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To cite this article: M. Faisal *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **557** 012074

IOP Conf. Series: Materials Science and Engineering **557** (2019) 012074 doi:10.1088/1757-899X/557/1/012074

Determination of Mung Bean Seed Viability Change in Vacuum Packaging during Storage in Different Temperatures

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Abstract. Mung bean seeds decreased their viability very fast during storage, especially in tropical area without proper packaging. The purpose of this study is to observethe decrease in viability of mung bean seeds during storage and to build a mathematical model for shelf life prediction. Dried mung bean seeds were vacuum packed and stored in Accelerated Shelf-life Testing chamber at five different temperatures of 30° C, 35° C, 40° C, 45° C, 50° C with approximately 80% in relative humidity, to facilitate rapid deterioration with different rate of physical and chemical change in the seed. The results show that there was an increase in moisture content and the germination decreased in different rates. Changes in values for some parametersof the seeds affected the viabilty of the seeds during rapid deterioration. The value of constant (k)for viability decreasing was calculated and used to build the mathematical model for predicting the shelf life of mung bean seeds during storage at any temperature. Using the developed model, it was observed that the shelf life of the Sriti variety of mung bean seeds in vacuum packaging could maintain 90% of germination level for 421 days or 14.3 months if stored at20 °C, and for 173.37 days or 5.78 months if stored at 25 °C.

1. Introduction

Mung bean (*Vigna radiata (l.)* Wilczek*)* is the third important legumes crop in Indonesia after soybeans and peanuts. However, the production of mung bean in Indonesia is not stable and small if compared to its consumption, and Indonesia needs to import the rest from other caountry. During the ten years of 2006-2015, average mung beans production was 288,199 ton/year, ranging from 204,670 ton/year to 342,342 ton/year **[1]**. Until now, many efforts to increase mung bean production in Indonesia have been introduced, but the result is still under expectation.

One important aspect fora success mung beans production is the use of good quality seeds.The availability of high quality seeds is very important for a success business in agriculture, including cultivation and production of mung beans **[2]**. Quality seeds of superior varieties are one key factor for high productivity in mung beans production. According to **[3]** production of seeds is usually carried out some time before the planting season begins, so that the seeds will experience chemical changeduring storage. Therefore, the seed must be stored properly so that it will experience minimum chemical change so that the seeds would have optimal viability when they were planted. It needs a long several steps processes to produce good quality seeds, from selection of cultivating resourcesincluding seeds, and a good facility to store seeds in packaging. Since the seeds reach physiological maturity stage until they are planted, the seeds will be in the storage period. During this storage period the seeds will experience decline in viability because of chemical change.

The decrease in the quality of mung bean seeds is usually caused by the change of relatively high protein and fat content in the seeds, increasing of moisture content of the seeds if the temperature and humidity of the storage environment are relatively high. This decrease in quality is a gradual and cumulative process of deterioration in viability, and cannot be reversed due to physiological and biochemical changes **[4-5]**. The process of decreasing seed viability cannot be stopped, but might be slow down to get a longer shelf life. The rate of chemical change in seeds during the storage period is influenced by two factors, internal and external factors. The internal factor is related to the seeds characteristics that influence germination process, a genetic trait called physiological decline. The external factor isrelated to seed packaging, storage temperature and humidity which is called physical factors, and biological causing damage to the seeds such as bacteria, fungi, insects and mouse **[6].**

There are two methods to determine shelf life of storing food or agricultural products, namely conventional methods and ASLT (Accelerated Shelf-life Testing) method **[7]**. Conventional method is carried out by storing products in normal conditions as they should be. This method is accurate but it takes a long time and is expensive **[8].** To facilitate a faster chemical change, the ASLT method is applied by storing the product in a modified environment so that the stored product will undergoa quick chemical change under the spesific condition of temperature and humidity of the modified space. Under the condition of storage temperature and humidity, the acceleration method with the Arrhenius model is considered suitable for estimating the shelf life of stored seeds. Therefore, seed shelf life can rich their limit for a relatively short time, without having to wait for the seed shelf life in the actual storage conditions **[9]**. The purpose of this study is to examine the parameters of viability of mung bean seeds during storage under vacuum packaging and estimate the shelf life of mung bean seeds at several storage temperatures using the ASLT (Accelerated Shelf-life Testing) method.

2. Research Methods

2.1. Time and Research Place

The study was conducted from February 2018 - September 2018, at the Food and Agricultural Products Processing Engineering Laboratory, Mechanical and Biosystem Engineering Department, Faculty of Agricultural Engineering and Technology, and Seed Science and Technology Laboratory, Faculty of Agriculture, Bogor Agricultural University.

2.2. Materials and tools

The mung bean seeds used as the material in this study are Sriti varieties. As much as 40 kg mung beans are used, packed in a vacuum pack each contains 200 g. The material used for packaging was vacuum plastic packaging (hermetic), aquades, paper, and chemicals for analysis of free fatty acids. The equipment used was Eyela Environmental Chamber, an Oven of 2120 Isuzu Seisakusho, a Vacuum sealer, germinator,scissor, scales, petri dish, desiccator, soxhlet device, camera, container (plastic tray).

2.3. Material Preparation and Seed Packaging

Mung bean grainsare obtained from aseeds producer in Bolo District of Bima Region in West Nusa Tenggara (NTB) Province. The grains were sorted manually from separated from dirtand broken grains, and then sundried until itsmoisture content reached 10% wet base or lower.Then the mung bean seeds were packed in a vacuum plastic package (hermetic,13 x 21 cmin size, and thickness of 0.08 mm)according to the mung bean seed capacity of 200 g with a packaging, then sealed using a vacuum sealer. Mung bean seeds that have been packed were put in the Eyela Environmental Chamber with different temperatures, namely temperature settings for each treatment (30°C, 35 °C, 40 °C, 45 °C, 50 °C) and 80% relative humidity.The number of samples needed with two replications as much as two packs for each observation at each temperature treatment, so that the sample needed were 10 packs of each temperature parameter. Since the observations are made once before storage and four times during the storage period, then total sample needed for the whole experiment was 50 packs of each temperature treatment. Storage period was determined based on the estimated storage time until the quality of the seeds of mung beans decreases significantly.

2.4. Moisture Content Analysis

Moisture content measurement was carried out using the oven method **[10]**, with a petri dish as a container that was dried in an oven 150ºC for 1 hour before using. The petri dish was cooled in the desiccator for 15 minutes ,then it was weighed using an analytical balance. A sample of 5 g was added to the cup and then weighed and put the sample into the oven, drying with a temperature of 105ºC for 24 hours. After that the sample was taken out form the oven, cooled again in a desiccator, then weighed. Drying is carried out until the weight of the material is constantand moisture content was calculated using a formula :

$$m = \frac{a-b}{a} x\ 100\% \quad (1)$$

where :

m = moisture content (% wb)
a = sample initial weight (g)
b = final sample weight (g)

2.5. Germination Analysis of Between Paper Test Methods

The germination level test used in this study was based on the ISTA standard [**11**], a between paper test method. Mung bean seeds were germinated in moist substrate conditions for a certain period of time with the purpose of distinguishing between normal sprouts (*seedling*) and abnormal ones. The measurement procedure is that the seeds were prepared as many as 400 grains taken randomly from the components of pure seeds as a result of physical purity analysis. Next, the paper media to be used is moistened with aquades and 25 seeds were sprinkled repeatedly (the seeds were arranged in a zigzag position). Seeds that have been sown thenclosed with the paper that has been moistened and and rolled neatly. The paper roll containing the seed was put into plasticbag, given a name or experimental code on each roll of paper. The paper rolls, then put into the *germinator* and then observed. Observations were carried out according to the schedule set by [**11**], namely for the first count mung bean seed was done on the 5th day and the final count was held on the 7th day. Percentage of germination can be calculated by formula:

$$V = \frac{NSp}{NSd}\ x\ 100\% \quad (2)$$

where :
V= viability level (%)
NSp =number of sprouting seeds
NSd = number of seed

2.6. Mung bean Seed shelf-life estimation with ASLT Method

Germination becomes a critical parameter in determination of mung bean seeds shelf life estimation using the Arrhenius model of the *Accelerated Shelf-Life Testing* (ASLT) method **[12]**. ASLT was carried out using five different temperature treatments namely 30 °C, 35 °C, 40 °C, 45 °C, 50 °C. The percentage of germination during storage was plotted on a semi logarithmic chart. The degradation kinetics can be calculated based on zero order reactions as follow:

$$A_t - A_0 = -kt \tag{3}$$

where A_0 and A_t were the initial condition (germination level) and condition at time t, and k is the constant decrease in viability, and t is time. When referring to a first-order reaction it can be replaced with another equation as follow:

$$\ln A_t - \ln A_0 = -kt \tag{4}$$

Correlation between storage temperatures and seed viability degradation was calculated by the Arrhenius method, by plotting $^1/_T$ (temperature in the Kelvin scale) on ln k (reaction constants that have been converted to ln forms). From this new linear regression equation curve the value of constants and $^{Ea}/_R$ can be used to find the Arrhenius constant (k). From the curve obtained the following equation:

$$\ln k = \ln k_0 - \frac{Ea}{RT} \tag{5}$$

where k_0 is the initial constant, R is the ideal gas constant (1,986 cal/mol.K), T is the temperature (K) and Ea is the activation energy (kal/mol.K).

In this research, the shelf life prediction was determined based on minimum germination level of 90% which was then calculated at each storage temperature using the next equation:

$$t = \frac{A_0 - A_t}{k} \tag{6}$$

where t is time (day). This equation is used for zero-order reactions, whereas for first-order reactions, the initial and final conditions need to be converted into ln forms, so that equation 7 is obtained as follows:

$$t = \frac{\ln A_0 - \ln A_t}{k} \tag{7}$$

3. Results and Discussion

3.1. Moisture Content

In this research, moisture content was measured at various storage temperatures, from five treatments in the experiment. The moisture content was believed not to directly influence the shelf life of mung bean seeds during the storage process, however this parameter is believed having contribution in such away to the viability of seeds. The moisture content of the mung bean seeds during storage related to predetermined seed quality standards. The graph of changes in moisture content during storage is shown in Figure 1.

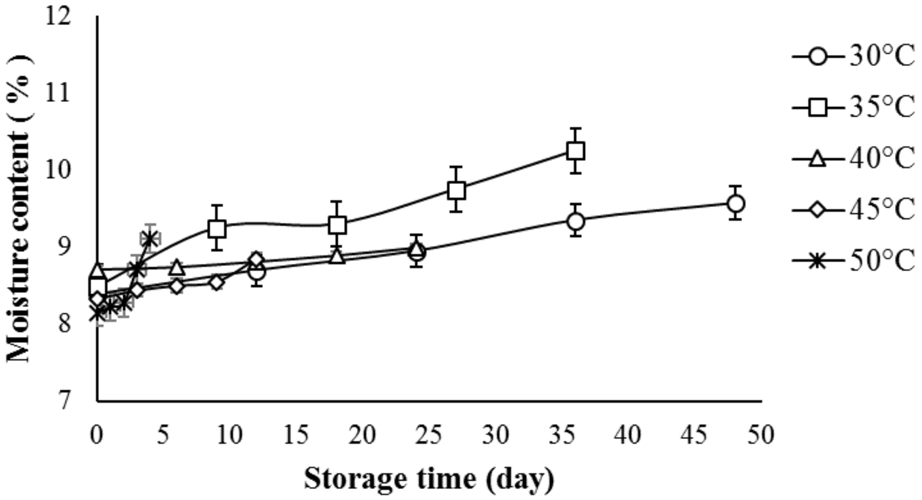

Figure 1. Changes in mung bean seed moisture content at five temperatures during storage period (30 °C, 35 °C, 40 °C, 45 °C and 50°C).

Moisture content for each temperature treatment slightly increased day by day. Allegedly the higher temperature during storage results in higher increasing in moisture content of mung bean seeds. This prooved that mung bean seeds are hygroscopic so they decrease (deterioration) in viability depending on the relative humidity of the air and temperature of the environment in which the seeds were stored. One factor that causes seeds to absorb water from the environment is the chemical composition of seeds, including protein. According to **[4]**, proteins are hygroscopic so they will absorb more water if the seeds are stored in humid conditions, and respiration, even slow enough, is also a factor that can cause a decrease (deterioration) in seeds. Respiration increases with increasing seed moisture content and temperature **[2]**. If the seeds are stored at low temperatures, the enzyme activity can be minimized so that respiration will be slow down. When stored at high temperatures, enzyme activity takes place faster so that respiration is faster, which causes the process of reforming food reserves faster. In this research, the results show that during storage of mung bean seeds at various temperature, the percentage of seed moisture content has increased but still within the limits of seed quality requirements. The quality requirements of mung bean seeds is a moisture content not higher than 11% **[13]**. Therefore the level of moisture content during storage is very important to minimize the reduction of mung bean seeds. Previous research has examined the effect of various water levels and storage temperature as stated in **[14]** that mung bean seeds with moisture content 9.4, 10.6 and 12.9 stored at 29 with 75% humidity for three months has a different vigor index, namely for water content 9.4 (23.46), 10.6 (21.55) and 12.9 (20.35).

3.2. Germination

In this research, changes in germination of mung bean seeds during the storage at five different temperatures of 30 °C, 35 °C, 40 °C, 45 °C and 50 °C are shown in Figure 2. In general, Figure 2 shows a decrease in the percentage of germination running faster at high temperatures than at low temperatures. This is similar to [**15**] that the seed has decreased the percentage of germination below 80% after being stored at temperatures above 50 °C for one month. While according to **[16]** seeds when stored at room temperature 30 °C the germination of seeds was still relatively high, which was above 80% after two months.

Another possibility for the reason lower germination level due to storage at high temperatures is the increase in mung bean seed respiration activity so that the food reserves in the seeds run out quickly. This phenomena is almost the same as the opinion expressed by [**5**] that storage of seeds at high temperatures can accelerate the activity of respiration enzymes.

Remodeling food reserves in the form of carbohydrates, protein and fat due to the process of respiration can produce metabolites. Some of these metabolites are inhibiting or poisoning other metabolism resulting in lost of germination before the energy supply in the seeds runs out.

The quality of germinating mung bean seeds in all temperatures setting after storage was still relatively good. Germination level was still above the average minimum threshold for seed quality requirements. Seed size was relatively small, protein and fat content was lower than average soybean seeds and the water content that ws at the safe limit, combined with vacuum packaging (hermetic) for storage were thought to be some factors that maintains the shelf life of the quality of mung bean seeds during storage. This fact is according to [**17**], with study which examined the effect of seed size on water absorption and germination of lentil seeds (*Lens cullinaris* Medik.), states that large-sized seeds absorb water more and faster than medium and small-sized seeds. According to [**18**] Boiyd and Van Acker (2004) the imbibition speed is influenced by the size of the seed and the diffusivity of water into the seed. With the storage of vacuum packaging (hermetic), it is suspected as important factor that maintains the germination level of mung beans was quite high. According to [**19**] Shu *et al.* (2018) vacuum packaging can inhibit changes in the morphology of mung bean seeds and maintain the quality of germination and combination of vacuum packaging with low temperatures further increasing the maintenance of germination level. This is because low temperatures decreases enzymes activities especially those contributes in respiration, can be suppressed. Meristematic cell death and decreased food reserves and degradation of enzymes can be slowed so that seed viabiality is higher.

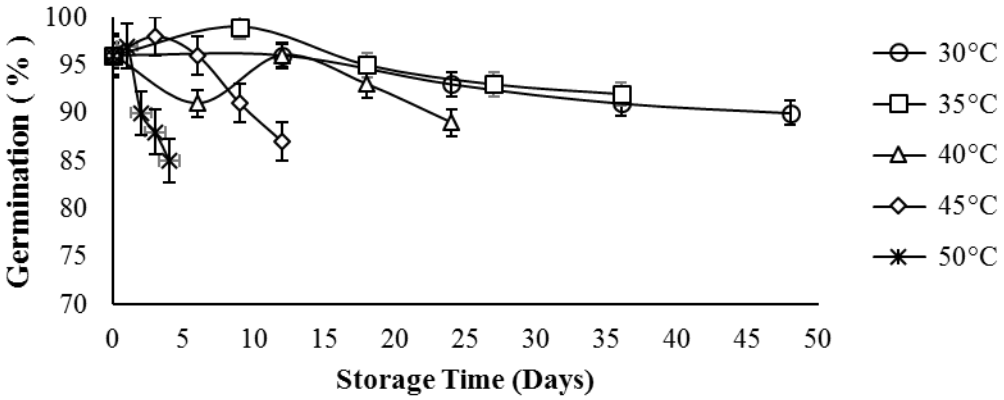

Figure 2. Changes in germination of mung bean seeds at five temperatures during storage time (30 ° C, 35 ° C, 40 ° C, 45 ° C and 50 ° C).

Figure 2 also shows that at 35 °C, 40 °C, 45 °C and 50 °C there is a slight change (fluctuation) of germination level, but after that it kept decreasing if compared to the one stored at room temperature (30 °C) that had no change (fluctuation) during storage. Allegedly mung bean seeds have a dormancy stage, but they can absorb water during the dormancy stage. However it will experience inhibition mechanism in the embryo causing the radicle to not appear and an imperfect growth or immature embryo and obstruction of water entering the seed so that causing seeds to fail to germinate.

3.3. Decrease in Germination

Long storage life seeds is very important for farmers and seed growers, nad they often having trouble in determining the expiration date or shelf life of seeds they stored. Almost similar to agricultural products such as fruit and vegetables which is quickly damaged and decay when stored at room temperature, but the decay in the seeds tend to happen much slower and take months until they really damaged. According to [**12**] shelf life estimation on storage condition, can be predicted based on material degradation which depends on the reaction order, reaction constant and activation energy. The kinetic model or order of reaction parameters is selected based on the regression equation with the highest coefficient of determination (R^2). The zero order indicates a quality change that occurs in a linear with storage time, whereas in order one the changes are exponential. Reaction order, rate of decline in quality and the coefficient of determination of the germination power of various types of mung bean seeds is shown in Table 1.

The slope values for all temperatures setting during storage show that the higher the temperature treatment results in the greater reaction rate, which means that the quality of mung bean seed quality decreases faster at higher storage temperatures (Table 1). It can also be seen that the coefficient of determination (R^2) for decreasing of the quality of mung bean seeds in all temperature treatments, for the first order reactions were higher than that of zero order reactions. Thus the kinetics reaction that decrease the quality of mung bean seeds during storage can be found, following the order pattern of the first order. The higher the coefficient of determination, the more accurate results of the data analysis. Declining quality of seeds tends to follow a first order reaction pattern [**9**].

Table 1. Order of reactions, rate of deterioration, and regression coefficient of germination for mung bean seeds after storage at five different temperatures.

Parameters	Temperature (°C)	Order 0		Order 1	
		Slope	R^2	Slope	R^2
Germination	30	0.1417	0.9383	0.0015	0.9395
	35	0.1556	0.6533	0.0016	0.6620
	40	0.2000	0.3789	0.0022	0.3805
	45	0.8333	0.7697	0.0090	0.7707
	50	3.1000	0.8998	0.0341	0.9049

After determining the reaction order, the first order reaction was chosen for the next step in the effort to determine shelf life by transforming the graph, plotting between 1/T as the absis and ln k as the ordinat so that a new equation can be developed, as shown in Figure 3. Figure 3 shows that the correlation value (R^2) for ln k and 1/T was 0.8505. This indicates that the variable reaction constant (y) was influenced by the storage temperature for germination level above 80%. The value of R^2 is approaching one ($R^{2=}1$) shows a very strong correlation between the constant reaction of mung bean seeds and storage temperature. Therefore the effect of temperature to provide changes in germination level parameters is obvious. Besides that the activation energy relationship tends to be inversely proportional to the rate of reaction. The smaller the activation energy, the faster the reaction rate because the minimum energy for the occurrence of a reaction gets smaller. On the other hand, the greater the activation energy, the reaction rate will be slower because the minimum energy for the reaction to occur is greater. Activation energy value can be calculated from the slope of the curve on the graph for relation of ln k and 1/T. The amount of activation energy obtained was 30769.098 kal/mol. Activation energy means that to reduce the germination of mung bean seeds during storage requires a minimal energy molecule of 30769.098 kal/mol. In this case it refers to the collision theory and movement of molecules of a material due to activation energy capable of initiating a reaction on the material (Rahmi *et al.* 2016).

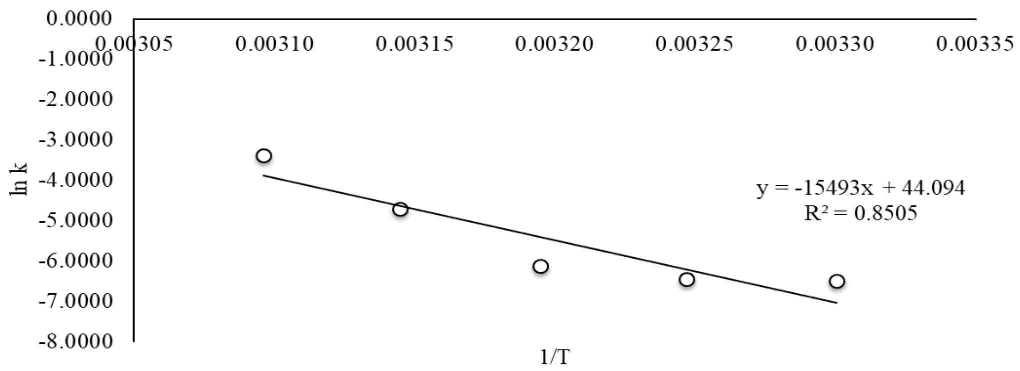

Figure 3. Arrhenius plot of order 1 reaction based on the relationship between the rate constant of germination and storage temperature.

3.4. Estimation of Mung Bean Seeds Shelf Life

One parameter that is able to describe seed viability directly is germination, not to mention mung bean seeds. The shelf life test of the Arrhenius method was chosen because the germination of mung bean seeds was sensitive to extreme temperature changes in a short time, with the equation for sheld life predicition was ln k = 44.094 + 15493/T. It is assumed, changes in the quality factor of mung bean seeds are only caused by one type of reaction and other factors do not result in significant quality changes, and the shelf life of mung bean seeds is determined from the critical limit of the germination level of 90%. Mung bean seeds germination at two storage conditions can be seen in Figure 4.

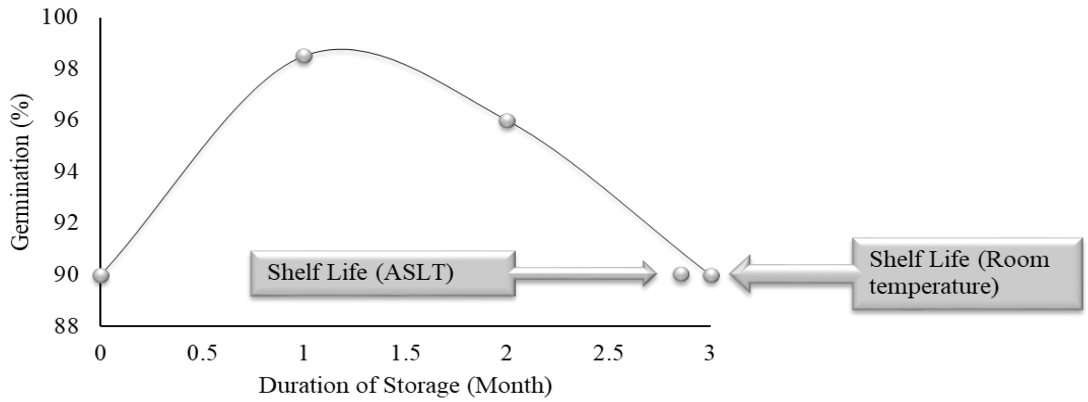

Figure 4. Germination of mung bean seeds in two storage conditions at room temperature 29 °C [**15**].

Figure 4 shows that mung bean seeds that stored naturally at room temperature 29 °C with RH 80% were able to maintain germination above 90% for 3 months at 29 °C [**15**]. In this experiment, the shelf life estimation results using the Accelerated Shelf-life Testing (ASLT) method with 90% germination level was predicted up to 2.9 months. According to [**18**] that seeds stored at 30 °C within 6 months of germination of mung bean seeds are above 90%. The same in the study of [**20**] that the storage of mung bean seeds at room temperature 30 °C with 75-80% RH, was able to maintain germination above 90% for 6 months. Besides that, if mung bean seeds are stored at room temperature 27 °C and RH 44% can only maintain 80% germination [**21**]. Allegedly different types of varieties can be seen in terms of seed physical size including medium and small seeds. So the smaller the seeds, the better the storability will be. Estimation of mung bean seeds shelf life results in various treatments at several storage temperatures with tolerance of viability levels above 90% are shown in Table 2.

Table 2. Estimation of the shelf life of mung bean seeds as a result of various treatments at several storage temperatures with tolerance of viability levels above 90%.

Parameters	Temperature (°C)	Shelf Life	
		(Days)	(Month)
Germination level ≥ 90%	20	421.00	14.03
	25	173.37	5.78
	30	73.52	2.45
	35	32.06	1.07
	40	14.35	0.48
	45	6.59	0.22
	50	3.10	0.10

Table 2 shows that the higher the storage temperature of seeds, the shorter the shelf life of mung bean seeds. The higher the storage temperature treatment results in a greater reaction speed which means a decrease in the quality of mung bean seeds occurs faster at temperatures higher than low temperatures. According to [**9**] that rapid method using high temperatures storage can reduce enzyme activity at embryo and cotyledon and reduce the amount of protein dissolved simultaneously within great shorter period. Decreasing enzyme activity and the amount of dissolved protein can increase the accumulation of peroxide which can inhibit the appearance of the radicle due to the formation of hydroxyl radicals. If there is a continuous increase in lipid peroxide, the seed germination and vigor will decrease over time.

4. Conclusion

The results of the study showed that during the storage moisture content of the seeds increased while their germination level were found decreased but still above the 80% as minimum limit of seed quality requirements. Activation energy value (Ea) to reduce the germination of mung bean seeds was found to be 30769.098 kal/mol, and this value can be used to calculate shelf life with the Arrhenius equation $\ln k = 44.094 + 15493/T$. using the developed equation, the estimation of the shelf life of the mung beans of the Sriti variety was found able to maintain 90% of germination level for 421 days or 14.03 months when stored at 20 °C, and for 173.37 days or 5.78 months when stored at 25 °C.

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