



STUDY OF STORAGE PERIOD AND PACKAGING TYPES AGAINST PHYSIOLOGICAL ASPECTS OF SOYBEAN (*GLYCINE MAX* (L.) MERRILL) SEEDS

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ABSTRACT

Soybeans are one of the agricultural commodities that are economically strategic sectors in Indonesia. The main limiting factor for soybean production is the availability of quality seeds because soybean seeds rapidly deterioration in storage. This study aimed to determine the effect of packaging type and storage period on the physiological aspects of soybean seed viability. The study was conducted at the Balai Benih Induk Laboratory, Medan, North Sumatra, Indonesia from January to September. The study used factorial Randomized Completely Design with 3 replications. The first factor is the packaging type (P) consisting of 4 levels: container (P1); plastic bags (P2), pots (P3), wooden box (P4). The second factor is the save period (S) consists of 3 levels: 2 months (S1), 4 months (S2), 6 months (S3). Variables observed were seed water content, germination, germination rate index, anaphase index, and chromosome aberration. The results showed that soybean seeds stored in container were able to maintain the quality of their seeds for 6 months in the storage period based on a decrease in water content, germination, and anaphase index. Soybean seeds stored in container can reduce the rate of deterioration of seed viability based on the germination rate index. Although the germination of soybean seeds stored in container for 6 months is still above 90%, they have experienced chromosome aberration.

KEYWORDS: Soybean seed, packaging, storage period, water content, seed viability.

INTRODUCTION

Soybeans (*Glycine max* (L.) Merrill) are one of the agricultural commodities that are economically strategic sectors in Indonesia. Soybeans are also a major source of vegetable protein for the people of Indonesia.^[1] Increasing population and awareness of nutritious food cause soybean demand is also increasing, but production from year to year is insufficient domestic demand.^[2]

Soybean production in Indonesia in 2015 reached 963,183 ton/ha.^[3] This soybean production is only able to meet around 65.61% of domestic consumption.^[4] The instability of soybean production in Indonesia is caused by a decrease in soybean harvest area which is not offset by an increase in soybean productivity.^[5] The low level of productivity and profits of soybean farming compared

to other commodities such as rice and corn, causes farmers to be less interested in growing soybeans and move to other more profitable farming.^[6] In addition, the main limiting factor for soybean production is the availability of quality seeds. The use of certified soybean seeds by farmers is still very low at around 5%.^[7]

Seed is one of the main factors that determines the success of farming and should be treated seriously in order to be provided with good and affordable by farmers.^[8] Provision of adequate quantities of soybean seeds in a timely manner is often a problem because of the low shelf life. Meanwhile, the provision of high quality seeds is an important element in efforts to increase crop production. Provision of seeds is often prepared some time before the planting season arrives so

that the seeds must be stored properly so they have high sprout power when planted again the following season.^[9] Quickly deterioration into a soybean seed in storage due to fat and protein content is relatively high, respectively 16% and 37%.^[10] In addition, Indonesia's tropical climate conditions with high temperature and humidity can also trigger the rate of deterioration of soybean seeds in storage.^[11] Seed deterioration is a seed physiological quality pullback process that causes changes in the seed thorough physical, physiological, and biochemical result in reduced seed viability.^[12] The deterioration of soybean seeds during storage is faster than other seeds with a rapid loss of seed vigor which causes a decrease in seed germination. Seeds that have low vigor seedlings in the field led to the appearance of the low, especially in the less than ideal soil conditions. So that the soybean seeds to be planted must be stored in a favorable environment (low temperature) in order to the quality of the seeds is still high until the end of storage.^[13]

According to,^[14] the use of packaging plays an important role in trying to maintain seed viability during storage. Suggests of,^[15] that the use of container and how to save seeds is highly dependent on the type, number of seeds, packaging techniques, long storage, store room temperature and humidity of disk space. For seed storage, the effectiveness of a package is determined by its ability to maintain seed moisture content and seed viability during storage. In low temperatures, respiration runs slowly compared to high temperatures so that seed viability can be maintained.^[16]

Based on the above, this study aimed to determine the effect of packaging types and storage period on the physiological aspects of soybean seed viability.

MATERIALS AND METHODS

The research was carried out at the Balai Benih Induk Laboratory, Gedung Johor, Medan, North Sumatra, Indonesia from January to September. This study uses factorial Randomized Completely Design with 3 replications. The first factor is the packaging type (P) consisting of 4 levels, namely: container (P1); plastic bags (P2), pots (P3), and wooden box (P4). The second factor is the storage period (S) which consists of 3 levels, namely: 2 months (S1), 4 months (S2), and 6 months (S3). Variables observed were seed water content, germination, germination rate index, anaphase index, and chromosome aberration.

Seed water content is calculated by equation.^[17]

$$WC (\%) = \frac{(M2 - M3)}{(M2 - M1)} \times 100\%$$

Where WC= seed water content (%); M1 = vessel weight before oven (g); M2 = vessel weight + seed before oven (g); M3 = vessel weight + seed after oven (g).

Seed germination is calculated by equation^[17]

$$\text{Germination } (\%) = \frac{\text{Number of normal seedling}}{\text{Total seed sown}} \times 100\%$$

Germination rate index is calculate by equation^[14]

$$GRI = \frac{G1}{D1} + \frac{G2}{D2} + \frac{G3}{D3} + \dots + \frac{Gn}{Dn}$$

Where: GRI = Germination rate index, G = The number of seeds that germinate on certain days, D = The time corresponding to a certain amount, n = Number of days in the last calculation.

Calculation of anaphase index begins with making preparations using the squash method. Soybean seeds are cut \pm 2 mm and fixed immediately in a 2-24 hour farmer solution. Then hydrolyzed in 3N HCL solution \pm 2-5 minutes and aceto orcein staining \pm 5 minutes.^[18] Observations were carried out using a L301 type light microscope. Data on the anaphase phase division are calculated, recorded and photographed. Then the anaphase index is calculated using equations^[18]

$$\text{Anaphase Index } (\%) = \frac{\text{Number of cells dividing}}{\text{Number of cells in one field of view}} \times 100\%$$

To determine the presence of chromosome aberration, a number of chromosomal aberration cells are taken into account in the preparation. Cells are calculated in the anaphase phase with 1000x magnification.

RESULTS AND DISCUSSION

Water Content (%)

The water content of the seeds is related to the relative humidity of the air because the seeds are hygroscopic. The seeds absorb water from the air or release water into the air so that the balance between the seeds and air humidity is reached.^[19] According to,^[15] water content is the factor that most influences the deterioration of seed quality, where the deterioration in seed quality increases with decreasing seed water content.

The results of variance analysis showed that the packaging type and storage period significantly affected the water content of soybean seeds (Table 1). The highest water content of seeds was found in the treatment of wooden box and pot packaging types, which were 12.23% and 12.04% respectively. The lowest water content of soybean seeds is found in the type of container packaging, which is 10.21%. The storage period also significantly affects the water content of soybean seeds, where the longer the storage period, the water content of soybean seeds is also lower, namely 12.92% in the storage period of 2 months, to 11.46% in the 4 month storage period, and 9.99% in the storage period 6 months.

According to,^[8] during storage, the water content of seeds is one of the factors that greatly affects the storability of seed, so that when harvested, harvested

seeds are cultivated which are physiologically ripe seeds with low water content. The decrease in seed water content aims to suppress seed respiration rate. The lower the water content of the seed, the lower the respiration

rate, so the seeds can be stored longer because the rate of deterioration is slow. However, the water content of seeds that are too low can actually cause the seeds to break or become easily damaged.^[20]

Table 1: Average water content, germination, germination rate index, and anaphase index of soybean seeds with the treatment of packaging type and storage period.

Treatments	Water content (%)	Germination (%)	Germination rate index	Anaphase index (%)
Packaging Type (P)				
Container (P1)	10.21c	95.68a	10.37a	10.40a
Plastic Bags (P2)	11.33b	92.20b	8.67b	7.60b
Pot (P3)	12.04a	81.07c	7.80c	6.40c
Wooden Box (P4)	12.23a	59.20d	5.27d	4.60d
Storage Time (S)				
2 month (S1)	12.92a	97.98a	10.13a	9.85a
4 month (S2)	11.46b	82.22b	8.03b	7.25b
6 month (S3)	9.99c	65.91c	5.93c	4.65c
Interaction				
P1S1	11.65cd	97.34b	11.17a	11.40a
P1S2	10.21ef	95.48c	10.37b	10.40b
P1S3	8.77g	94.22d	9.57c	9.40c
P2S1	12.23b	100.00a	9.87c	10.20b
P2S2	11.31d	92.20e	8.67d	7.60e
P2S3	10.46ef	84.40f	7.47e	5.00g
P3S1	13.95a	100.00a	10.60b	9.40c
P3S2	12.08bc	81.99g	7.80e	6.40f
P3S3	10.10f	61.20h	4.99f	3.40h
P4S1	13.85a	94.60cd	8.87d	8.40d
P4S2	12.23b	59.20i	5.27f	4.60g
P4S3	10.61e	23.80j	1.67g	0.80i

Note: Rows in the same column followed by unequal letters differ significantly at the 5% level based on the LSD test

During the storage period, soybean seed decreased water content, both in the soybean seeds stored in container, plastic bags, pot, as well as the wooden box (Figure 1).

Figure 1 shown that soybean seeds stored in the packaging have decreased water content with increasing storage period. In line with the results of the^[21,17,22,23] which also showed that there was a decrease in seed water content during storage. However, it is different from the results of,^[24] which showed an increase in water content in soybean seeds during storage. According to,^[25] the same water content of seeds at the beginning of storage could vary during storage depending on the humidity of the storage space and the tightness of the packaging material used in storage. Further,^[26] states that the initial water content of seeds and packaging types is very influential in maintaining the water content of the seeds during storage. According to,^[27] the use of appropriate packaging materials can be protect the seeds from changes in storage environment conditions, namely relative humidity and temperature.

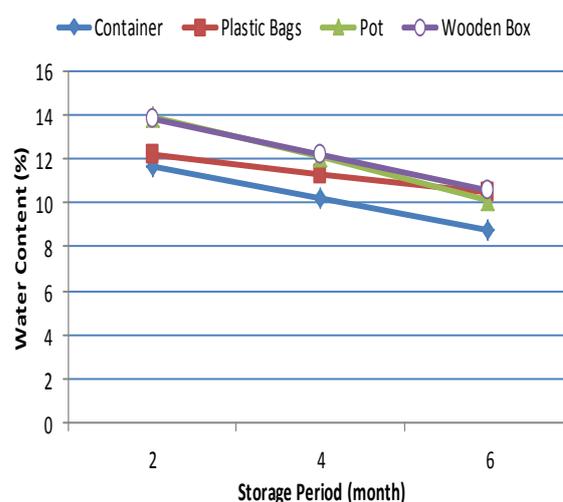


Figure 1: Changes in water content of soybean seeds during the storage period with various types of packaging.

Soybean seeds stored in pots and wooden boxes have decreased water content slowly with increasing storage period. The soybean seeds stored in the pot decreased

water content from 13.91% in a storage period of 2 months, to 12.20% in a storage period of 4 months and 10.58% in a storage period of 6 months. Similarly, soybean seeds stored in wooden boxes have decreased water content from 13.95% in a storage period of 2 months, to 12.08% in a storage period of 4 months, and 10.10% in a storage period of 6 months.

Soybean seeds stored in container experienced a significant decrease in water content during the storage period, ie from 11.69% in a storage period of 2 months, to 10.24% in a storage period of 4 months, and 8.75% in a storage period of 6 months. This is due to the humidity in the container is lower than other types of packaging. According to^[23], during storage, the water content of the seeds will increase if the surrounding air humidity is high enough. In addition to air humidity, the storage time factor and room temperature also affect the high and low water content of the seeds in the packaging. Changes in water content can not be separated from the influence of the relative humidity (RH) of each type of packaging. Where relative humidity is the ratio of the partial pressure of water vapor to the pressure of saturated water vapor at the same temperature.

Germination (%)

One of the most important things to know the quality of seeds in addition to water content is through germination. Germination is the potential ability of germinating seeds after optimal handling so that they can reflect the expected sprout yield during the nursery.^[28] The results of variance analysis showed that the type of packaging and storage period significantly affected the germination of soybean seeds (Table 1). Soybean seeds stored in container have higher germination than soybean seeds stored in plastic bags, and pots, which are 95.68%, 92.20%, and 81.07% respectively. The lowest germination of soybean seeds is soybean seeds stored in a wooden box, which is 59.20%.

Table 1 shown that the higher the water content of soybean seeds, the lower the germination of soybean seeds. In line with the results of the study of^[2] which showed that the high water content of soybean seeds and stored for 4 months resulted in a low percentage of germination. Similarly, the results of,^[24] which also showed a decrease in germination of green bean seeds with higher seed water content. The seed water content is the dominant factor in the process of seed deterioration.^[29] Similarly, according to,^[15] water content is the most influencing factor for seed deterioration, where seed deterioration increases with increasing seed water content. Decreasing the germination of seeds during storage and decreasing seed quality depends on the temperature and relative humidity of the storage space, the storage period, and the initial water content of the seed. Increased seed water content causes an increase in enzyme activity, including hydrolysis enzymes. The enzyme is already inside the seed and will be active if the seeds absorb water. Its activity increases when more

water is available.^[30] One of the hydrolysis enzymes is lipase which hydrolyzes fat into fatty acids and glycerol. Thus there is an increase in fat change into free fatty acids and glycerol,^[31] and increasing levels of free fatty acids, causing seed viability to decrease.^[14] Increased free fatty acids is related to an increase in water content in seeds,^[32] while^[33] states that increasing the temperature and water content of seeds in the storage space stimulates an increase in free fatty acids.

Table 1 also shown that the use of container and plastic bags as soybean seed storage vessel is able to maintain high germination of soybean seeds (95.68% and 92.20%, respectively). The basic principle of seed packaging is to maintain seed viability and vigor. Good and proper packaging can create a good storage space ecosystem for seeds so that seeds can be stored longer. Storage of seeds with airtight containers such as container and plastic bags can protect seeds from the influence of the surrounding environment such as relative air humidity and temperature. In addition, airtight storage containers reduce the availability of oxygen, which inhibits the activity of seed respiration.^[27] Respiration uses a substrate from the food reserves in the seed, so that the food reserves in the seed are reduced for embryo growth when the seeds are germinated. Respiration results in seed deposits in the form of heat and water vapor. Heat that arises as a scattering of energy in a seed that should be stored during storage directly can cause seed viability and vigor to decline.^[34]

As the water content of the seed, soybean seed germination was also decreased with increasing storage period (Figure 2). Figure 2 shown that with increasing storage periods, the germination of soybean seeds decreases. The storage period will affect seed viability, where the decrease in viability is in line with the increase in storage time.^[35]

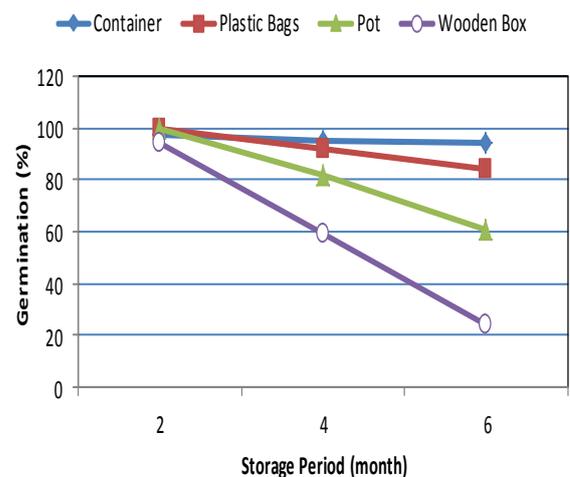


Figure 2: Decrease in germination of soybean seeds during the storage period with various types of packaging.

Soybean seeds that experience a significant decrease in germination are soybean seeds stored in a wooden box, while soybean seeds that experience a relatively low germination are soybean seeds stored in container. The results of the study by^[36] showed that the type of airtight packaging was able to maintain soybean seeds up to 4 months with a germination of 70%. From the results of his research, it was shown that the seeds that decreased their viability were caused by an increase in the content of significantly higher free fatty acids. High fat content allows deterioration. The free fatty acid levels of cocoa seeds increased in seeds stored at high water levels due to increased lipase activity.^[37] According to^[38] the increase in lipase activity in *Oryza sativa* seeds will cause the release of free fatty acids from triglycerides.

Free fatty acid levels increase with the high initial water content of the seed and the storage period. This occurs faster on soybean seeds stored in wooden boxes (Table 1 and Figure 2). Wooden boxes are porous packaging, allowing seeds to absorb water from their environment. When the seed water content increases, the formation of free fatty acids increases, so the seed will deteriorate which is indicated by a decrease in germination and germination rate index (Table 1, Figure 2 and Figure 3). According to^[15] changes in fat to fatty acids and glycerol occur rapidly on seeds that have deteriorated. Furthermore^[39] stated that the level of free fatty acids increased when soybean seeds were stored with a water content of 11.3% to 17% at a temperature of 25 °C for 140 days. Furthermore, it is said that the increase in levels of free fatty acids is in line with the longer the seeds are stored and negatively correlated with the appearance of seeds and seed viability.^[2]

Germination Rate Index

The germination rate index is a fast reactivation process when the surrounding conditions to grow optimally and the metabolic process is not inhibited. The germination rate index can be expressed as a measure of the vigor of the strength of seed growth. Seeds that have a high growth rate and simultaneous growth have high levels of vigor.^[40]

The results of variance analysis showed that the type of packaging and storage period significantly affected the germination rate index of soybean seeds (Table 1). Soybean seeds stored in containers and plastic bags have a higher germination rate index compared to soybean seeds stored in pots and wooden boxes, which are 10.37, 8.67, 7.80 and 5.27 respectively.

Packaging types and storage periods significantly influence the germination rate index (Table 1), where the storage period increases, the germination rate index of soybean seeds stored in containers, plastic bags, pots and wooden boxes also decreases (Figure 3).

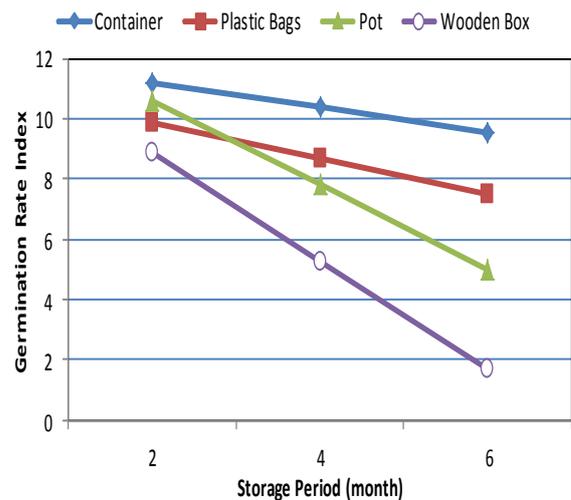


Figure 3: Decrease in the germination rate index of soybean seeds during the storage period with various types of packaging.

The rate of decline in the germination rate index of soybean seeds varies based on the type of packaging (Figure 3). Soybean seeds stored in containers and plastic bags have a lower rate of decline germination rate index than the soybean seeds stored in pots and wooden boxes. According to^[41] wheat seeds stored using porous packaging will accelerate deterioration because it will increase the water content of the seeds, thereby reducing the germination rate index of seeds. According to^[42] the high temperature and water content of the seeds are the contributing factors to the declining germination rate index of seed. In line with the results of the study by^[43] which shows that soybean seeds with an initial water content of 10.4% or lower which are packed with airtight vessels such as containers and polyethylene plastic can maintain a germination rate index of seed of more than 80% for 18 months. According to^[44] the germination rate index of soybean seeds will decrease from the initial germination rate index, which is above 90% to 0% depending on species and water content during storage. Furthermore^[45] stated that soybean seeds stored with a water content of 6% and 8% for 4 months at a temperature of 15 °C had a germination velocity index above 7.

Anaphase Index and Chromosome Aberration

Anaphase index is used to determine the rate of deterioration of a seed. According to^[35] before the seeds show symptoms of growth, the deterioration of seed viability can be seen by looking at the anatomical structure of the cell, both by looking at the nucleus of cells and organelles in the nucleus. One of the core organelles is the chromosome. In the normal mitotic process there is a doubling of chromosomes and then chromosome separation. The chromosome separation starts from the anaphase phase so that the telophase phase is formed 2 new cells.^[46]

The results of variance analysis showed that the type of packaging and storage period significantly affected the soybean seed anaphase index (Table 1). Table 1 shown that cell division in soybean seeds stored in containers is higher than cell division in soybean seeds stored in plastic bags, pots and wooden boxes. Anaphase index of soybean seeds stored in containers is higher than soybean seeds stored in plastic bags and pots, which are 10.40%, 7.60%, and 6.40% respectively, while the lowest anaphase index of soybean seeds is found in soybean seeds stored in wooden box, which is 4.60%. Soybean seeds also decreased the anaphase index by increasing the storage period, which is from 9.85% in the storage period of 2 months, to 7.25% in the storage period of 4 months, and 4.65% in the storage period of 6 months.

The low anaphase index in seeds stored in wooden boxes is due to the low germination and the germination rate index of soybean seeds stored in wooden boxes (Table 1). The low anaphase index is caused by the seeds having retreated once, so that the ability to anaphorize the germinated seeds is less or no equal. In line with the results of the study of,^[47] and,^[48] which showed that the seeds that experienced severe deterioration, the ability of the cells to anaphase was less than that, even the seeds did not grow at all. According to,^[47] seeds that are downturn or germinate and the germination rate index is

low, the anaphase index that occurs is also small. If the seed is damaged, the cell's mitotic division is blocked.

The high anaphase index in soybean seeds stored in containers and plastic bags is due to water content, germination and soybean seed germination rate indexes are also high (Table 1). In cell development, there are indications that the water content in the seed can increase osmotic pressure and cell permeability. This results in a reduction in pressure on the cell wall, increases protein synthesis and plasticity and development of cell walls.^[49,50] This process causes the cell wall to loosen, so that water can enter and the turgor pressure will rise. The rising turgor pressure will cause the cell to expand and if the cell development goes in the same direction for example towards the vertical it will cause cell elongation. In mitotic division there is a process of cell elongation. Water plays a role in activating enzymes in the seeds, thus affecting the process of mitotic division in soybean seeds.^[51]

Another event that occurs in seeds that experience deterioration is the occurrence of chromosome aberration. Chromosome aberration occurs because of the inability of the chromosomes to separate completely in the anaphase phase.^[46] In this study chromosome aberration occurred after 4 months of storage (Table 2).

Table 2: The average aberration of soybean chromosomes stored in various types of packaging in a storage period of 4 months.

Aberasi Kromosom	Packaging Type			
	Container	Plastic Bags	Pot	Wooden Box
	24.1	31.2	41.4	0.0

Table 2 shown that the highest chromosome aberration is found in soybean seeds stored in pots, which is 41.4 followed by soybean seeds stored in plastic bags (31.2), and containers (24.1), while soybean seeds stored in wooden boxes do not experience chromosome aberration. This is due to the soybean seeds stored in wooden boxes, the water content of the seeds is higher than the water content of soybean seeds stored in containers, plastic bags, and pots. In addition, the wooden box is hygroscopic which is able to absorb water vapor from the outside environment, thus creating a saturated state of water for the seeds inside. According to,^[48] seeds stored in a dry state are more likely to experience chromosome aberration compared to seeds stored in full imbibed. This is due to the dry condition of fat outolipoxidation in the seed which produces carbonyl compounds. Carbonyl compounds are very active so they can damage proteins. If DNA proteins are damaged, chromosome damage occurs, resulting in chromosome aberration.^[14]

CONCLUSION

Soybean seeds stored in containers are able to maintain the quality of their seeds for 6 months in the storage

period based on a decrease in water content, germination, and anaphase index.

Soybean seeds stored in containers can reduce the rate of deterioration of seed viability based on the germination rate index.

Although the germination of soybean seeds stored in containers for 6 months is still above 90%, they have experienced chromosomal aberration.

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