

Effect of hermetic storage on end-use quality of mungbean

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DOI: xx.xxxx/xxx.2014.xxx.xxx.xxx

Abstract

This research was carried out to evaluate the suitability of hermetic storage for mungbean. Mungbean samples were stored in airtight PET bottle (hermetic sample) and woven polyethylene bags (control sample) at room temperature either uninfested or infested with *Callosobruchus chinensis*. Another set of mungbean samples were obtained directly from field and stored in gas-wash bottles as hermetic samples. These samples were stored for 6 months to monitor the gas concentration in every four days intervals. Percentages of grain weight loss, grain damage, moisture content and germination were evaluated from the stored field grain samples. At 2-month intervals during 6 month storage period, development of hard to cook (HTC) characteristics of hermetic and uninfested control samples were evaluated in terms of grain hardness and cooking time. These grain characteristics were also compared with the initial samples. The oxygen content of hermetic samples was dropped to 0.7% and carbon dioxide content was increased to 8% within 28 days of storage. Live insects of *C. chinensis* were not found in hermetic samples after 30 days. After 6 months, germination decreased from 93 to 87% in the hermetic samples, whereas it was decreased from 93 to 46% in control sample due to grain damage. Percent weight loss and grain damage of hermetic sample was only 4.8% and 5% respectively, compared to the heavy insect damage in the control samples. Moisture content of hermetic samples remained unchanged in comparison to the control. At 6-months, cooking time and grain hardness of the control samples had increased by about 40% and 10% respectively. However, cooking time and grain hardness of the hermetic samples were similar to the initial samples. These data indicated that, insect infestation and HTC characteristics can be effectively controlled by hermetic storage of mungbean while maintaining its desirable market quality.

Keywords: hermetic, mungbean, germination, hard to cook, *Callosobruchus chinensis*

1. Introduction

Legume grains such as mungbean are mainly stored in polysack bags resulting insignificant postharvest loss of 10-15% within 3-4 months of storage due to improper storage techniques (Sartaj and Ekanayake, 1991). Nearly 30-40% of cereals and grain legumes harvested in Sri Lanka are stored by farmers for consumption, seeds and future sale for a period of three to nine months (Adhikarinayake, 2006). This kind of on-farm storage provides about 70% of the total food requirements of the farm families and it is a substantial contribution to income during the off-season. However, on-farm storage contributes to the largest postharvest losses of gains due to lack of proper storage techniques and facilities in Sri Lanka in comparison to the rest of the world.

Grain legume is an inexpensive source of dietary protein supplement for more than 67% of Sri Lankans as an alternative to animal protein. Due to their high nutritional value, they are often being used in various dishes such as soup, porridge, curry and some traditional

confectioneries. Coconut mixed boiled mungbean dishes are considered as one of the most popular breakfast diet among Sri Lankan next to the bakery products and rice/ rice based products. Three mungbean (*Vigna radiate* (L.) Wilczek) varieties (MI-6, MI-5, Ari) are cultivated in Sri Lanka. Mungbean is cultivated mainly by dry-zone farmers in Sri Lanka due to the fact that the crop can be managed throughout the year with limited investment of fertilizer and water (Kumararathna et al., 2014). According to the present statistics mungbean is cultivated over 8790 ha and yielding 10,472 MT per annum. The domestic production is insufficient for local consumption and nearly about 11% of the total production has been imported to Sri Lanka in 2010 - 2011 to compensate the gap between domestic production and the local demand (AESD, 2011).

In Sri Lanka harvested grain legumes are highly susceptible to damage by Bruchids such as *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (Fabricus), commonly known as southern cowpea weevil and cowpea weevil respectively. However, *C. chinensis* is the widespread bruchid species that infests stored mungbean in Sri Lanka. Bruchid attacks on stored grains cause considerable quantity and quality losses when storage methods do not arrest the initial infestations, re-infestations and cross-infestations. In fact grain infestation by bruchids appears under poor storage condition but the initial infestation can be started even from the field. Therefore, farmers treat mungbean with many hazardous insecticides to protect their stored grains soon after harvesting which creates both health and environmental problems. Phosphine fumigation is not recommended at the farm level because of the non-availability of skill and safety required in the application. As an alternative to the considerable storage loss, farmers sell their grains soon after harvesting at low price prior to the infestation by those insects. However, during the off season price of mungbean increases significantly in the retail market, ranging between \$1.00-2.50 per kilogram. Grain quality deterioration is unavoidable under common storage conditions. Hard-to-cook (HTC) defect is another problem of mungbean associated with poor storage at high temperature and high humidity. HTC is characterized by extended cooking times for softening of grains (Liu and Bourne, 1995), increase in the grain hardness and changing the colour of the seed-coat. The extended cooking time and poor textural quality reduce the consumer preference and market value of mungbean. The other main problem is the loss of stored grain viability due to development of HTC characteristics and insect attack which hinders the percentage of seed germination. Therefore, an effective storage method should be able to prevent the growth of pest and molds, while maintaining the physico-chemical and functional properties of grains. Physical controlled techniques such as high pressure, vacuum, controlled atmosphere or modified atmosphere (CA/MA), radio frequency waves and high temperature possess advantages of being free of chemical residues and environmental hazards (Mbata and Reichmuth, 1996; Fleurat-Lessard and Torc'h, 2001; Wang et al., 2002; Johnson and Zettler, 2009).

Hermetic storage is an airtight MA method that creates high carbon dioxide (CO₂) and low oxygen (O₂) in the storage environment while inhibiting the growth of pests and fungi during the storage time (Seck et al., 1996; Quezada et al., 2006; Murdock, 2012). Murdock et al. (2012) found that hypoxia leads to cessation of larval feeding activity of *C. maculatus* in cowpea leading to death due to desiccation during hermetic storage of cowpeas. This reflects the importance of hermetic storage where early infestation can be avoided without significant damage to the stored legume grains.

It has been shown that hermetic storage is the most effective method to store paddy in Sri Lanka due to its simplicity and low-cost (Donahaye et al., 1991; Adikarinayake, 2006; Hafeel et al., 2008; Prasantha et al., 2014) similar to the other countries (Caliboso and Sabio, 1998; Villers et al., 2006; Quezada et al., 2006). Several scientists have shown that hermetic

systems are effective method for storage different types of grain legumes (Seck et al., 1996; Chankaewmanee et al., 2001; Murdock et al., 2012; Mutungi et al., 2014). However due to practical difficulties and inappropriateness of current technology such as structural weakness and handling, implementation of hermetic storage system has not yet been well adopted among many farmers. The other major problem is the lack of information on final quality of stored mungbean such as germination and cooking quality. Therefore, it is important to study the applicability and effectiveness of hermetic storage on preservation of mungbean. This research was carried out to evaluate the suitability of hermetic storage for mungbean to minimize postharvest losses and thereby to improve the end-use qualities of mungbean such as seed germination and minimizing the HTC characteristics of mungbean.

2. Materials and Methods

The research was carried out at the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Common cultivated varieties of mungbean MI-6 (*Vigna radiate* (L.) Wilczek) were selected for the study. Grains were obtained directly from the field 2-3 weeks after harvesting and sun dried to moisture content (m.c.) of $11\pm 2\%$ (w.b) before storage. Mungbean samples were stored in airtight PET bottle (hermetic sample) and woven polyethylene bags (control sample) similar to the common aerated storage. All experiments were conducted in laboratory conditions at room temperature and relative humidity (r.h.) of $28\pm 2^\circ\text{C}$ and $77\pm 6\%$ respectively. Adult *Callosobruchus chinensis* (L.) were obtained from a culture that maintained on mungbean at the Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka for several years.

2.1. Biological tests

Prior to the experiment, grain samples were stored under frozen conditions (-18°C) for 4 weeks to destroy any hidden infestations of insects. About one kilogram of mungbean sample was stored in a woven polyethylene bag (four replicates) and considered as the uninfested “control sample”. Grain quality characteristics of mungbean were measured in the control samples before storage (initial samples) and during the storage in every two months interval up to six months.

Another experiment was carried out by storing 200 g of uninfested mungbean obtained from control sample in 750 ml PET bottle. This sample was artificially infested with 20 unsexed freshly emerged (1-3 days) adults of *C. chinensis*. All the PET bottles were hermetically closed using thread seal with airtight PVC caps and covered with high vacuum silicon grease. Samples were stored under the same laboratory conditions as above. Mortality of insects was determined by sifting three samples at 4, 8, 12, 16, 20, 24, 28, 30 and 32 days after exposure to the hermetic condition. In each sampling, the number of alive and dead insects was recorded. Oxygen (O_2) and carbon dioxide (CO_2) concentrations were monitored in each sample prior to counting of the mortality of insects. A rubber septum was glued onto the centre surface of the hermetic PET bottle and pierced with a needle connected to the gas analyzer (Quantek model-902D, USA) to determine the O_2 and CO_2 contents.

2.2. Hermetic storage

In the second experiment, a mungbean sample (variety MI-6) was obtained directly from the farm fields and sun dried to m.c., of $11\pm 2\%$ (w.b) prior to storage. A 500 ml gas-wash bottle was filled with 450 g of grains and two arms of the bottle were sealed using rubber stoppers to ensure the development of hermetic condition. The long arm was attached with a rubber septum to withdraw the air sample from the bottle. Gases were measured (Quantek model-902D; USA) in four day intervals for first 30 days and thereafter in one week interval for a period of six months. Gas samples were obtained and analyzed from four replicates. The

initial O₂ and CO₂ concentrations (atmospheric) of the hermetic bottles were 20.5% and 0.1% respectively. Volumes of the bottles were measured end of the experiment. Altogether four gas-wash bottles of hermetic samples were arranged randomly at room temperature of 28±2°C. Another set of mungbean sample (580 g) was stored in airtight 750 ml PET bottle as hermetic sample to test the grain quality 0, 2, 4 and 6 months after storage.

2.3. Evaluation of storage losses

About five kilogram of mungbean sample (variety MI-6) was obtained two weeks after harvesting from five different fields (field sample) to identify the major insect pest species (Haines, 1991), percent weight loss and percent grain damage. Each field sample was reduced to a 50 g sub-samples (Boxall, 2002) and stored in glass jars covered with muslin cloth. Samples were stored at room temperature of 28±2°C and 77±6% r.h. The amount of grain damage due to fungal attack, insect attack and undamaged was counted and weighed in every two months intervals. Percent weight loss and percent grain damage were estimated during the storage periods up to six months using equations (1) and (2) respectively (Adams and Schulten, 1978; Boxall, 2002). Altogether 20 replicates were used in this experiment.

$$\text{Weight loss \%} = \frac{Nd \times Wd - Nu \times Wu}{(Nd + Nu) \times Wu} \times 100 \quad (1)$$

$$\text{Grain damage \%} = \frac{Nd}{(Nd + Nu)} \times 100 \quad (2)$$

Where; Nd = Number of damaged grains in the sample

Nu = Number of undamaged grains in the sample

Wd = Weight of damaged grains in the sample (g)

Wu = Weight of undamaged grains in the sample (g)

2.4. Germination percentage

Grain germination was evaluated according to the standard method of ISTA (2006). Samples were obtained from initial, control or hermetic samples and after 2, 4 and 6 months of storage. To determine the percentage of grain germination, samples of 100 mungbeans from each storage method were germinated on wet paper towels. Percent germination was calculated as the number of grains showing plumule and radicle emergence after 24 h of incubation at room temperature.

2.5. Proximate composition

The m.c., of the initial and stored control or hermetic samples was determined (% w.b) by forced-air oven drying at 105°C for 24 h. Crude fat, crude protein and total ash of the grains were determined according to AOAC (1995). All experiments were carried out in triplicates at the end of 6 months storage period.

2.6. Hardness of grains

Fifty undamaged grains without any cracks were selected from each treatment conditions (initial, control and hermetic) and placed in the hardness tester (Model GW J-II; China) to measure the grain hardness. Plunger of the hardness tester was carefully pushed down against the grain and force at first rupture of the grain was measured as the yield point (N).

2.7. Minimum cooking time

Minimum cooking time was evaluated using the method describe by Singh et al. (1991). Two grams of grains were taken into a boiling tube and cooked by adding 20 ml of distilled water in a boiling water bath. The cooking time was determined by removing few grains at different

time intervals during the cooking. The gains were pressed in between two glass slides until uncooked core was disappeared. This experiment was repeated for ten times.

2.8. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SAS (1990) statistical package by using PROC GLM procedure. Prior to the statistical analysis, the percentage data of mortality, weight loss, grain damage and germination were transformed into square root format. Duncan's multiple range tests was used to separate means when ANOVA showed significant treatment effect at $P < 0.05$. Other descriptive statistics and graphical methods were used to present change of parameters with time and storage conditions where appropriate.

3. Results and Discussion

Mungbean samples obtained directly from the farmers' fields have already been infested. After 30 days of storage insects were emerged from the field samples were identified as *C. chinensis*. It is important to note that, there was no any other species of bruchids found in the samples. Emerging of insects was not observed in the hermetic field samples during the storage period of 6 months. Legume grains are highly susceptible to damage by bruchids and ineffective method of storage cause a substantial loss in quality and quantity of grains (Mutungi et al., 2014). Grain weight loss and damage were increased up to $56.8 \pm 4.4\%$ and 68.8 ± 0.7 respectively, in the field samples after 4 months of storage. However, total destruction was observed after 6 months of storage (Table 1). These samples of mungbean were heavily damaged with high number of insect emergence holes. This indicates that the initial infestation or cross-infestation has occurred at the fields. Throughout the 6 months, weight loss and grain damage of the hermetic samples remained low as average of 4.8% and 5.0% respectively. Slight grain damage was observed in the hermetic samples indicating that hermetic storage could successfully protect the grains from initial infestation of insects.

Table 1 Percent weight loss, percent grain damage and percent germination of field and control mungbean samples stored under ambient and hermetic conditions.

Storage (months)	Weight loss (%)		Grain damage (%)		Germination (%)	
	Field ^s	Hermetic	Field	Hermetic	Control [#]	Hermetic _c
0	0.0	0.0	0.0	0.0	93 ± 2^a	93 ± 2^a
2	$23.6 \pm 1.3^{a*}$	4.8 ± 0.5^a	11.8 ± 0.4^a	5.1 ± 0.1^a	94 ± 2^a	94 ± 2^a
4	56.8 ± 4.4^b	4.9 ± 0.8^a	68.8 ± 0.7^a	5.0 ± 0.2^a	85 ± 2^b	94 ± 3^a
6	98.6 ± 1.2^c	4.8 ± 0.5^a	100 ^c	5.0 ± 0.4^a	46 ± 1^c	87 ± 3^a

*All data represent the mean \pm SD of five replicates. Values followed by the different small letters in each column are significantly different ($P < 0.05$)

^sDirectly obtained from the farmers' fields and stored under ambient conditions

[#]Mungbean samples were disinfested prior to storage and stored in woven polyethylene under ambient conditions

A significant drop ($P < 0.05$) of O₂ and increase of CO₂ to a critical limit was observed in the hermetic samples within first 21 days (Figure 1). The O₂ content dropped approximately to below $0.7 \pm 0.1\%$ and CO₂ increased to $7.7 \pm 0.4\%$ within first 28 days of storage. This amount was about 96% decrease from the atmospheric O₂ content. Thereafter O₂ and CO₂ levels remained more or less stable throughout the storage period of 6 months. There was no

any insects were found in the hermetic samples after 6 months of storage. This indicated the successful development of the hermetic condition and the sustainability of the condition within the grains samples during 6 months. The significant drop in O₂ and increase in CO₂ indicated that it was due to metabolic activity of the insects living within the grain stored in hermetic PET bottles. Similar results have been observed by Murdock et al. (2012). According to their experiment, feeding activity of the larvae of bruchids was fallen down when the O₂ levels drops to <5% and the CO₂ increased up to 15-20% in the infested cowpea grains. Respiration of insects, microorganisms and the grain itself were the reasons for lowering the O₂ and raising the CO₂ contents of inter-granular atmosphere of hermetic stored grains. Increase of CO₂ in hermetically stored grains help to destroy the insects, reduce the growth of fungi and reduce the grain respiration within 30 days while preventing mass losses of the stored grains (Moreno-Martinez et al., 2000; Adhikarinayake et al., 2006; Navarro, 2012; Prasantha et al., 2014). However drop of O₂ content was most critical factor for the death of bruchids than rise of CO₂ content in the inter-granular atmosphere of hermetically stored grains. Murdock et al. (2012) showed that cowpea grain was infested by immature stage (larval/ pupal) of *C. maculatus* (L.) consumed 8.9±0.4 ml of O₂ per insect within 21 days. Although the exact amount of O₂ consumption of *C. chinensis* has not been recorded, immature stages of *C. chinensis* could also be able to consume more or less similar amount O₂ in an air tight container within 21-28 days. Generally *C. chinensis* are smaller in body size than the *C. maculatus* but more active. However, they may have died as a result of low O₂ tension and/or partial pressure developed during the storage period (Mbata et al., 2005).

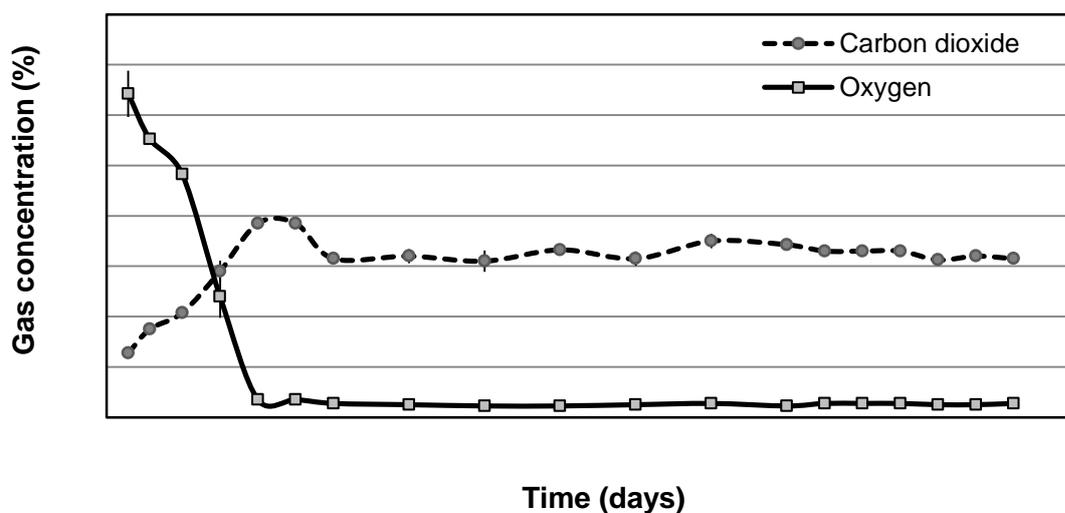


Figure 1 Changes in gas composition of hermetically stored field mungbean samples in gas-wash bottles. Data used are means ± SD of four replicates.

Similar result was observed in the artificially infested samples as well (Figure 2). No live insects were found in hermetic PET bottles, but they had significantly increased the CO₂ and reduced the O₂ in the artificially infested PET bottles.

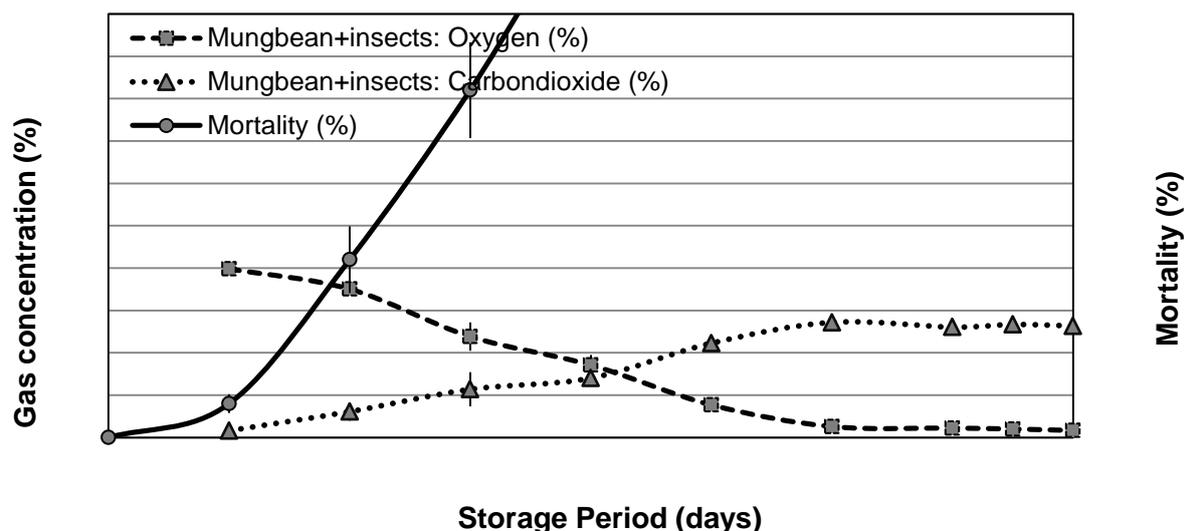


Figure 2 Solid line indicated the mortality of 20 adults of *C. chinensis* artificially infested with 200 g of mungbean in 750 ml hermetic PET bottle. Oxygen (\square) and carbon dioxide (Δ) levels in hermetic PET bottles over a six month storage period. Data used are means \pm SD of four replicates.

Under hermetic conditions, after 30 days of storage, mortality of *C. chinensis* was 100%. During this period CO₂ content has significantly ($P < 0.05$) increased up to maximum level of $13.6 \pm 1.0\%$ and O₂ had dropped down to the average of $1.1 \pm 0.3\%$. These final gas compositions have been reached to a plateau within 24-30 days after hermetic storage in PET bottles. Mortality of insects has significantly increased ($P < 0.05$) after 20 days where O₂ concentration was dropped $< 5\%$. Seck et al. (1996) found that infested cowpea stored under hermetic condition can completely be disinfested after 5 to 7 days. Calderon and Navarro (1980) showed the synergistic effect of O₂ depletion with increasing level of CO₂ supporting for insect control.

Germination of initial sample was $93 \pm 2\%$ and it was significant reduced ($P < 0.05$) in the control samples compared to the hermetic samples (Table 1). Throughout the 6 months of storage, the mungbeans stored in hermetic PET bottles retained significantly higher germination percentage compared to those stored as control samples in polyethylene bags. According to the statistical analysis, germination of mungbean stored in hermetic conditions did not decreased significantly (NS; $P = 0.08$) compared to the initial germination rate and following during 6 months of storage. In contrast germination of untreated controls dropped to $46 \pm 1\%$ after 6 months of storage. This could be due to development of HCT characteristics in the control samples. However, the hermetic sample showed slight reduction of grain germination to $87 \pm 3\%$ after 6 month of storage. This could be attributed to retardation of the physiological activity of grain due to low O₂ tension in the hermetic storage environment. Similar results have been observed by Mutungi et al. (2014). They found that mungbean stored in triple-layer hermetic bags retained high grain germination ability after 6 months of storage. Chankaewmanee et al. (2001) also showed that germination of mungbean stored in the airtight condition decreased slightly compared to control sample after one year of storage. Hamel, (1989) found that CO₂ can reduce the viability of wheat, rape seed, soy bean, and onion seeds.

Initial moisture content of mungbean was $12.0 \pm 0.1\%$ (w.b.) and there were no significant difference (NS; $P = 0.1$) in the moisture content between among hermetic and control

mungbean samples (Table 2). Results of the proximate composition of initial, control and hermetic samples are presented in Table 3. There was no significance difference (NS; $P = 0.1$) among crude fat and ash contents with the storage method.

Table 2 Changes in mean moisture content (% w.b.) with storage method and duration of storage.

storage (months)	Moisture content (Mean \pm SD%)	
	Control (%)	Hermetic (%)
0	12.0 \pm 0.4 ^{a*}	12.0 \pm 0.4 ^a
2	11.9 \pm 0.5 ^a	11.5 \pm 0.2 ^a
4	12.0 \pm 1.7 ^a	11.2 \pm 0.2 ^a
6	12.5 \pm 1.5 ^a	11.0 \pm 0.3 ^a

*All data represent the mean \pm SD of triplicates. Values followed by the different letters in each row are significantly different ($P < 0.05$)

Crude protein content of the control samples showed a slight but non-significant (NS; $P = 0.06$) reduction compared to hermetic samples. Antunes and Sgarbieri (1979) found that storage of dry beans at 37°C and 76% r.h., increased bean hardness and lowered the protein quality and the availability of amino acid. Shiga et al. (2009) reported that common beans stored at 30°C and 75% r.h., for 8 months showed development of HTC and subsequently decrease in the protein and ash contents with seeds ageing, with changes in physical properties of the carbohydrates. Proximate analysis of faba bean revealed that there was little or no effect on nutritive value of beans stored for 12 months at high temperatures at airtight condition (Nasar-Abbas et al., 2008).

Table 3 Crude protein, crude fat and ash content of hermetic and control samples of mungbean and cowpea.

Storage condition	Initial	Hermetic	Control
Crude protein (%)	23.5 \pm 0.1 ^{a*}	23.4 \pm 0.1 ^a	22.4 \pm 0.3 ^a
Crude fat (%)	0.9 \pm 0.1 ^a	0.9 \pm 0.1 ^a	1.1 \pm 0.1 ^a
Ash (%)	5.0 \pm 0.6 ^a	5.0 \pm 0.6 ^a	4.9 \pm 0.6 ^a

*All data represent the mean \pm SD of triplicates. Values followed by the different small letters in each row are significantly different ($P < 0.05$)

Control samples exhibited significant increased ($P < 0.05$) in their hardness compare to the initial samples (Figure 2a) but hermetic samples did not increase (NS; $P = 0.1$) their hardness compare to the hardness of initial sample (56.1 \pm 2.1 N). Average hardness after 6 months of storage in hermetic samples and controlled samples were 56.1 \pm 5.8 N and 60.4 \pm 1.7 N respectively.

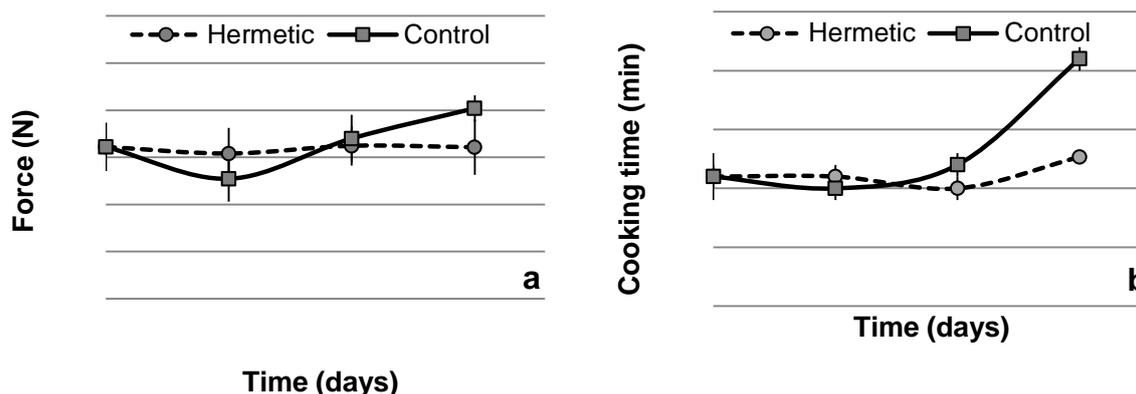


Figure 2 Changes in (a) hardness of grains and (b) cooking time with storage method vs. storage time.

Cooking time of hermetic samples did not show any significant increase (NS; $P = 0.07$) compared to the initial samples (Figure 2b) but control sample had significantly increased ($P < 0.05$) their cooking time by about 38.5% after 6 months. Cooking time of control samples has increased to 36 ± 1.7 min., compared to the initial cooking time of 26 ± 2.0 min., after 6 months of storage. Although, hermetic samples also showed a slight but non-significant trend of increasing in cooking time, the tendency was much less compared to the control samples. Extension of cooking time is a very important indication of grain hardness development and it is commonly described as HTC phenomenon. HTC defect is always associated with increase in cooking time and hardness of the grains. During ageing beans changed their carbohydrates (Shiga et al., 2009). Therefore HTC grains do not soften sufficiently during soaking and cooking. These types of HTC grains need additional energy for cooking which may affect nutritional qualities and acceptability by consumers (Deshpande et al., 1984). Kon and Sansuluck (1981) reported that the storage of dry common beans under high r.h., and high temperature conditions showed a 5-fold increase in their cooking time and significantly increased the hardness of the grains (Yousif et al., 2007). Nasar-Abbas et al. (2008) found that HTC characteristics of faba bean increased substantially with high storage temperature during 12 months of stored in airtight polyethylene lined aluminum foil bags. However, results of this experiment revealed that hermetic storage can successfully delay the development of HTC in mungbean at least for 6 months.

4. Conclusions

Storage damage of mungbean was mainly caused by *C. chinensis*. Hermetic conditions in stored samples were successfully maintained during the period of 6 months. Increase in CO_2 content and drop of O_2 in hermetic samples indicated the successful development of the hermetic condition within the hermetically stored grains. According to the study hermetic storage can prevent increase in cooking time and development of hardness in mungbean. It was revealed that hermetic storage can prevent the development of HTC characteristics and postharvest loss of mungbean.

Acknowledgement

This research (NRC 12-050) was mainly funded by National Research Council, Sri Lanka. Authors also like to thank for the International Research Center, University of Peradeniya and National Science Foundation Sri Lanka.

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