Vol.53, n. 6: pp.1469-1476, November-December 2010
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Efficacy of Modified Atmosphere Packaging to Control *Sitophilus* spp. in Organic Maize Grain

Marcelo De Carli[1], Bruna Bresolin[1], Caciano Pelayo Zapata Noreña[1*], Irineu Lorini[2] and Adriano Brandelli[1]

[1]Instituto de Ciência e Tecnologia de Alimentos; Universidade Federal do Rio Grande do Sul; Av. Bento Gonçalves, 9500; 91501-970; Porto Alegre - RS - Brasil. [2]Empresa Brasileira de Pesquisa Agropecuária; Rodovia BR 285; Km 174; 99001-970; Passo Fundo - RS - Brasil.

ABSTRACT

The effect of modified atmosphere packaging on the mortality of Sitophilus *spp. in organic maize grain was studied. Maize grains were packed with five different atmospheres consisting of 20% O_2, or 20, 40, 60, 80% CO_2 with remaining N_2. The packages were stored for 30 days at 26°C. CO_2 and O_2 concentrations were monitored inside the packages during the storage. The moisture content, titratable acidity and pH were analysed on the first and thirtieth day. At 5, 15 and 30 days of storage no significant differences were observed in the percentages of dead insects when CO_2 was used. The results of progeny indicated that from the fifth day the number of emerging insects was low at 20, 60 and 80% CO_2. Complete inhibition of the insects was achieved with 30 days of exposure in CO_2 atmospheres.*

Key words: Carbon dioxide; Modified atmosphere; Insect mortality.

INTRODUCTION

The explosion of human population in developing countries is creating an unprecedented demand for greater production of food grains (Moreno-Martinez et al., 2000). The postharvest losses due to infestation by insect pests have required the use of diverse procedures for the control of the arthropods (Gbaye and Odeyemi, 2005). *Sitophillus* spp. is a major pest of stored maize grain in Brazil and its control must be made efficiently to reduce the loss in grain.

In the recent past, the preservation of cereals and other durable agricultural products during the storage has relied heavily upon the insecticides to control the storage pests. However, the present trend is towards alternative non-toxic control methods that pose no threat to the health of operator or consumer, and which are environmentally friendly (Donahaye et al., 1996). Developing countries have resorted primarily to the use of insecticides (Moreno-Martinez et al., 2000) and most of the contact insecticides have been restricted for use on food materials (Shunmugam et al., 2005). Along with preventive practices, the farmers rely mostly on chemical control of stored products pests. However, this can be potentially harmful to human health in the form of chemical residues (Shunmugam et al., 2005).

The consumers today expect the food products that are pesticide free or with lower levels of residues (Conyers and Bell, 2007). In addition, pressure

*Author for correspondence: czapatan@ufrgs.br

from the international consumer groups to reduce the chemicals used in food products makes a non-chemical insect control practice highly desirable (Mitcham et al., 2006). It is demanding to develop the alternative methods that are economically feasible and ecologically adjusted to control the storage grain insects and fungi (Moreno-Martinez et al., 2000).

Aeration systems to cool the bulk grains and thereby prevent insect development are being widely used even in warm climates but are only applicable for the bulk grains. Other non-residual treatments such as mechanical impact, irradiation, biological control or heating are suitable for high-quality commodities or niche situations (Donahaye, 2000). CO_2 has considerable attraction as a means of disinfecting foodstuff in general and stored durable products in particular (Annis and Morton, 1997). This technology can be used in hermetic storage. The use of controlled atmospheres using either N_2 or CO_2 closely depends on well-sealed storage structures, which are rarely available in rigid silos, but easily obtainable with flexible plastic liners (Donahaye, 2000). Donahaye and Navarro (2000) reported the use of modified atmospheres (MA) as a non-toxic and environmentally benign alternative to fumigation for the control of insects in stored products. MA has been used in the agricultural and food industries for the protection and preservation of raw materials from harvest to the finished product (Conyers and Bell, 2007).

Disinfestation of stored grain using MA involves the alteration of the natural storage gases such as CO_2, O_2 and N_2 to render the atmosphere in the stores lethal to pests (Jayas and Jeyamkondan, 2002). As a method for insect control in bulk commodities, MA systems increased CO_2 or decreased O_2 atmospheres, or a combination of both (Bell, 2000; Donahaye and Navarro, 2000). MA may adversely affect the arthropods both through creation of a hypoxic (low O_2) environment, as well as from direct physiological effects of the gases (Held et al., 2001).

The maize is an agricultural product widely distributed in Brazil, and is commercialized natural form or with minimal processing to reduce the cost (Biazus et al., 2009). High value commodities, such as organic maize grain may be adapted for MA in flexible plastic containers for transporting overseas to extend its shelf life and to kill the insects. The aim of this study was to evaluate the effect of the atmosphere modified packaging on the mortality of *Sitophilus* spp. in organic maize grain.

MATERIALS AND METHODS

Samples

The organic maize grains (variety Fundacep 35) were supplied by Coperfamília (Tenente Portela, Rio Grande do Sul State, Brazil). The samples were cleaned, dried and kept in the fridge at 8°C.

Insect

Unsexed adults of *Sitophilus* spp. were obtained from the Laboratory of Brazilian Agricultural Research Corporation (Embrapa, Passo Fundo, Brazil). In order to obtain an insect population of know age, 550 adult weevils (both sexes) were transferred to 2 l plastic jar containing 1kg of maize grain. These were then covered with a lid of metal mesh. This procedure was repeated four times. The jars were kept at 26°C, 55% RH, in a 12-12 h light and dark photoperiod. After 15 days, all the insects were removed and the maize grains were again kept in the same condition for 56 days. After this period, the adults which emerged from these cultures were used in the experiments.

Experimental procedures

Fifty insects were placed in a plastic flask containing 250 g of maize grains and kept for 40 days as described above. After this period, all the contents (insect and maize) were transferred to the bags with barrier properties (Sealed Air – CRYOVAC). The air was evacuated and a food grade gas mixture (White Martins, Porto Alegre, Brazil) was introduced into the package before heat-sealing using a vacuum sealer (Selovac, model 200). The experiments were conducted for various modified atmosphere packaging: 20% (v/v) O_2, 20, 40, 60, 80% (v/v) CO_2 with remaining N_2 and 30 days exposure at 26ºC.

After the exposure, the packages were opened and all the materials were placed on a table in order to register the number of insects. Dead insects were recognized by their lack of movement despite a range of stimuli (prodding, subjecting to small breeze and leaving undisturbed). Special care was taken to ensure that death had really occurred (Annis and Morton, 1997). If they moved, they were scored as live. Recording of the dead insects were realized at 1, 2, 3, 4, 5, 15 and 30 days

exposure to modified atmosphere packaging at 26°C.
After registering the number of dead insects and removing the live ones, the grains were returned to the jars and kept again at 26°C, 55% RH for 45 days to observe the possible emergence of insects. After this period, the emergence of adult insects from any progeny was recorded.

Headspace gas composition
The CO_2 and O_2 concentrations were measured using a gas analyzer (MOCON, model Pac Check 65). To avoid the modifications in the headspace gas composition due to gas sampling, each package was used only for a single determination of the headspace gas composition (Del Nobile et al., 2006). The O_2 and CO_2 concentrations in the headspace of packages filled with the maize grains and the insects were measured after 1, 2, 3, 4, 5, 15 and 30 days exposure to modified atmosphere packaging at 26°C.

Moisture, titratable acidity and pH
The moisture content, titratable acidity and pH values were determined after one and 30 days exposure of the grains and insects in AM packaging according to AOAC methods 925.10, 942.15 and 943.02, respectively (AOAC, 1990).

Statistical analysis
A 5x7 factorial design was planned in order to study the influence of CO_2 concentration and storage period on the mortality insects. Tukey's test was used to determine the statistically significant differences between the treatment means at p=0.05. The experiments were repeated three times. Statistical analysis was completed using the SAS software (SAS Institute Inc., Cary, N.C. USA).

RESULTS

Physico-chemical analysis
The maize grain with moisture content of 10.43% (m.c.), 3.52 mg NaOH/100g of acidity and pH of 6.25 was used to the test. The results of moisture content, acidity titratable and pH are summarized in Table 1. The moisture content significantly increased (p<0.05) with exposure time at 20% O_2, 20 and 40% CO_2, but not when 60 or 80% CO_2 was used (p>0.05). The highest moisture content on the thirtieth day was observed at 40% CO_2.
Titratable acidity was almost constant in the time studied, except at 20 and 80% CO_2 (Table 1). In addiction, on the thirtieth day, no differences (p>0.05) were found among the different atmospheres tested. No significant changes in the pH values were observed at 20, 40, 60 and 80% CO_2 within the exposure time. In addition, on the thirtieth day, no significant changes in the pH values were found at different atmospheres.

Table 1 - Summary of means of moisture content, titratable acidity and pH values at different conditions of AM packaging.

	Time (days)					
	Moisture content %		Acidity mg 'NaOH/100g		pH	
Initial atmosphere	**1**	**30**	**1**	**30**	**1**	**30**
20% O_2	9.92±0.26 a*, (a)*	10.79±0.24 b, (a,b)	3.50±0.22 a, (a)	3.22±0.40 a, (a)	6.40±0.04 a, (c)	6.54±0.06 b, (a)
20% CO_2	10.21±0.22 a, (a)	10.85±0.10 b, (a,b)	3.48±0.22 a, (a)	2.90±0.32 b, (a)	6.52±0.05 a, (b,c)	6.55±0.06 a, (a)
40% CO_2	9.71±0.48 a, (a)	11.03±0.23 b, (a)	2.70±0.25 a, (b)	2.98±0.30 a, (a)	6.59±0.03 a, (a,b)	6.56±0.01 a, (a)
60% CO_2	10.21±0.24 a, (a)	10.52±0.27 a, (b)	2.80±0.16 a, (b)	2.86±0.38 a, (a)	6.66±0.10 a, (a)	6.58±0.02 a, (a)
80% CO_2	10.15±0.74 a, (a)	10.45±0.35 a, (b)	2.64±0.23 a, (b)	3.08±0.15 b, (a)	6.59±0.06 a, (a,b)	6.56±0.02 a, (a)

Means followed by different letters indicate significant differences (p<0.05) between *days or ( )* between concentrations.

Headspace atmosphere

The evolution of internal CO_2 and O_2 concentrations with exposure time can be observed in Figures 1 and 2 respectively. At 20% O_2, internal levels of CO_2 increased while O_2 concentrations rapidly decreased. After the fifth day, the levels of CO_2 started to decrease. When CO_2 was used, the internal CO_2 concentrations slightly decreased from the day 3 (Fig. 1). After the fifteenth day, CO_2 achieved a steady-state condition. The initial internal O_2 concentration was around 0.8% O_2, consequence to vacuum produced before inputting CO_2 into the package. On the first day the O_2 concentration decreased to 0.14%. However, O_2 levels slightly increased after the fifth day. On the thirtieth day, the O_2 levels significantly increased ($p<0.05$), except at 20% CO_2. This could be due to the package used, which was a O_2 low permeability film.

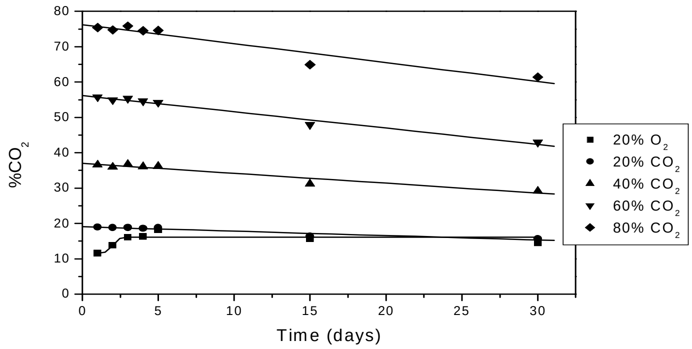

Figure 1 - Evolution of the internal CO_2 concentration inside of the package at different conditions of AM packaging.

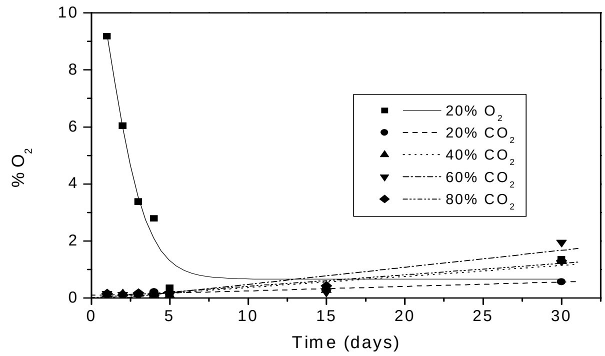

Figure 2 - Evolution of the internal O_2 concentration inside of the package at different conditions of AM packaging.

Effect on adult insects

The percentages of mortality under different MA conditions are presented in the Table 2. At 20% O_2, the mortality from the second day was significantly lower when compared with any CO_2 concentration. During all the exposure times studied at 20% O_2, the percentages of dead insects were lesser than 9%. When CO_2 was used, from the fifth day, the higher percentages of insect mortalities were obtained and no differences ($p>0.05$) in the percentages of dead insects were observed at 5, 15 and 30 days of exposure.

Effect on progeny

It was observed that under 20% O_2, the emergence of new insects was not repressed in the periods less than 30 days (Fig. 3). The highest amount of adult emergence from the progeny was observed at 20% CO_2 and two days of exposure. The insect emergences began to decrease from the fifth day, being the number of live insects lower than three using 20, 60 and 80% CO_2. On the fifteenth day, there were less than one insect at 20, 40, 60 and 80% CO_2. On the thirtieth day, no live insects were found at 20, 40, 60 and 80% CO_2 (Fig. 3).

DISCUSSION

In the experiments conducted in the absence of insects, no significant changes were found in the moisture content between the first and the thirtieth day. Moreno-Martinez et al. (2000) found that the moisture content of the maize grain stored under hermetic conditions remained almost the same as the initial values. When the tests were conducted in the presence of insects, a significant increase ($p<0.05$) of moisture content at 20% O_2, 20 and 40% CO_2 was observed. The increase in the moisture content in the grains was usually a result the metabolic activity of insects. Caneppele et al. (2003) found that the infestation of stored corn with the adults of *S. zeamais* elevated the seed moisture content, which favored the proliferation of molds by the ninetieth day. The grain stored with high moisture content is subjected to high losses caused by the attack of the insects and fungi (Vásquez-Castro et al., 2008).

Table 2 - Means (%) of mortality in adult insects at different conditions of AM packaging.

Initial atmosphere	Days						
	1	**2**	**3**	**4**	**5**	**15**	**30**
20% O_2	6.7±2.0 a, (b)	3.3±1.1 a,b, (c)	4.0±2.0 a,b, (c)	6.0±2.0 a,b, (b)	2.7±0.6 b, (b)	4.7±1.5 a, b, (c)	8.7±3.2 a, (b)
20% CO_2	6.7±3.0 d, (b)	14.7±6.4 d, (b)	40.0± 4.0 c, (a,b)	74.7±9.0 b, (a)	98.7±1.1 a, (a)	97.3±2.7 a, (a)	98.0±2.0 a, (a)
40% CO_2	22.0±7.2 d, (a)	23.3±1.1 d,(a)	38.7±2.3 c, (b)	84±2.0 b, (a)	100.0±0.0 a, (a)	96.0±2.0 a, (a,b)	98.0±2.0 a, (a)
60% CO_2	16.0±3.8 d, (a)	28.0±5.3 c,d, (a)	47.3±11.3 c, (a,b)	80.7±5.0 b, (a)	98.0±2.0 a, (a)	98.0±2.0 a, (a)	85.3±10.7 a,b, (a)
80% CO_2	10.7±4.0 e, (a,b)	27.3±10.2 d, (a,b)	52.7±9.0 c, (a)	77.3±8.3 a,b, (a)	92±7.8 a,b, (a)	82.7±12.5 a,b, (b)	98.0±2.0 a, (a)

Means followed by different letters indicate significant differences ($p<0.05$) between *days or ( )* between concentrations.

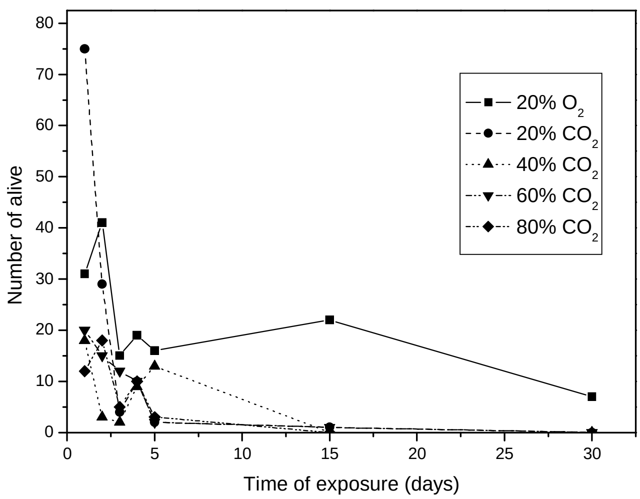

Figure 3 - Adult emergence from any progeny at different conditions of AM packaging.

However, at 60 and 80% CO_2 changes in moisture were not significant ($p \geq 0.05$). This behavior was due to the diminished respiration rates of both the insects and grains when they were in modified atmosphere or in low O_2 concentrations. In addition, low levels of moisture also decreases the respiratory rate of maize grain.

No significant changes in pH were observed when the atmosphere with 20 CO_2 was used, as well as to acidity at 40 and 60% CO_2. Rehman et al. (2002) reported that there was no change in pH and titrable acidity of maize grains kept at 10°C for six months storage. However, they observed a decrease in pH and increase in acidity after 30 days of storage at 25 and 45°C.

It was observed that in the atmosphere with 20% O_2, there was a high consumption of O_2 in the first five days, whereas CO_2 increased to 18.1%. This behavior was associated with the respiration of insect, grain and microorganisms in the package, a physiological process that occurred involving the O_2 and CO_2. Insects are the main consumers of O_2, followed by fungi and grains. Thus, insects and fungi deplete the O_2, creating a most unfavorable atmosphere for themselves (Moreno-Martinez et al., 2000).

When the experiments were conducted with CO_2, the O_2 concentration achieved was lower than 0.5% until the fifteenth day. After this period, O_2 level increased significantly to values up to 2%, except for 20% CO_2. ANOVA revealed that CO_2 concentration ($p<0.05$), time of exposure ($p<0.05$), and their interaction ($p<0.05$) were significant factors affecting the CO_2 concentration. Figure 1 depicted that the CO_2 concentration decreased with the time and initial levels of CO_2. On the contrary, the concentration of O_2 was independent of initial concentration of CO_2 and their concentration began to increase slowly after the fiftieth day (Fig. 1).

The consumption and production of O_2 and CO_2 by vegetable tissues are metabolically interrelated, making difficult to study the effect of one gas independent the other. The uncontrolled nature of the atmosphere in the package and the time interval required to reach the equilibrium are additional factors that complicate the interpretation of the results (Saltveit, 2003). Besides, there is the transfer of gases through the packaging, leading to an atmosphere richer in CO_2 and poorer in O_2 (Fonseca et al., 2002).

The mortality observed for *Sitophilus* spp. when high CO_2 concentrations were used could be associated with either O_2 depletion or CO_2 toxicity or the interaction of both (Annis and Morton, 1997). However, the natural permeability of cellular membranes appeared to be an important protection mechanism for the survival of arthropods under low O_2 or high CO_2 atmospheres (Mitcham et al., 2006). Hoback and Stanley (2001) described that arthropods were hypoxia-tolerant organisms, although their mortality increased with the lower O_2 concentrations. Mitcham et al. (2006) also showed that in the case of using controlled atmosphere, the insecticidal conditions generally contained concentration higher than 20% CO_2 and/or lower than 1% O_2, depending on the temperature, with the remaining of the atmosphere composed of N_2 gas.

ANOVA showed that time, CO_2 concentration, and their interaction ($p<0.05$) were significant factors affecting *Sitophilus* mortality, indicating that these two factors acted together on the mortality of insects. Similar behavior was observed by Annis and Morton (1997), who reported that the use of time-concentration relationship was not directly applicable to explain the effects of CO_2 on *S. oryzae* because of the interdependent effects of lowered O_2 and increased CO_2 concentrations. In addition, there was a particular time interval for each species after that an irreversible damage was done to their metabolism (Conyers and Bell, 1996).

In this work, it was shown that after five days of exposure in any initial CO_2 concentration studied, the number of insects diminished drastically. In addition to the effect of the concentrations of CO_2 and O_2 and time, there are other factors that may influence the effect of these atmospheres on the arthropods, such as temperature, grain moisture, insect species and life stage (Pearson and Sorenson, 1970; Mitcham et al., 2006).

In the experiments developed to evaluate the effect on progeny, total inhibition was achieved at 30 days using CO_2 atmospheres. This time corresponded to O_2 concentrations lower than 2%. Conyers and Bell (2007) mentioned that an increase of CO_2 to 10 or 20%, reducing O_2 to 5% was sufficient to eliminate the emergence of *Sitophilus granarius* at 20ºC, but a few individuals emerged at 25ºC. In addition, *S. granarius* exposed to atmospheres containing 1 to 1.6% O_2 with a balance of N_2 at 20°C and 70% RH were killed within seven days if the atmosphere also contained 10% or more CO_2 (Krishnamurthy et al., 1986). Other studies have shown that the minimum concentration of CO_2 useful for the complete disinfestation of *S. oryzae* was lower than 40% but substantially above 20% CO_2 (Annis and Morton, 1997). Arthropod mortality is generally greater in response to elevated CO_2 atmospheres when compared with low O_2 atmospheres (Mitcham et al., 2006).

This study showed the possibility of using MA packaging in order to protect the maize grains produced organically against insect attack. It was observed that the relationship between the initial CO_2 concentration and time was important to achieve the higher percentages of mortality for adult insects. Atmospheres containing higher than 20% CO_2 and lower than 2% O_2 and higher than five days of exposure were necessary to eliminate most adult insects. However a period of 30 days was necessary to achieve the total inhibition of the progeny.

RESUMO

O efeito da embalagem em atmosferas modificadas sobre os insetos *Sitophilus* spp. nos grãos de milho orgânico foi estudado. Os grãos de milho foram empacotados em cinco atmosferas iniciais diferentes de 20% de O_2, ou 20, 40, 60, 80% de CO_2 com balanço de N_2. As embalagens foram armazenadas por 30 dias a 26°C. As concentrações de CO_2 e O_2 foram monitoradas dentro das embalagens durante o armazenamento. As análises de teor de umidade, acidez e pH foram também realizados no primeiro e trigésimo dia. Os resultados mostraram que no quinto, décimo quinto e trigésimo dia de estocagem não foram encontrados diferenças significativas nas porcentagens de insetos mortos quando CO_2 foi usado. Os resultados relativos à progênie indicaram que no quinto dia o número de insetos emergentes foi menor a 20, 60 e 80% CO_2. A completa inibição dos insetos foi atingida com 30 dias de exposição em atmosferas de CO_2.

REFERENCES

Annis, P.C.; Morton, R. (1997), The acute mortality effects of carbon dioxide on various life stages of *Sitophilus oryzae*. *J. Stored Products Res.*, **33**, 115-124.

AOAC (1990), *Official Methods of Analysis*. 13th edn., Washington: Association of Official Analytical Chemists.

Bell, C .H. (2000), Fumigation in the 21st century. *Crop Prot.*, **19**, 563-569.

Biazus, J.P.M.; Souza, R.R.; Márquez, R.R.; Franco, T.T.; Santana, J.C.C.; Tambourgi, E.B. (2009), Production and Characterization of Amylases from *Zea mays* Malt. *Braz. arch. biol. technol.*, **52**, 991-1000.

Conyers, S.T.; Bell, C.H. (1996), The effect on the mortality of adult *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae), *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) of interrupting low oxygen exposures with periods of elevated oxygen. *J. Stored Products Res.*, **32**, 187-194.

Caneppele, M.A.B.; Caneppele, C.; Lázzari, F.A.; Lázzari, S.M.N. (2003), Correlation between the infestation level of *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera, Curculionidae) and the quality factors of stored corn, *Zea mays* L. (Poaceae). *Rev. Bras. Entomol.*, **47**, 625-630.

Conyers, S.T.; Bell, C.H. (2007), A novel use of modified atmospheres: Storage insect population control. *J. Stored Products Res.*, **43**, 367-374.

Del Nobile, M.A.; Baiano, A.; Benedetto, A.; Massignan, L. (2006). Respiration rate of minimally processed lettuce as affected by packaging. *J. Food Eng.*, **74**, 60–69

Donahaye, E.J.; Navarro, S.; Rindner, M.; Azrieli, A. (1996), The combined influence of temperature and modified atmospheres on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J. Stored Products Res.*, **32**, 225-232.

Donahaye, E.J. (2000), Current status of non-residual control methods against stored product pests. *Crop Prot.*, **19**, 571-576.

Donahaye, E.J.; Navarro, S. (2000), Comparisons of energy reserves among strains of Tribolium castaneum selected for resistance to hypoxia and hypercarbia, and the unselected strain. *J. Stored Products Res.*, **36**, 223-234.

Fonseca, S.C.; Oliveira, F.A.R.; Brecht, J.K. (2002), Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *J. Food Eng.*, **52**, 99–119.

Gbaye, O.A.; Odeyemi, O.O. (2005), Evaluation of hypercarbia atmosphere on the mortality of dried-fruit beetle, *Carpophilus hemipterus* (Linnaeus). *J. Food Agr. Environ.*, **3 (3-4)**, 43-46.

Held, D.W.; Potter, D.A.; Gates, R.S.; Anderson, R.G. (2001), Modified Atmosphere Treatments as a Potential Disinfestation Technique for Arthropod Pests in Greenhouses. *J. Econ. Entomol.*, **94**, 430–438.

Hoback, W.W.; Stanley, D.W. (2001), Insects in hypoxia. *J. Insect Physiol.*, **47**, 533–542.

Jayas, D.S.; Jeyamkondan, S. (2002), Modified Atmosphere Storage of Grains Meats Fruits and Vegetables. *Biosystems Eng.*, **82**, 235–251.

Krishnamurthy, T.S.; Spratt, E.C.; Bell, C.H. (1986), The toxicity of carbon dioxide to adult beetles in low oxygen atmospheres. *J. Stored Products Res.*, **22**, 145-151.

Mitcham, E.; Martin, T.; Zhou, S. (2006), The mode of action of insecticidal controlled atmospheres. *Bull. Entomol. Res.*, **96**, 213–222.

Moreno-Martinez, E.; Jiménez, S.; Vázquez, M.E. (2000), Effect of *Sitophilus zeamais* and *Aspergillus chevalieri* on the oxygen level in maize stored hermetically. *J. Stored Products Res.*, **36**, 25-36.

Person, Jr N.K.; Sorenson, Jr J.W. (1973), Use of gaseous nitrogen for controlling stored-product insects in grains. *Cereal Chem.*, **27**, 679–686.

Rehman, Z.U.; Habib, F.; Zafar, S.I. (2002), Nutritional changes in maize (Zea mays) during storage at three temperatures. *Food Chem.*, **77**, 1.97–201.

Saltveit, M.E. (2003), Is it possible to find an optimal controlled atmosphere? *Postharvest Biol. Tec.*, **27**, 3-13.

SAS. (1992), Statistical Analysis System, v. 6.08. The SAS Institute, Cary, N. C.

Shunmugam, G.; Jayas, D.S.; White, N.D.G.; Muir, W.E. (2005), Diffusion of carbon dioxide through grain bulks. *J. Stored Products Res.*, **41**, 131-144.

Sivertsvik, M.; Rosnes, J.T.; Bergslien, H. (2002), Modified atmosphere packaging. In: Ohlsson, T.; Bengtsson, N., *Minimal processing technologies in the food industry*, (CRC Press, Boca Raton), 61-86.

Vásquez-Castro, J.A.; Baptista, G.C.; Gadanha, C.D.J.; Trevizan, L.R.P. (2008), Effect of spray volume on the moisture of stored corn and wheat grains. *Braz. arch. biol. technol.*, **51**, 3, 453-456.

Received: January 08, 2009;
Revised: October 05, 2009;
Accepted: August18, 2010