Effect of modified atmospheric conditions for soybean seeds under hermetically sealed large capacity storage

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Abstract: This study was carried out to evaluate the effect of modified atmospheric conditions (MAC) created in large capacity

hermetically sealed soybean storage in comparison to ambient warehouse soybean storage. Change of physio-chemical properties of soybean such as hardness, 1000 seed mass, bulk density, color, moisture content (MC) under sealed hermetic condition was evaluated. As well, change of biological condition such as germination percentage (GMP) and change of the nutritive property such as protein content (PC) of soybean stored under MAC (sealed hermetic condition) was evaluated. Similarly, change of those all physiochemical, biological and nutritive properties under ambient warehouse conditions were evaluated and these values were compared with their initial values after 8 months' storage. All above mention soybean properties were measured initially and after 8 months' storage under both storage conditions. Complete Randomized Design (CRD) was used as an experiment designed and Duncan Multiple Range Test (DMRT) was adopted for mean seperation of treatments. Results revealed that hardness, bulk density, 1000 seed mass and GMP were changed significantly under both storage conditions in comparison to their initial values. MC and b color values of the soybean stored under ambient warehouse condition was changed significantly compared to its initial value and MAC storage. However, MC of soybean stored under MAC was not changed significantly. PC, L and a color values did not change significantly under both storage conditions. Hardness and GMP significantly reduced under MAC in comparison to warehouse storage. As well, 1000 seed mass & b color value significantly reduced under ambient warehouse storage condition in comparison to MAC storage. Soybean weight losses and color losses can be prevented by MAC in comparison to ambient warehouse storage. Quality and outer appearance of soybean seed can be maintained throughout the storage period under hermetically sealed MAC storage. However, the germination ability of soybean seed significantly reduced under MAC.

Key words: Modified atmospheric storage, hermetically sealed, soybean properties

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1 Introduction

Soybean is one of the most important protein rich food

Received date: 2019-02-14 Accepted date: 2019-07-31 *Corresponding author: Champathi Gunathilake, D.M.C., Principle Research Scientist, Department of Agricultural Engineering & Machine Development, National Engineering Rsearch & Development Centre of Sri Lanka, Ekala, Ja-Ela 11380, Sri Lanka.Tel: +94 11 2236284. Fax: +94 11 2233153. Email: champathi@hotmail.com. crop among legume crops. Soybean can be used as supplementary for animal protein because the amino acid profile of soy protein is nearly equivalent in quality to meat, milk and egg protein (Miglani and Sharma, 2017; Narayan et al., 1988). Further, it is also the most economically important oilseeds in the world market (Duarte et al., 2004). Generally, soybeans are ingredients for hundreds of food products such as infant & breakfast formulas, tofu and Textured Vegetable Protein (TVP).

Soybean is cultivated as a seasonal crop in most

countries. Therefore, it will require storage for consumption or processing until next season. Generally, most of local farmers store soybean under ambient conditions and ambient environment conditions such as temperature and relative humidity are subjected to change frequently. In addition, stored soybean seeds are biologically alive. Therefore, respiration and other metabolic reactions take place continuously as a result, Physio-chemical, biological and nutritional properties can be altered during storage, that is not only ambient warehouse storage, but under other storage conditions also. These changes directly affect physical & organoleptic qualities and processing parameters of soybean seeds (Gunathilake et al., 2019; Hou and Chang, 2004).

Hermetically sealed storage that grains and legumes stored under sealed container or bag where air and moisture transmission fully restricted, is one of the suitable storage method for grains and legumes (durable crops) because it can overcome most critical storage problems such as insect pest damage. However, seed should be dried upto a moisture content (MC, 13%- 12%) before hermetic storage (Gunathilake et al., 2018; Donahaye et al., 1991). Previous Research studies revealed that the growth of fungus, insects and pests can be inhibited under the modified atmospheric condition created inside sealed hermetic storage that inside oxygen is reduced due to respiration of seeds and other living organisms (as explain above, inside outside gas transition fully restricted under sealed hermetic storage) as a result, inside carbon dioxide was gradually increased. Growth of living organisms and metabolic rate of stored seeds were decreased due to lack of oxygen inside (Donahaye et al., 1991; Villers et al., 2006).

Gunathilake et al. (2019) showed physical properties of soybean are changed during ambient warehouse storage under tropical climatic condition. Therefore, evaluating Physio-chemical, biological and nutritional properties under sealed hermetic conditions is also very important because it is helpful to identify cost effective long term storage method for legume storage under local climatic conditions in order to reduce losses and protect Physio-chemical, biological and nutritive qualities of the seed during storage. As well, very few studies reported in this field. Therefore, this research study was conducted to evaluate seed's biological nutritive and physio-chemical changes such as germination percentage (GMP), protein content, MC, bulk density (BD), thousand seed mass (TSM), kernel hardness and seed color under commercial scale hermetically sealed modified atmospheric storage in comparison to ambient conventional warehouse storage for the eight months' storage period.

2 Methodology

This research was conducted at Institute of Post-Harvest Technology, Sri Lanka. Locally grown soybean variety PB-01 was used for this study. The seeds were dried up to 12% MC before storage. Sealed hermetic (modified atmospheric) stored conditions were given using the Grain Pro cocoon bag manufacture by Grain Pro Inc. USA. As shown in the Figure 1, 1220 kg of soybean seeds bagged in 22 poly-sack bags and they were storaged in the Grain Pro cocoon bag, sealed and placed in the warehouse. Same amount of soybean seeds bagged in same poly-sack bags and placed in a warehouse under ambient storage condition.



Figure 1 Filling sealing and placing of cocoon bag

Inside oxygen level of the hermetically sealed cocoons was measured using an oxygen meter manufactured by Grain Pro Inc, USA once a two week till the end of the experiment. Inside temperature of hermetically sealed cocoon and outside warehouse temperature were measured using thermos-couples placed inside sealed cocoon and outside warehouse.

2.1 Measuring of grain weight loss during storage

Total weight loss percentage under different storage methods was calculated by dividing final weight losses from initial weight (Equation 1). The final total weight of soybean bags under two different storage method was recorded for calculating weight loss percentage.

$$Weight Loss \% = \frac{Initial weight(g) - Final weight(g)}{Initial weight(g)} \times 100$$
(1)

Biological, nutritive and physio-chemical properties of soybean such as GMP, protein content, MC, BD, TSM, kernel hardness and seed color were measured initially and after eight months' storage by following methods.

2.2 Measuring of germination percentage

Standard germination test, 50 soybean seeds placed in germination paper wrapped with adequate water and covered with polyethylene sheet and kept room temperature for seven days was performed.

2.3 Measuring of protein content

Analyzed by Kjeldahl method, based on the digestion of the sample with concentrated H_2SO_4 . as catalyst to covert organic nitrogen to ammonium ions. After digestion alkali was added and the liberated ammonia was distilled with an excess of boric acid solution which was titrated with hydrochloric acid to determine the absorbed boric acid and protein content was determined

2.4 Measuring of moisture percentage

The method used by American Oil Chemists Society (AOCS) was adapted for measuring MC (percentage) of soybean samples. Soybean sample of 10 g heated in a laboratory oven at 130°C for 3 hours and dry mass of the sample was measured. Accordingly, MC of seeds was

calculated using Equation 2. It was replicated three times.

MC (wet basis) =
$$\frac{W1-W2}{W1} \times 100$$
 (2)

W1 = Initial mass (g)W2 = Final mass (g)W2-W1 = mass loss (g)

2.5 Measuring of bulk density

Bulk density is defined as the mass of the sample per unit bulk (seed + air space) volume. The volume of laboratory, BD apparatus was filled by soybean. Mass of filled soybean was measured and BD was determined using Equation 3. It was replicated three times.

Bulk Density =

Mass of the sample (kg)	(3)
Total volume of bulk density apparatus (cubic m)	(3)

2.6 Measuring of 1000 seed mass

Ten seeds were selected from the sample and their mass were measured. It was replicated ten times and average mass of 10 seeds was taken. Thousand (1000) seed mass (g) value obtained by multiplying the average mass value of 10 seed by 100. Precision laboratory top loading balance was used to measure the mass of the seed.

2.7 Measuring of seed hardness

A compression test was carried out to measure seed hardness (yield stress). The Instron, TA, XT2 texture analyzer has been adapted to perform a compression test. Force at rupture(kg) was considered as the seed hardness. Three replicate were considered for each measurement and the measured compression force was averaged.

2.8 Measuring of seed color

Soybean seed color was measured by using Mini-scan XE plus Hunter Lab Colorimeter. Hunter scale 'L' 'a' 'b' values were measured. 'L' stood for lightness (black=0, white=100), 'a' represented for greenness and redness (+a, redness; -a; greenness), 'b' indicated blueness and yellowness (-b, blueness; +b, yellowness).

2.9 Statistical analysis

Minitab statistical software was used for analyzing the data. Each treatment was replicated two times. Analysis of variance (ANOVA) on Complete Randomized Design (CRD) by General Liner Model (GLM) procedure was performed and also treatment means were separated by the Duncan's Multiple Range Test (DMTR) at $\alpha = 0.05$ level of significance.

3 Results and discussion

3.1 Change of oxygen percentage inside hermetically sealed soybean storage

Figure 2 shows the reduction of the oxygen percentage inside the hermetic cocoon that creation of modified atmospheric condition. Oxygen percentage of hermetic cocoon reduced from 20.5% (ambient environment O_2 percentage) to 9.4% during 8 months' storage.



Figure 2 Change of oxygen percentage inside of the hermetically sealed cocoon

3.2 Change of temperature inside hermetically sealed bag and outside warehouse

Figure 3 is shows on the temperature change inside of the hermetically sealed soybean storage and outside warehouse where cocoon was placed. Temperature was recorded in between 13.30 - 14.30 hours in which

maximum temperature reported time for the day. The results revealed that the temperature inside hermetically sealed soybean storage had less fluctuation in comparison to outside warehouse. Generally, environment relative humidity (moisture) is also fluctuated according to the temperature fluctuation.



Figure 3 Change of temperature inside cocoon and outside (warehouse)

3.3 Weight loss during the storage

Figure 4 shows the weight losses in different storage methods for 8 months. Higher weight losses observed in warehouse stored soybean in comparison to the hermetically sealed modified atmospheric stored soybean. Generally insect past damage was one of the major reasons for weight losses of stored grain and legumes. Insect pest damage was minimum under hermetically sealed condition.

In addition, under sealed hermetic storage condition, moisture removal was less and due to low respiration/metabolic rate seed mass loss was also less (Chin and Kieu, 2006; Villers et al., 2006). Therefore, weight-loss of long time storage was low under hermetically sealed, modified atmospheric condition in comparison to ambient warehouse storage.





Table 1 shows the mean comparison of MC, GMP, protein content, BD, TSM, kernel hardness and color values between initial and after 8 months' storage under two storage methods i.e. hermetic cocoon and warehouse. Soybean seed MC was significantly changed in ambient warehouse samples in comparison to its initial MC. Further, MC of warehouse storage samples was significantly lower in comparison to hermetically sealed stored sample's. During the experimental period, dry weather (low relative humidity) was at surrounding area, therefore seed moisture was evaporated under ambient warehouse storage. But seed moisture was unable to evaporate under sealed hermetic condition, hence initial MC of sealed cocoon samples was changed slightly from its initial value. GMP was significantly reduced in the sealed cocoon samples after eight months' storage from its initial values and it is also significantly less hermetically sealed samples in comparison to warehouse samples. Warehouse samples have shown higher GMP than cocoon samples. This result proves previous results obtain by Narayan et al. (1988), that seed viability can be lost under low oxygen storage conditions. Protein content was not significantly reduced in both storage methods for 8 months' storage in comparison

to its initial value. TSM significantly reduced in ambient warehouse storage in comparison to hermetically sealed cocoon. Hermetically sealed, modified atmospheric storage, preserved soybean seed mass due to low metabolic rate of seeds and less water evaporation. Gunathilake et at. (2019) showed changes of seed mass directly proportional to seed's MC change. The seed BD was significantly increased in both cocoon and warehouse samples from its initial value. However, seed BD of hermetically sealed sample was not significantly different from ambient warehouse samples, both were changed in similar pattern. This result proved that BD was increased even under modified atmospheric storage and soybean seed volume was highly decreased in comparison to seed mass. Previous research studies reported that BD was increased under ambient storage condition mainly due to reduce of the seed volume in comparison to mass (Gunathilake et at., 2019). Seed hardness (SH) was significantly reduced from its initial value under both storage methods. Further, highest reduction of seed hardness was observed from hermetically sealed storage samples. The 'L' and 'a' color values of soybean seed were reduced slightly (not significant) from its initial value in both storage methods. However, 'b' color (yellowness) value (-b, blueness; +b, yellowness) was becoming significantly lower in warehouse stored soybean in comparison to modified atmosphere storage (cocoon) after 8 months of storage. It indicated, that under ambient storage condition caused for lowering yellowness of seed in comparison to modify atmospheric condition. Gunathilake et al. (2019) investigated that soybean seed yellow color reduces with ambient warehouse storage.

Table 1 The results of the DMRT for biological, I	Physia_chamical and nutritiva i	nronarties change during	i different storage conditions
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Storage method	MC	MC PRO	GMP	TSM	BD	SH	Seed color		
Storage method MC	WIC					511	L	а	b
Initial Mean values	11.2 ^a	36.31 ^a	96.12 ^a	146.5 ^a	775.18 ^b	7.04 ^a	54.15 ^a	3.70 ^a	15.34 ^a
Cocoon Mean values	11.1 ^a	36.04 ^a	44.45 ^c	141.7 ^b	790.88 ^a	5.85°	53.34 ^a	3.25 ^a	15.30 ^a
Warehouse Mean values	9.92 ^b	35.12 ^a	70.32 ^b	130.4 ^c	789.15 ^a	6.35 ^b	51.98 ^a	4.14 ^a	14.07 ^b

Note: a-Columns having same letter are not significantly different at $\alpha = 0.05$ by DMRT

MC-moisture percentage, PRO-protein percentage, GMP-germination percentage, TSM-thousand seed mass, BD-bulk density, SH-seed hardness.

4 Conclusion

Oxygen percentage of inside cocoon bag (after creating of modified atmospheric conditions) storage reduced from 20.5% to 9.4% for 8 months. Temperature fluctuation inside the hermetic cocoon was low in comparison to ambient environment temperature fluctuation. Change of seed moisture percentage was significant under ambient warehouse storage. GMP of soybean seed significantly reduced under hermetically sealed, modified atmospheric condition. Protein percentage was not changed with different storage methods. Seed mass was significantly preserved in hermetic storage and seed hardness was reduced (texture got soften under sealed hermetic condition). BD of soybean was increased during storage and it was highly increased in hermetically sealed cocoon. Soybean seed color (yellowness) was preserved under modified atmospheric condition. Finally, it can be concluded that hermetically sealed modified atmospheric (large commercial scale) storage was given storage benefits in terms of weight loss, seed moisture loss and preserving seed color in comparison to warehouse storage for soybean.

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