120.10
Tukey HSD (0.01)	2.29

Table 7b: The effect of storage periods on 1000 seed weight (g) of soybean seeds

Storage periods	Seed Weight (g)
No storage	116.76
Three months	119.14
Six months	124.29
Mean	120.10
Tukey HSD (0.01)	2.29

IV. CONCLUSIONS

The research findings from the study revealed that:

1. Relative humidity ranged from 61.7% to 86.6% whereas temperature ranged between 22.6 ºC and 28.8 ºC.
2. There was significant variety x storage period interaction (P≤0.01) for seed germination capacity. Nangbaar seeds without any storage recorded the highest germination percentage (92.17%) though it was not significantly different from that of the control. Jenguma at six months of storage recorded the lowest germination percentage (34.75%). Seeds in the control recorded the highest germination percentage whereas those stored for six months recorded the least.
3. There were no significant interactions for seed vigour. Jenguma obtained the highest electrical conductivity value (37.87 µS $cm^{-1}g^{-1}$). Both Anidaso and Nangbaar recorded the lowest (35.20 µS $cm^{-1}g^{-1}$ and 35.56 µS $cm^{-1}g^{-1}$ respectively). Seeds stored for six months recorded significantly the highest conductivity value (47.19 µS $cm^{-1}g^{-1}$) than those stored for three months.
4. There was significant variety x storage period interaction (P≤0.01) for seed moisture content. Anidaso seeds without any storage registered significantly the least (7.55%) moisture content. However, Nangbaar seeds stored for six months had the highest (9.27%) moisture content. Seeds without storage registered the lowest (7.80%) whereas seeds stored for six months recorded significantly the highest percentage moisture content (9.20%). The results indicated that the seed moisture content for the entire storage duration ranged between 7.55 and 9.27.
5. There was significant variety x storage period interaction such that Nangbaar and Anidaso stored for six months produced significantly the highest seed protein content (30.51% and 30.40% respectively). Nangbaar seeds without storage (28.08%) produced the lowest seed protein content. Storing soybean seeds for six months recorded the highest mean percentage protein content (30.14%) while seeds without storage (28.37 %) obtained the least. Across the storage periods, Anidaso produced the highest protein content whereas Jenguma and Nangbaar registered the lowest. Nangbaar seeds without storage had an average protein content of 28.08 %, Anidaso had 28.68 % and Jenguma obtained 28.36%.
6. There was significant variety x storage period interaction for seed oil content (Table 6). Anidaso seed without storage contained significantly the highest oil content (18.63%) whereas Nangbaar and Jenguma seeds stored for six months contained the lowest oil content (18% and 18.06% respectively). Soybean seeds without storage contained significantly high oil content (18.61%) as compared to seeds stored for six months which recorded the least (18.17%). Anidaso had the highest oil content (18.53%). Nangbaar registered the least oil content (18.21%).
7. There were no significant interactions for one thousand seed weight. However, among varieties, Jenguma registered significantly the heaviest seed weight (126.30 g). Nangbaar and Anidaso (116.92 g and 116.97 g, respectively obtained the least seed weight.

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