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Effects of Low-Pressure Storage of Food Commodities on the Mortality of Adult Stored Product Insects

Rohitha Prasantha B.D.*
Department of Food Science & Technology, Faculty of Agriculture, University of Peradeniya, 20400, Sri Lanka

Abstract

The food industry faces serious problems regarding pest infestation leading to high storage loss of food commodities. Due to adverse impacts of chemical preservation methods, a promising physical method of food protection could be considered as an urgent need. This study investigated the effect of low-pressure (vacuum) on the mortality of adults of three stored product pests of drug store beetle (*Stegobium paniceum* L.), southern cowpea weevil (*Callosobruchus chinensis* L.) and red flour beetle (*Tribolium castaneum* Herbst). The pests were exposed to 20 kPa and 10 kPa vacuum at 37 ºC and 75% relative humidity. Mortality of the insects was recorded at 8 h and 32 h after vacuum treatments. There were significant differences ($p<0.05$) in the susceptibility to the vacuum treatment among the tested pests. Adults of *C. chinensis* showed comparatively less susceptibility to the vacuum treatment compared to *S. paniceum* and *T. castaneum*. Mortality of insects exposed to two low-pressure treatments did not show 100% mortality at the two exposure periods, irrespective of the type of food materials and species. The mean percentage mortality increased by about 66.0±5.7% when vacuum pressure increases from 20 kPa to 10 kP irrespective of the species, exposure time and the products. The oxygen concentration decreased to >70% inside the vacuum chamber during treatments.

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*Corresponding author:
bdrp@pdn.ac.lk
https://orcid.org/0000-0003-3134-0756

1. Introduction

Cosmopolitan insects such as the Indian meal moth (*Plodiainter punctella* Hübner), Angoumois grain moth (*Sitotroga cerealella* Olivier), weevils (*Sitophilus spp.*), red flour beetle (*Tribolium castaneum* Herbst), southern cowpea weevil (*Callosobruchus chinensis* L.), lesser grain borer (*Rhyzopertha dominica* L.), cigarette beetle (*Lasioderma serricorne* F.), drug store beetle (*Stegobium paniceum* L.) and saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) are some of the stored product pests found in Sri Lanka, causing considerable economic loss to the food industry. Adult insects fly towards food manufacturing facilities and warehouses for oviposition and feeding. Adults would later be present in the packaged foods products such as flour, pet food, biscuits, nuts, dried fruits, chocolates, powdered milk and other food commodities (Riudavets *et al.*, 2009; Noomhorm *et al.*, 2009). Under some circumstances, the larvae of moths about to pupation emerge through the packages for pupation (Mullen & Pedersen, 2000). Such damage to the packaging materials later incurs many insect and fungal infestations. Other larval stages grow inside the food commodities without being seen from the outside and cause heavy damage to the stored products.

Stored product pest management heavily relies on chemical pesticides applied topically and as a fumigant. Fumigation with phosphine is effective to a certain extent; however, insects inevitably develop resistance to phosphine, which is reported from all over the world (Adler*et al.*, 2000). Low-pressure (vacuum) treatment is a non-chemical modified atmosphere technique that kills most of the stored product insects within a short period of exposure compared to chemical fumigation. Low-pressure storage or vacuum packaging of foods is considered an effective and portable modified atmospheric (MA) technique. It provides an environment with low oxygen (O_2) (2-5%) and relatively high CO_2 (>30%), within relatively a short period of time after removing the atmospheric gasses within the package or storage containers (Johnson & Zettler, 2009; Johnson, 2010; Kučerová *et al*., 2013). It is an alternative storage technique for refrigeration of shelf-stable and some perishable food products. The anaerobic environment of low-pressure treatments prevents the growth of spoilage microorganisms and insects thus averts the development of off-odor, off-flavor, undesirable color and textural changes. Storage of shelf-stable foods at an ambient storage condition leads to significant changes in physical, biochemical and physiological properties of foods within a short period of time. Postharvest loss of ambient condition grain storage, spices and nuts are high (about 15%) due to improper storage techniques and the lack of proper storage facilities in Sri Lanka (Sartaj & Ekanayake 1991). Therefore, producers and farmers apply many hazardous pesticides or fumigate with phosphine to protect their stored grains soon after harvesting and also several times during storage. Therefore, resistance may develop among insect pests and additionally pesticide residues could cause a grave problem to the environment and health of the end-users. Pest control measures are costly when insects heavily infest stored products while the quality of the products would also suffer. Most of the grains, spices, dry herbs and nuts today are organically grown and exported in bulk to Europe and Mediterranean countries as organic products. In some instances, export restrictions are imposed to Sri Lankan commodities due to records of pest infestation, fungal infections and pesticide residues in the product. Therefore, the food industry is encouraged to search for an alternative non-chemical pest control technique for the protection of stored food. Physical and low-risk control techniques such as controlled or modified atmospheric storage (CA/MA), diatomaceous earth, radio frequency, microwave and high temperature have advantages as physical protection methods (Fields & White 2002). Despite the efficacy of CA technique used to control the stored pests of shelf-stable commodities, hermetic and low-pressure/vacuum treatment is a fairly inexpensive system for local industries. On the other hand, operational cost is comparatively lower than for CA and chemical fumigation. This type of treatment is good for high value organic shelf-stable commodities such as spices, nuts and herbs, which can fetch a high income in international markets. Therefore, the aim of the present research project was to evaluate the effect of a low-pressure technique on the mortality of three pests species drug store beetle (*S. paniceum*), southern cowpea weevil (*C. chinensis*) and red flour beetle (*T. castaneum*). These pests are regularly found in the stored and/or package grains, spices, flour and dry food products causing considerable economic losses.

2. Materials and Methods

2.1 Treatment Bioassay

The study was conducted at the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya. The samples of selected grains and spices were obtained directly from the market and sun-dried to moisture content (MC) of 11.5±2 % wet-basis (w.b) before the low-pressure storage test. Rice and wheat flour samples were obtained from the market and dried to 9±0.5 % (w.b) MC in an airflow oven. Adults of the drug store beetle (*S. paniceum*), southern cowpea weevil (*C. chinensis*) and red flour beetle (*T. castaneum*) were obtained from the seed stores of 'Pelwehera' seed farm, Department of Agriculture, Sri Lanka. Prior to the experiment, all species were bred separately in glass jars at laboratory conditions (28±1 °C and 80±2% RH) for about 3-4 months.

All experiments were carried out at 37±1 ºC temperature and 75±2% relative humidity (RH) in a vacuum oven having an approximate chamber capacity of 18 L (Fisher Vacuum Oven-28, USA). About 500 mL of the saturated salt (NaCl) solution was kept inside the vacuum oven to obtain 75% equilibrium RH. Temperature and vacuum pressure were monitored and controlled by the temperature and vacuum gauge indicators were fixed to the vacuum oven. Another thermo-hygrometer type data logger (Omega-OM-EL-USB-5, USA) was placed inside the vacuum oven to record the changing RH and temperature levels during the study. Perforated plastic lids attached to 200 mL glass jars were used as the test sample holders. About 100 g of groundnut seeds, coriander seeds and nutmeg seed kernels (pieces) were placed separately in the jars. The samples were artificially infested with 25 adults of mixed sexes of *S. Paniceum* and the jars were closed tightly with the perforated lids. About 150 g of mungbean and cowpea samples were artificially infested with 25 adults of mixed sexes of *C. Chinensis* (2-3 days old). About 150 g of rice flour and wheat flour samples were infested with 50 adults of mixed sexes of *T. castaneum*.

Vacuum oven chambers were connected to a vacuum pump (Fisher vacuum pump LAV-3, USA) and simultaneously pumped down to the target vacuum pressure of 20 kPa (*ca*. 150 mmHg) and 10 kPa (*ca*.75 mmHg) which was equivalent to *ca*. 4.1% and *ca.* 2.0% oxygen content inside the vacuum chamber. Oxygen (O_2) and carbon dioxide (CO_2) concentrations were monitored inside the vacuum chamber at the end of the test period using a gas analyzer (Quantek-902D, USA). All the experiments were repeated 3 times and the percentage mortality was assessed at 8 h and 34 h after the low-pressure treatments. An insect was considered dead when it failed to move after being prodded gently with a needle for 30 s. Control samples were also tested inside the vacuum oven at 37±1 °C and 75% RH under ambient pressure. The MC (w.b%) of the initial, control and low-pressure treated samples were determined by forced-air oven drying at 105 °C for 24 h.

2.2 Statistical Analysis

Mortality data (mean±SD) were corrected using the Abbott's formula (Abbott, 1925). If mortality in the control samples exceeded 10%, the complete bioassay was discarded and repeated. The mortality values of insects were analyzed using one-way ANOVA at 95% confidence interval (p<0.05). The two low-pressure vacuum conditions and two exposure periods considered as treatment levels (20 kPa/8 h, 20 kPa/32 h, 10 kPa/8 h and 10 kPa/32 h) under each food commodity and species of insects. The MC and mortality values of insects were analyzed using the least significant difference (LSD) test at p<0.05 (SAS Institute 1990).

3. Results

The average RH levels and temperature recorded during low-pressure treatments in the vacuum chamber are shown in Fig. 1. This study revealed that RH levels dropped initially during the low-pressure application, but returned to equilibrium levels shortly after (*ca.* 2 h) the vacuum application was stopped. Subsequently RH and temperate did not change significantly inside the vacuum chamber during the rest of the 32 h of exposure.

The mean moisture contents of the product remained constant (p>0.05), even after 32 h of vacuum treatments at 20 kPa and 10 kPa pressure compared to the initial sample (Table 1). Maximum and minimum moisture contents were observed in initial, 20 kPa vacuum and 10 kPvacuum pressure treated samples, indicating slight decreases in MC with increasing vacuum pressure and exposure period. The differences in the average moisture contents of 10 kP/8 h and 10 kPa/32 h stored samples were statistically not significant (p>0.05).

Mortality of the test insects exposed to 20 and 10 kPa vacuum pressure treatments is presented in Table 2. There were no significant differences (p>0.05) in motilities between the two exposure periods (8 h and 32 h) at 20 kPa vacuum pressure treatment. However, vacuum pressure treatments at 10 kPa showed significant differences (p<0.05) in mortality between the two exposure periods. In addition, the mortality of all tested insects has significantly increased (p<0.05) with increasing vacuum pressure (from 20 kPa to 10 kPa). It was observed that with a two-fold increase of vacuum pressure, the percentage mortality increased by about 66.0±5.7%, irrespective of species, exposure time and the tested food products.

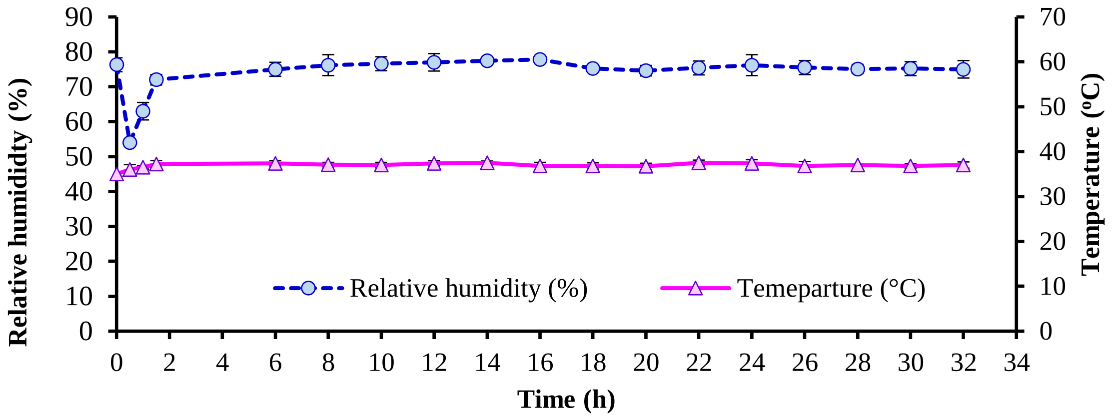

Figure 1. Average relative humidity and temperature fluctuations recorded during two low- pressure treatments at 37 ºC when a vacuum oven humidity level was controlled by saturated salt (NaCl) solutions.

Table 1. Effect of low-pressure treatments on moisture content (wet basis %) of the tested food commodities at temperature 37±1 ºC and 75±2% relative humidity.

Products	Initial moisture content (w.b%)	Exposure time (h)	Moisture content (w.b%) after low-pressure treatments	
			20 kPa	10 kPa
Groundnut	11.6±1.1	8.0	11.4±0.8	11.1±0.6
		32.0	10.6±0.5	10.1±0.8
Coriander	10.2±0.6	8.0	10.0±0.7	9.4±0.6
		32.0	9.5±0.4	9.4±0.7
Nutmeg kernels	10.5±1.3	8.0	10.1±1.2	9.6±0.9
		32.0	9.4±0.8	9.1±0.6
Mung bean	12.7±0.7	8.0	12.4±0.6	12.1±0.8
		32.0	12.0±0.4	11.7±0.5
Cowpea	12.2±0.5	8.0	11.6±1.0	11.4±0.6
		32.0	11.0±0.5	10.4±0.7
Rice flour	10.6±0.5	8.0	10.0±0.7	9.4±0.5
		32.0	9.6±0.4	9.2±0.2
Wheat flour	9.8±0.3	8.0	9.3±0.5	9.1±0.5
		32.0	9.0±0.4	8.8±0.3

Means ± SD values between the rows of 20 and 10 kPa for each product are not significantly different (p>0.05).

The observations showed that mortality in *C. chinensis* was significantly lower than in *T. castaneum* and *S. paniceum* at each low-pressure treatment level. Mortality of insects exposed to the two low-pressure treatments did not result in 100% mortality in any of the treatments.

The O_2 concentration decreased by more than 70% inside the vacuum chamber during the treatment. However, O_2 concentration significantly increased (p<0.05) inside the vacuum chamber after the 32 h of low-pressure treatment.

4. Discussion

Common storage pests such as the cowpea weevil (*C. chinensis*), drug store beetle (*S. paniceum*), and red flour beetle (*T. castaneum*) cause considerable economic losses to the grains, spices, nuts and herbal industries. The adult beetle damages the foods and the female laid many eggs during its life-time. The larvae hatching from the egg and feeds on the stored product while contaminating it with frass, larval skins and feces. The final instar larvae tunnel through the produce/ packaging materials to pupate, causing indirect damage. Adults and larvae may later be present in the final packaged products (Riudavets *et al.,* 2009; Noomhorm *et al*., 2009). Although stored product insects are relatively more susceptible to the MA treatments, their eggs are relatively tolerant to the high CO_2 environment (Adler, 1999; Adler *et al*., 2000; Johnson, 2010).

Changes in the quality of stored food can be predicted by the knowledge of storage temperature, product moisture, storage atmosphere condition and properties of the food. The moisture content of food products play an important role as a quality indicator and it is the limiting factor for growth of insects and fungi. Documenting information on changes in moisture, gas diffusion, isothermal characteristics, physico-chemical and structure of the low-pressure treated commodity is essential to establish proper control measures for stored product pests. In order for protective measures to be effective under low-pressure storage, the CO_2 content in the container must reach levels >30% to create an effective MA condition. Therefore, low pressure may also be used in combination with high CO_2 in the containers or packages. Johnson & Zettler (2009) and Johnson (2010) showed that vacuum treatment could be effectively used to control storage pests of tree nuts.

The RH in the vacuum chamber and moisture content of the product play a significant role during the low-pressure treatments. As a result, low-pressure stored foods are subject to excessive dehydration, but this may not be a problem for shelf-stable food commodities compared to perishable foods.

Table 2. Percentage mortality of adult drug store beetle (*S. paniceum*), cowpea weevil (*C. chinensis*) and the red flour beetle (*T. castaneum*) exposed to low-pressure conditions (20 and 10 kPa) at temperature 37±1 ºC and 75±2% relative humidity.

Test insect and seeds	Exposure time (h)	Mortality (%) of adult insects at low-pressure treatments		Low-pressure O_2 content (%)	
		20 kPa	10 kPa	20 kPa	10 kPa
Stegobium paniceum					
Groundnut	8.0	6.3±3.5	18.3±7.5*†	4.7±0.2	2.5±0.2
	32.0	14.7±7.2	47.6±6*†	5.2±0.4	2.4±0.1
Coriander	8.0	5.6±2.6	15.4±5.2*†	5.0±0.4	2.9±0.1
	32.0	14.5±4	45.0±8.7*†	5.3±0.3	2.5±0.4
Nutmeg kernels	8.0	7.6±3.7	20.2±7.0*†	5.5±0.1	2.8±0.2
	32.0	16.6±5.4	43.0±10.5*†	5.7±0.5	3.5±0.5
Callosobruchus chinensis					
Mungbean	8.0	2.3±3.3	10±3.3*†	4.5±0.2	2.0±0.0
	32.0	12±8.2	34.5±3.4*†	4.3±0.2	2.3±0.2
Cowpea	8.0	4.1±4.2	10.7±5*†	4.4±0.1	2.2±0.1
	32.0	11.3±5.5	36.5±2.3*	4.3±0.3	2.3±0.2
Tribolium castaneum					
Rice flour	8.0	6.5±2.2	18±7.0*†	5.4±0.1	4.6±0.1
	32.0	10±3.0	46.5±7.5*†	5.7±0.3	3.5±0.2
Wheat flour	8.0	14±2.5	33.5±5.2†	4.6±0.1	3.8±0.1
	32.0	18±4.5	45.4±8.5†	5.2±0.3	3.3±0.2

*Means± SD values between the 20 kPa and 10 kPa low pressure treatments (in a column) are significantly different for each commodity (p<0.05)

†Means ± SD values between the each commodity are significantly different (p<0.05) for each low pressure treatment and exposure time (in a raw).

Our study indicated that the moisture content of the product remained unaffected due to constant RH and temperature levels because it was maintained at near constant levels throughout the study period. However, long-time storage foods may reduce the moisture due to the low boiling point of water and change in the latent heat of evaporation under constant vacuum pressure.

Mortality rates of insects under low-pressure storage is dependent on the size of the insects and their developmental stages, CO_2 susceptibility, product type, temperature and whether infestation occurs inside or outside of the commodity (Johnson, 2010; Kučerová *et al.,* 2013). Most *Callosbruchus* spp. are internal feeders and damage to the legume grains in their larval stages. Only the adult stage can be seen to the outside because of the active mating and egg laying behavior of adults. Therefore, immediate control of adults is very important and effective than the control of larval or pupal stages, to control progeny production. According to Mbata *et al*. (2005), 99% mortality of adult cowpea weevils (*Callosbruchus maculatus*) was achieved within an hour at low-pressure (32.5 mmHg) and 30-35 ºC although the pupae and eggs were tolerant to the low-pressure treatment. A recent study by Mortazavi & Ferizli (2014) found that total mortality in adults of *C. chinensis* can be obtained 192 h, after low-pressure treatment at 8.88 kPa/ 25 ºC temperature. The results of this study showed *ca*. 35% mortality of *C. chinensis* adults at 10 kPa/ 37 ºC treatment levels. This indicates that *C. chinensis* was less susceptible to low-pressure than other species probably due to their larger body size. Mbata *et al*. (2004) reported that the lethal (LT_{99}) time for the lesser grain borer (*R. dominica*) was 79 h at 30 ºC and 50 mmHg (*ca*.6.7 kPa) vacuum treatment. According to Kučerová *et al*. (2013), lethal time (LT_{99}) of adult *T. castaneum* was 15 h at 25 °C and the vacuum pressure of 1 kPa created in an airtight bag. In another study, Finkelman *et al*. (2006) have observed that the LT_{50} values of adult stored product insects *Trogoder* *magranarium* and *L. serricorne*, and *O. surinamensis* were less than 3 h when subject to low-pressure treatment (*ca*. 6.7 kPa) at 30 ºC and 55% RH. In contrast, the present findings showed that mortality of both *T. castaneum* and *S. paniceum* reached mortality levels of 45% at 10 kPa treatment after 32 h of exposure time. The reason for this might be the differences between the species of insects and treatment conditions. Even though both *L. serricorne* and *S. paniceum* have similar morphological characteristics, different treatment conditions such as O_2 content and RH, and the differences in age, biological strain and metabolic activity may have affected the mortality of these insects.

It has been reported that the mortality of stored product insect increases with increasing temperature in vacuum pressure treatments (Mbata & Phillips, 2001). Therefore, vacuum, temperature (37±1 ºC) may cause a synergetic lethal effect in insects. The lethal effect of low-pressure treatment is mainly due to the reduction of O_2 and gradual evaporation of moisture through the spiracles, under low O_2 tension (Mbata *et al*., 2005). Comparatively high O_2 levels were detected inside the vacuum chamber at the end of the exposure period (O_2 content from 4.1% to 5.7%) in comparison to that at the initial stage, due to the slow release of O_2 from inter-granular space to the outside of the products. Although the intensity of the selected low-pressure treatment and exposure period may not be able to destroy 100% of the insects, low-pressure treatments < 10 kPa and longer exposure (> 34 h) may have better control over the pests. Therefore, further studies are needed to evaluate the efficacy of different low-pressure levels, temperature, exposure period, amount of commodity and packing material for the control of stored product pests.

5. Conclusions

Adults of *S. paniceum*, *C. chinensis*and *T. castaneum* beetles displayed mortality levels exceeding 35% at 10 kPa and 37 ºC. Adult stages of *T. castaneum* and *S. paniceum* were more susceptible to vacuum treatments than the adults of *C. chinensis*. This study indicated that low-pressure can effectively control the stored product insects at the adult stage.Therefore, vacuum treatment before packaging may help to increase the market shelf-life of packaging dry food products. However, longer exposure periods and high vacuum pressure treatments are necessary for maximum control of stored product pests. This indicates low-pressure is a potential alternative to chemical disinfestation of stored product insects, especially for extended shelf-life of dry food commodities.

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