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# Deterioration of quality soybean seeds (*Glycine Max* (L.) Merr. AGS 190) at harvest stages, seed moisture content and storage temperature in Malaysia

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**Abstract**

Soybean [*Glycine max* (L.) Merill AGS 190) is an important vegetables and oil in the Asian communities like Malaysia. Seed deterioration is a harmful feature of agriculture crops which hindered quality seed of Soybean. Thus, the purpose of the study is to determine the effect of non-ultra-dry and ultra-dry seed moisture content stored in room temperature and cold room conditions for seed harvested stages on the quality or seed deterioration of soybean seeds (AGS 190) which grown under the humid tropical region. This study was conducted at Faculty of Agriculture, University Putra Malaysia in three harvested stages such as R6 (Full seed stage), R7 (Commencement of maturity period), and R8 (Fully maturity stage), moisture content (12% non-ultra-dry and ≤5% ultra-dry) and storage temperature (room storage at 25 to 30°C and cold room storage at 10°C). The result of this study showed that the seed deterioration rate was less in harvest stage R7 compared to R6 and R8 especially for ultra-dry seeds. In addition, seed deterioration can decrease at room temperature by the ultra-dry treatment compared non-ultra-dry but deterioration was higher for non-ultra-dry seed during storage at room temperature than cold room.

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## Introduction

Soybean is the world's leading legume (Weiss, 1983) with oil seed crops and protein content, widely produced in the tropical, sub-tropical as well as the temperate region (Akter *et al.*, (2014). Soybean seed is structurally weak, inherently short-lived and easily subject to damage (Delouche *et al.*, 1973). Being demanded soybean seed in worldwide as well as Malaysia, it is necessary to preservation of quality soybean seed for a long period which is widely recognized. As per the finding by scholars (Jyoti & Malik, 2013) that seed deteriorations simply damage of seed quality, viability and vigor and it affect by unfavorable environments factors. Likewise Ghassemi-Golezani *et al.*, (2010) commented that environmental factors such as temperature, relative humidity and seed moisture content extremely affected to the speed of deterioration of seed quality. Also seed deterioration is one of the key factor for productivity of soybean (Shelar *et al.*, 2008). Deterioration is noticeable as reduction of percentage germination of seed quality.

Furthermore, harvesting and storage activities also responsible for the damage seed quality (Farhadi *et al.*, 2012). A long storage condition had to the decrease in seed feature that resulted from changes that occur in seeds during aging (McDonald, 1999). This remarkably affects seed quality and longevity. Proper storage ensures inhibition of not desired biological processes eliminating harsh environmental factors that limits safe storage duration. Seed longevity is not only determined by seed moisture and storage temperature but it also is influenced by interactions of the genetic environment at seed maturation and harvesting stages (Walter *et al.*, 2010). Biochemical processes in grain seeds are directly influenced by the presence of moisture and higher air temperature (Azadi & Younesi, 2013). The grain seeds should be ultra-dried for the proper seed keeping. The ultra-dry is a seed storage technique that aids in reduction the seed moisture content to less than 5% at ambient temperatures. In addition, Li *et al.*, (2008) argued that storage ultra-dry seed is benefited by both as like used to maintain the quality of seeds and improves the storability of seeds.

Crop seed quality parameters solely depend on the stage at which the seed crop is harvested. Therefore, seed should be harvested at the maximum level of quality traits. Early harvesting has resulted poor seed quality parameters and thereby it gives rise to a greater number of immature and under developed seeds. Seed maturation considered as a component of seed quality and a prerequisite for improved seed germination and emergence should be taken into consideration for any seed development programme. If the harvest is delayed however the seed yield and then quality are negatively affected by the vagaries of environmental condition in the field. Therefore, the crop should be harvested when it reaches physiological maturity (Kamotho *et al.*, 2014). Thus, seed quality is influenced by harvesting stage in relation to germination, vigor, viability and also storability (Khatun *et al.*, 2009).

Generally, soybean seed have weak structure, short life period and easily expose to damage. Thus, the quality of seeds usually relay on the maturity stage which is have relation with longevity of seed during storage. However, maturity, moisture content and storage temperature are significant factors which play critical role of soybean seed longevity storage.

Therefore, this study aimed to determine the effect of non-ultra-dry and ultra-dry seed moisture content stored in room temperature and cold room conditions for seeds harvested at different in maturity stages which is full seeds stage (R6 stage), commencement of maturity period (R7 stage) and full maturity stage (R8 stage) on the quality or seed deterioration of the semi temperate soybean seeds (AGS 190) that produced and grown under the humid tropical region.

## Materials and methods

*Experimental site and design*

Field experiment of this study was conducted at Ladang 2, Faculty of Agriculture, University Putra Malaysia on March 12, 2015 with variety of Soybeans AGS 190. Seeds were harvested at different stages which are at full seeds stage (R6 stage) in June 2015, commencement of maturity period (R7 stage) in July,

2015 and full maturity stage (R8 stage) in July, 2015 respectively. These R6, R7 and R8 are the first factor in this study. An equally balanced NPK compound fertilizer (15: 15: 15) of 75 kg per hectare was applied before seeding as basal and after 21 days seedling emergence. Sprinkler irrigation was used to prevent plant to water stress.

The moisture content (MC) of seeds harvested at R6, R7, and R8 were 60.6%, 31.8% and 20.3%, respectively. The seeds were air dried under shade at 30°C ± 5°C. The seed moisture was checked at two days interval until the moisture content 12% is obtained. These seeds were categorized as non-ultra-dry seeds and labeled as moisture content one (MC1) which is the second factor is moisture content in this study. Some of seeds were dried in silica gel (2:1) in a desiccators at normal atmospheric temperature at 25°C and the moisture content was checked at regular interval until less than 5%. These seeds were categorized as ultra- dry seeds and labeled as moisture content two (MC2) and for this study moisture content is the second factor in this experiment.

The ultra and non-ultra-dry seeds then were kept in plastic bags and stored in two different temperatures at 25 to 30°C with relative humidity of 65-70% storage one (S1) and cold room storage at 10°C with relative humidity of 80-85% storage two (S2) for a period of 12 months. This is the third factor in this experiment. Thus, the experiment was a 3-factoiral experiment with, factors of harvest stages R6, R7 and R8, moisture content 12% non-ultra- dry and ≤ 5% ultra-dry and storage room temperature at 25 to 30°C with relative humidity of 65-70% and cold room storage at 10°C with relative humidity of 80-85%.

**Data collection and statistical analysis**

*Measurement of moisture content, germination percentage, and electrical conductivity*

*Moisture content test*

A 20 soybean AGS 190 seeds were harvested at three harvest stages, R6, R7, and R8 with three replicates to measure moisture content. Seeds were oven dried at 130°C for 1 hour (ISTA 2006). Moisture content was calculated according to the formula below and expressed on weight basis. Moisture Content= (Fresh seed weight – Dry seed weight) x 100 Fresh seed weight

*Germination percentage test*

Germination percentage was carried out from seeds stored for 0, 4, 8, 10 and 12 months. Standard germination test was conducted according to the method as described by (ISTA 2006). A 50 seeds per replication from each experimental unit of harvest × MC × storage were germinated in the tray (size 28×22 cm) containing sterilized sand. Seeds were considered germinated when the cotyledons completely emerged from the sand surface. Final germination percentage was calculated on day 7 according to the formula below:

$$\text{Germination percentage} = \frac{\text{Number of seed germinated}}{\text{Total number of seed}} \times 100$$

*Electrical conductivity test*

The electrical conductivity was determined from seeds stored for 0, 4, 8, and 12 months. Electrical conductivity of seed leachates was performed on 25 seeds obtained from each experiment unit plot according to ISTA procedure (ISTA, 1995). Seeds were weighted and socked in 50ml deionized water at room temperature. Results were expressed as $\mu S\ cm^{-1}\ g^{-1}$.

*Measurement of Super oxide dismutase (SOD), Catalase (CAT) activities and Lipid per oxidation*

Extraction of enzyme was done according to Bradford (1976) with slight modification soybean embryo axes samples (0.1g) from each harvest were soaked in distilled water at 25°C for 12 h. Samples were mixed with 1ml of ice cold water 50mM phosphate buffer solution (pH 7.0). The mixture was centrifuged at 13500rpm for 20min at 4°C and the supernatant was collected for measurement of enzymes activities.

*Superoxide dismutase (SOD)*

Superoxide dismutase (SOD) activity was measured by the reduction performance in the absorbance of superoxide nitro blue tetrazolium complex by the enzyme (Gupta *et al.*, 1993).

*Catalase (CAT) activities*

Catalase **(**CAT) activity was measured according to (Aebi 1984). Enzyme activity was determined by calculating the amount of decomposed $H_2O_2$.

*Lipid peroxidation*

Lipid peroxidation was determined by estimating malondialdehyde (MDA) content as described by Stewart & Bewley (1980).

*Statistical Analysis*

Data analysis was conducted using completely randomized design with three replications. An analysis of variance (ANOVA) and mean grouping (least significant different $p \leq 0.05$) was carried out by using SAS Window Version 9.4 accordingly.

**Results**

*Germination percentage of soybean (AGS190) seed at different harvest stages, moisture content and storage temperature*

The germination percentage was used as an indicator of deterioration in different types of grains during storage (de Alencar *et al.*, 2011). As can be seen in Fig. 1, that the germination percentage of AGS 190 seeds are affected by harvest stages, moisture content and storage temperature and storage time. At 0 month storage, the highest germination percentage (96 %) was shown by R7 prior stored in both room and cold condition. R6 and R8 had low germination percentage (50-70 %). For seed harvested at R6 stage, at 0 month storage, the germination of AGS 190 seed of S1MC1 and S2MC1 higher than S1MC2 and S2MC2. After 4 months storage, the germination seed S2MC1 increased drastically and decreased slowly for 8 and 12 months storage. The germination of S1MC1, S1MC2 and S2MC2 AGS 190 seed after 4, 8 and 12 month storage declined drastically. For seed harvested at R7 stage, the germination percentage is different with R6 stage. At 0 month storage, S1MC1 and S2MC1 show significantly higher percentage (96 %) than S1MC2 and S2MC2 (70%). However, the germination percentage of S1MC1 stored for 4, 8 and 12 months declined drastically. The germination of S2MC1 and S2MC2 remain constant. From Fig. 1, the lowest of germination percentage of AGS 190 seed is shown by seed harvested at R8 stage. All seeds have the same percentage at 0 month storage. After 4, 8 and 12 months storage, the germination S2MC1 and S2MC2 remain constant but S1MC1 and S1MC2 decreased drastically.

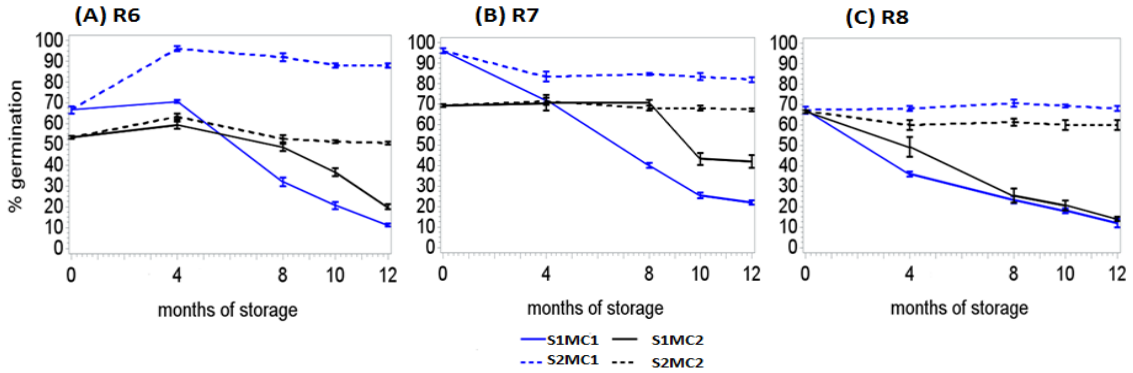


**Fig. 1**. Germination percentage of sown soybean AGS 190.

As per shown in Fig. 1 that seeds stored for 0, 4, 8, 10, and 12 months for different harvesting stages, seed moisture content, and storage conditions. R6, R7, and R8 are the reproductive stages of the full seed stage, commencement of maturity period and full maturity respectively. MC1 and MC2 are the seed moisture content of 12% and ≤ 5%, respectively, while S1 and S2 are the respectively room and cold room storage conditions. Each point is given as mean ± standard error.

*Electrical conductivity soybean (AGS 190) seed at different harvest stage, moisture content and storage temperature*

Electrical conductivity is an indicator of mechanical destruction such as mechanical injury that leads to loss of integrity of seed coats, particularly in large seed legumes (AOSA 2002). The results on Fig. 2 shows that electrical conductivity (EC) of soybean AGS 190 seeds at different harvest stages are significantly different. The level of EC for 12 month

storage is R6>R7>R8. EC of R6, S2MC1 and S2MC2 of AGS 190 seeds have the lowest EC and not significantly change for 12 months storage but S1MC1 and S1MC2 increased for 8 and 12 months storage. EC of R7, S2MC1 shows the lowest EC and remains constant for 12 month storage. EC of S1MC2 and S2MC2 higher than other and do not changes for 12 month storage. Compared with EC of S1MC1 which is similar with S2MC1 at 0 month storage and increased at 4, 8 and 12 month storage. At R8 harvest stage, S1MC1 and S2MC1 lower than S1MC2 and S2MC2 at 0 month storage. EC of S1MC1 and S1MC2 increased after 4, 8, 12 month storage but EC of S2MC1 and S2MC2 do not significantly declined.

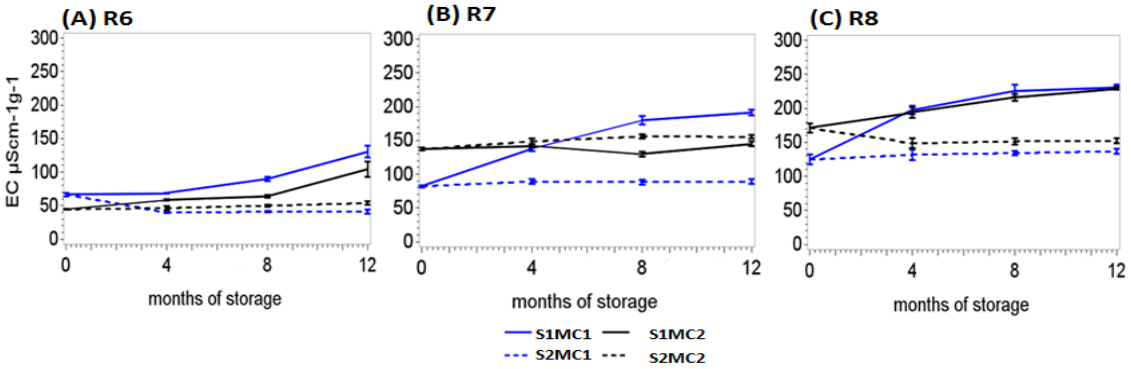


**Fig. 2.** Electrical conductivity of soybean AGS 190**.**

Above at the Fig. 2 denote that soybean AGS 190 seeds from seeds stored for 0, 4, 8, and 12 months at different harvesting stages, moisture content, and storage conditions. R6, R7, and R8 are the reproductive stages of the full seed stage, commencement of maturity period, and full maturity respectively. MC1 and MC2 are the seed moisture content of 12% and ≤ 5%, respectively, while S1 and S2 are the respectively room and cold room storage conditions. Each point is given as mean ± standard error.

*Super oxidase dismutase (SOD) activity of soybean (AGS 190) seed at different harvest stage, moisture content and storage temperature*

Super oxidase dismutase (SOD) activity of AGS 190 seeds is presented on Fig. 3. SOD activity of R6 is similar for S2MC1 and S2MC2 for 0 month until 12 month storage, but SOD activity of S1MC1 and S1MC2 declined after 8 month storage.

For R7, SOD activity of S1MC1 and S2MC1 higher than S1MC2 and S2MC2 until 4 month storage but declined after 4 month storage. SOD activity of S2MC1 and S2MC2 remain constant for 8 and 12 month storage. For R8, SOD activity of S2MC1 and S2MC2 not change for 12 month storage, on other hand, SOD activity of S1MC1 and S1MC2 keep declining from 0 month until 12 month storage.

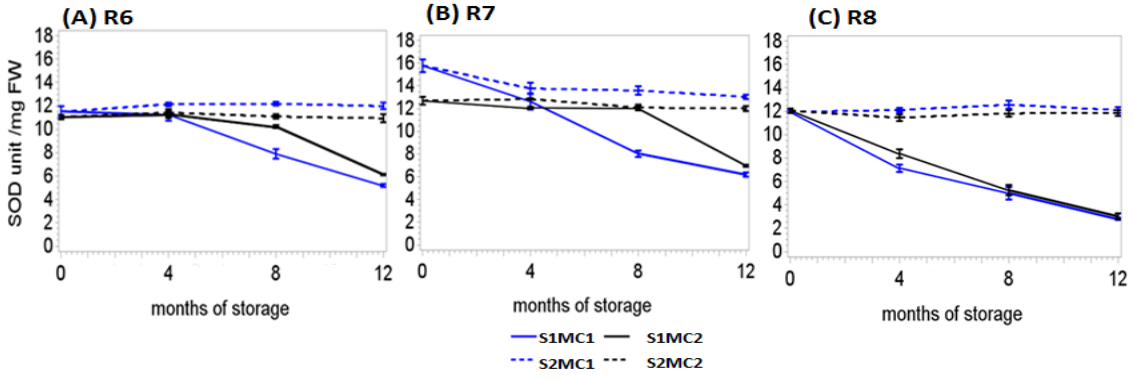


**Fig. 3.** Super oxidase dismutase content (SOD) of soybean AGS 190.

At the Fig. 3 shown that soybean AGS 190 seeds stored for 0, 4, 8, and 12 months at different harvesting stages, seed moisture content, and storage conditions. The R6, R7, and R8 are the reproductive stages of the full seed stage, commencement of maturity period, and full maturity respectively. The MC1 and MC2 are the seed moisture content of 12% and 5%, respectively, while S1 and S2 are the respectively room and cold room storage conditions. Each point is given as mean ± standard error.

*Catalase activity of soybean (AGS190) seed at different harvest stage, moisture content and storage temperature*

The results shows that catalase activity of Soybean AGS 190 seeds can be written as R7>R8>R6. For R6, the catalase activity of S2MC1 and S2MC2 not change for 12 month storage but the activity declined for S1MC1 and S1MC2 after 4 month storage. For R7, the activity of S1MC1 and S2MC1 higher at 0 month but decline drastically for S1MC1. The activity of S2MC1 do not decline drastically. The activity of S1MC2 and S2MC2 do not changed drastically for 8 month storage, only S1MC2 shows declined activity for 12 month storage. For R8, Fig. 4 shows the catalase activity of S2MC1 and S2MC2 do not change for 12 month storage, compared with S1MC1 and S1MC2 the activity of catalase declined until 12 month storage.

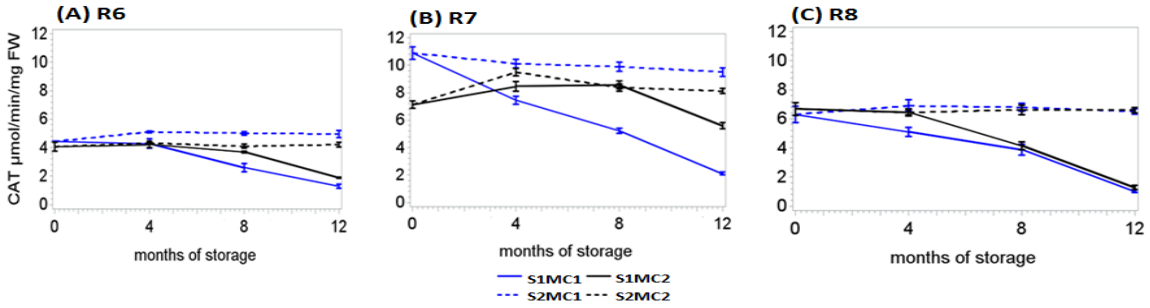


**Fig. 4.** Catalase content (CAT) of soybean seeds (AGS 190).

At the Fig. 4 shown that soybean AGS 190 from seeds stored for 0, 4, 8, and 12 months for different harvesting stages, seed moisture content, and storage conditions. The R6, R7, and R8 are the reproductive stages of the full seed stage, commencement of maturity period, and full maturity respectively. The MC1 and MC2 are the seed moisture content of 12% and ≤ 5%, respectively, while S1 indicated room storage condition as well as S2 indicated the cold room storage conditions. Each point is given as mean ± standard error.

*Malondialdehyde (MDA) content of soybean (AGS190) seed at different harvest stage, moisture content and storage temperature*

Malondialdehyde (MDA) is the compound that gradually accumulated during seed deterioration different development stage conditions from the process of lipid peroxidation (McDonald 1999). Fig. 5 shows that MDA content of S2MC1 and S2MC2 at R6, R7 and R8 stages do not change for 12 month storage, but, for S1MC1 and S1MC2 of all stages increased until 12 month storage.

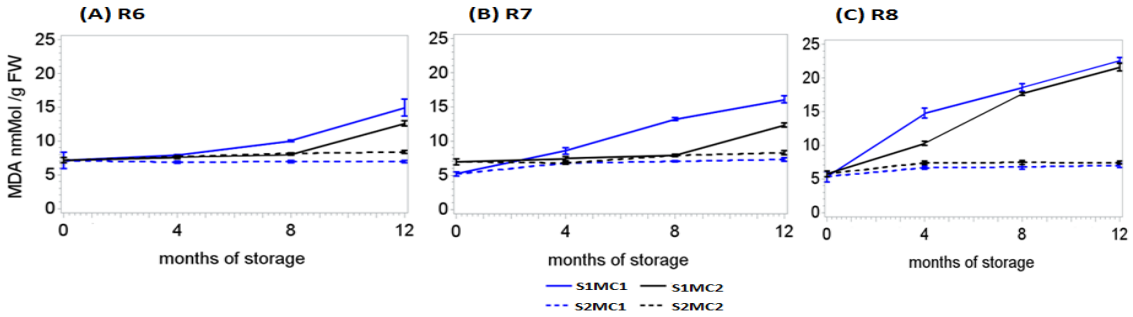


**Fig. 5.** Malondialdehyde (MDA) content of soybean seeds (AGS 190).

At the Fig. 5 shown that from seeds soybean seeds (AGS 190) stored for 0, 4, 8, and 12 months for different harvesting stages, seed moisture content, and storage conditions. The R6, R7, and R8 are the reproductive stages of the full seed stage, commencement of maturity period, and full maturity respectively. The MC1 and MC2 are the seed moisture content of 12% and ≤ 5%, respectively, while S1 and S2 are the respectively room and cold room storage conditions. Each point is given as mean ± standard error.

**Discussion**

Seed damage under storage, is unavoidable and reduction of quality (Adebisi *et al*., 2004; Fabrizius *et al*., 1999). Seed filling conditions, harvesting and handling operations are some of the factors that affect seed quality. During storage, seeds germination declined significantly as storage temperature increased. The storage process is determined by temperature and moisture content, might be linked with different chemical and metabolic modifications in seeds.

The germination of grain seed deteriorated with the increase in the seed moisture and storage period. But the deterioration in germination of seeds clearly show that damaged seed coat was very severe at room temperature. This leads to fast seed deterioration of soybean as the result of lipid peroxidation, thereby leading to loss of seed viability (Shelar *et al*., 2008). Moreover, result from the experiment of during storage soybean seed at 5°C and 25°C temperature, reveled aging militate against seed germination and promote lipid peroxidation. Likewise Sung & Chiu (1995) showed from their study that accelerated seed deterioration linked with lipid peroxidation with increasing temperature. In an experiment by Surki *et al*., (2012), commented that reduction in seed germination is due to storage temperature and harvest moisture which had greater effects on deterioration rate, then incremental changes in electrical conductivity which is being associated to lipid per oxidation. The trends of chemical and membrane changes confirmed the results. These are similar to what was observed in the present study as presented in Fig. 1.

The membrane system is important for the regulation of material exchange, owing to the damage of the membrane system; many materials move out of cells due to turgor pressure consequent to this, the vigor of seed is decreased (Wang *et al*., 1999). In an experiment by Goel & Sheoran., (2003) seen that reduction in seed germination and antioxidant enzyme with increase electrical conductivity of seed leachates and malondialdyhade content. This explained seed deterioration through seed storage and causes membrane perturbation. In this finding, the leakage rate of soybean seed electrolyte increased at different harvest stages and different moisture content especially non ultra-dry with increase storage period in room temperature condition compared to cold room storage condition as can be shown in Fig. 2. This result could be as the result of reduction in cell membrane permeability that probably occurred as the result of temperature alterations than differences in moisture content.

Antioxidant enzymes such as SOD, CAT and POD in plant cells are able to remove active oxygen resulting from plant stress (Amirjani 2010). The damaging of antioxidant enzymes or the decrease in their biochemical activity will accelerate seed aging. Reactive oxygen species (ROS) such as super oxide radicals ($O^{2-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH^{\cdot}$), play a key role during seed storage that accumulate in ageing seed tissues and have a vital role in seed vigor reduction (Mansouri-Far *et al*., 2015). Accumulation of ROS leads to their reaction with unsaturated fatty acids and causes changes in cell membranes, such as lipid peroxidation and finally its destruction.

The results of this study showed that the enzymes activities of SOD and CAT decreased at different harvest stages and different moisture content especially non ultra-dry with increase storage period in room temperature condition compared to cold room storage condition which in Fig. 3 and 4. In this study, the enzymes activities of SOD and CAT start decreased after 8 months in seed harvested (R7) under ultra-dry room temperature storage condition.

It was observed from this study that the activity changes of antioxidant enzymes had influence on desiccation tolerance, and the ultra-drying process does not degrade the enzymes. Deterioration of seeds is in most cases associated with accumulation of active forms of oxygen, including superoxide radical ($O^{2-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (.OH) (Li *et al.*, 2010).

Lipid peroxidation processes are considered as the main cause of soybean seed deterioration for the period of storage, as the high lipid and protein content in soybean seeds make them prone to oxidative stress and is related to fast seed vigor decline with seed age (Lisjak, *et al.*, 2009).

Malondialdyhad (MDA) is the end product of lipid peroxidation. The content of this product in seeds is consequently indicative of the degree of deterioration. In our study, this content increased at different harvest stages and different moisture content especially non ultra-dry with increase storage period in room temperature condition compared to cold room storage condition like in Fig. 5.

During this study, the content of (Malondialdyhad) MDA increased and was more clearly in seeds harvested (R8) with ultra-dry and non- ultra-dry room temperature storage condition. This content increased after storage was correlated with the reduction in activities of SOD and CAT like Fig.s 3 and 4.

**Conclusion**

Seeds of soybean deteriorate affected by the time of the harvest; relative humidity, storage temperature and moisture content. Results showed that ultra-dry treatment of soybean seeds can reduce deterioration in seeds at room temperature compared to non-ultra-dry seed. In cold room storage, there is no statistically significant difference between ultra-dry treatment and non-ultra-dry treatment in deterioration rate. The deterioration in room temperature storage condition was higher than cold room storage condition especially in non-ultra-dry seeds. The seeds deterioration rate was lowest in R7 compared to R6 and R8 especially ultra-dry seeds.

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