

## The use of carbon dioxide in big bags and containers for the control of pest in food products

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DOI: 10.5073/jka.2010.425.142

### Abstract

Modified atmospheres (MA) based on high carbon dioxide (CO<sub>2</sub>) contents offer an alternative to synthetic chemical fumigation for insect pest control in food commodities during storage and shipment processes. The present study aimed to establish the efficacy of using CO<sub>2</sub> in big bags and containers to prevent pests' development. Four trials were conducted with gastight big bags (900 x 900 x 1000 or 1600 cm). Two of these trials were conducted with polished rice and samples of *Stiophilus oryzae*, one trial with chamomile infested with *Lasioderma serricornne* and one trial with cocoa and samples of *Tribolium confusum* and *Ephestia kuehniella*. Initial contents of CO<sub>2</sub> were higher than 75%, which decreased depending on exposure time (13 to 90 d) and food product. In all four trials the insects present in the infested samples were controlled with the MA. An additional trial was conducted in a 9 m container containing dried herbs in boxes, big bags and other packaging formats. Twelve infested samples of *L. serricornne* and *Plodia interpunctella* were distributed uniformly at the bottom and top of the container. A concentration between 70% and 15% CO<sub>2</sub> was maintained for an exposure time of 18 d. In spite of the decrease in CO<sub>2</sub> content, the treatment was also effective to control all insects present in the samples. Our results confirmed that CO<sub>2</sub> could be applied to food products during the storage in big bags and containers to control the occurrence of pests.

Keywords: Modified atmosphere, Carbon dioxide, Pest control, Stored-product pests.

### 1. Introduction

Food commodities can be affected by insect pests during the storage period and therefore contamination due to storage insects or their remains may be present in the final product. On the other hand, there are increasing restrictions on the use of pesticides and on the number of active chemical compounds officially registered for pest control. Therefore, implementation of alternative methods of control is necessary. Among alternative methods, Modified Atmosphere (MA) treatments are safe and environmentally friendly ways of controlling pests that affect a large number of raw and manufactured food products. MA treatments have been adopted as feasible alternative treatments since the Montreal Protocol decided to phase out the use of methyl bromide. MA with high carbon dioxide (CO<sub>2</sub>) content has been tested for many years for the control of various different pest species (Fleurat-Lessard, 1990; Adler et al., 2000; Navarro, 2006). The use of CO<sub>2</sub> offers several advantages for the food industry: there is no accumulation of toxic residues after the treatment in the final product, no safety interval following treatment is necessary for consumption of the product and it is currently approved as a food additive, E-290 (FAO and WHO, 2010). However, high CO<sub>2</sub> MA requires the use of gastight structures to keep the correct gas concentration throughout the treatment. The objective of the present work was to demonstrate the efficacy of using high CO<sub>2</sub> MA in gastight bags specifically manufactured for this application and in a 9 m container to prevent insect pests' development during the storage of rice, cocoa beans, chamomile and different dried herbs.

## 2. Materials and methods

Five trials were conducted: two with polished rice in big bags and samples of *Sitophilus oryzae* (L.), one with chamomile in big bags infested with *Lasioderma serricorne* (F.), one with cocoa beans in big bags and samples of *Tribolium confusum* du Val and *Ephesia kuehniella* Zeller, and the last one with various dried herbs in a 9 m container with samples of *L. serricorne* and *Plodia interpunctella* (Hübner).

Insect species tested in this study were obtained from stock colonies maintained at the IRTA (Cabrils, Barcelona). *Sitophilus oryzae* was reared on brown rice, *P. interpunctella* on wheat bran, brewer's yeast and glycerine, and *L. serricorne*, *T. confusum* and *E. kuehniella* on wheat flour and yeast. Rearing was conducted in a climatic chamber at 25±1°C, 75±10% r.h. and at a photoperiod of 16:8 h (L: D).

Standard big bags (900 x 900 x 1000 cm for rice and cocoa beans trials and 900 x 900 x 1600 cm for the chamomile trial) of woven polypropylene with an internal plastic liner were used for the MA treatment (AG Protectpack S.L.) (PRO COEX PACK 3.85 TRIK multy layer) (Figure 1). The experiments were conducted with food grade Carbon Dioxide CO<sub>2</sub> (S.E. Carburos Metálicos S.A. - Air Products Group).



**Figure 1** Chamomile in big bags (900 x 900 x 1600 cm).

### 2.1. Rice in big bags.

Two trials were conducted with polished rice. In the first trial, 750 g of brown rice infested with eggs of *S. oryzae* were distributed throughout the top, middle and bottom levels of a big bag filled with polished rice. In the second trial, 125 g of infested rice were distributed in the big bag. The big bags were then purged with CO<sub>2</sub> and kept in a warehouse at ambient conditions. Gas concentrations were determined with a gas analyzer (Abbiss, TOM-12), during the exposure, to verify the contents of CO<sub>2</sub> and O<sub>2</sub> inside the big bags. At the end of 48 d in trial 1 and 90 d in trial 2, the bags were opened and insect samples were collected from each big bag. The samples were placed in a climatic chamber at 25±1°C and 75±10% r.h. until the emergence of adults. The number of *S. oryzae* adults which emerged was then recorded. In both trials, a control treatment with a big bag of rice infested with the same number of samples of *S. oryzae* was conducted in order to compare the results obtained with and without CO<sub>2</sub>.

### 2.2. Cocoa beans in big bags

The trial was conducted with 2 gastight big bags which were purged with 680 kg of cocoa beans. Samples, with 50 g of wheat flour and yeast infested with 50 eggs of *E. kuehniella* and 500 eggs of *T. confusum* were prepared using paper envelopes. Three samples with *T. confusum* and three samples with *E. kuehniella* were distributed at each level of the big bag (bottom, middle and top). One of the big bags was then purged with CO<sub>2</sub> and the not treated was kept as a Control. The big bags were kept in a warehouse at ambient conditions for 13 d. Gas concentrations were determined with a gas analyzer (Abbiss, TOM-12) at the start and at the end of the exposure time, to verify the contents of CO<sub>2</sub> and O<sub>2</sub> inside the big bag. At the end of the treatment, big bags were opened and all samples with the insects were collected and placed in the climatic chamber for 7 wk. Afterwards, the number of emerged adults of *T. confusum* and *E. kuehniella* were counted.

### 2.3. Chamomile in big bags

The trial was conducted with 28 gastight big bags of chamomile infested with a natural population of *L. serricornis* and purged with CO<sub>2</sub>. In nine randomly selected big bags, gas content was determined at the start and after 16, 18, 21, 22 and 24 d of exposure, with a gas analyzer (Abbiss, TOM-12) to verify CO<sub>2</sub> and O<sub>2</sub> levels inside the big bags. At the end of the MA treatment, four samples of approximately 100 g each of chamomile were collected randomly and placed in the climatic chamber for 2 mo. Three Control samples of 100 g of chamomile, not treated with CO<sub>2</sub> were used in order to assess insect infestation. The number of insect adults emerged was recorded in all samples at the end of the trial.

### 2.4. Dried herbs treatment in container

The experiment was conducted in a 9 m container connected to a cylinder of CO<sub>2</sub>. The container had 11 pallets with various dried herbs such as chamomile and fennel in boxes, big bags and in other packaging formats. Samples with 80 g of wheat flour and yeast infested with, approximately, 50 eggs and 50 larvae of *L. serricornis*, and with 20 g of wheat bran infested with, approximately, 100 larvae of *P. interpunctella* were prepared using paper envelopes. Six infested samples of *L. serricornis* and 6 of *P. interpunctella* were distributed uniformly at the bottom and top of the container. The container was purged with the MA until CO<sub>2</sub> content reached 70%, approximately. Gas content inside the container was measured with a gas analyzer (Abbiss, TOM-12) to verify CO<sub>2</sub> and O<sub>2</sub> levels throughout the exposure time. Due to a decrease in the concentration of CO<sub>2</sub>, additional CO<sub>2</sub> was introduced inside the container during the trial. After 18 d of exposure, the container was opened and the samples with the insects were placed in a climatic chamber for 2 months. Control samples of each insect which were not treated with CO<sub>2</sub> were maintained in a climatic chamber in order to assess insect infestation. The number of live insects was recorded at the end of the experiment.

## 3. Results and discussion

### 3.1. Rice in big bags

In the trial 1, the CO<sub>2</sub> and O<sub>2</sub> contents within the sealed big bag just after it was purged with the MA were 100% and 0.8%, respectively. Twenty one d after dosing, CO<sub>2</sub> content decreased to 59% and O<sub>2</sub> content increased up to 10%. At the end of the 48 d treatment, CO<sub>2</sub> content decreased to 45% and O<sub>2</sub> content increased up to 14%. At the end of the trial, *S. oryzae* adults were only recorded in samples of the Control big bag, since they were on the top layer of the big bag where the number of *S. oryzae* adults was higher (Table 1).

**Table 1** Rice in big bags. Trial 1. Number of *S. oryzae* adults after 48 d of exposure to CO<sub>2</sub>. Samples were collected at three different levels (top, middle and bottom).

Big bag	Level	Sample	Number of <i>S. oryzae</i>
Treated (CO <sub>2</sub> )	Top	1.1	0
		1.2	0
		1.3	0
	Middle	2.1	0
		2.2	0
		2.3	0
	Bottom	3.1	0
		3.2	0
		3.3	0
Control	Top	1.1	160
		1.2	118
		1.3	61
	Middle	2.1	36
		2.2	17
		2.3	26
	Bottom	3.1	7
		3.2	4
		3.3	0

In the second trial, when the big bag was purged, the CO<sub>2</sub> content reached 78% and residual O<sub>2</sub> remained around 0.7%. After 45 d, CO<sub>2</sub> content decreased to 45% and O<sub>2</sub> increased to 10%. At the end of the 90 d exposure, CO<sub>2</sub> content was reduced to more than half compared to the initial values. The O<sub>2</sub> content increased to 12%. No insects survive in the big bag treated with CO<sub>2</sub>, while in the Control big bag a large number of insects were recorded in all three levels sampled (Table 2).

**Table 2** Rice in big bags. Trial 2. Number of *S. oryzae* adults after 90 d of exposure to CO<sub>2</sub>. Samples were collected at three different levels (top, middle and bottom).

Big bag	Level	Sample	Number of <i>S. oryzae</i>
Treated (CO <sub>2</sub> )	Top	1	0
	Middle	2	0
	Bottom	3	0
Control	Top	1	56
	Middle	2	22
	Bottom	3	27

### 3.2. Cocoa beans in big bags

Just after the big bag was purged, CO<sub>2</sub> and O<sub>2</sub> contents were 99% and 0.6%, respectively. At the end of the treatment, 13 d after dosing, CO<sub>2</sub> content decreased up to 10% and O<sub>2</sub> concentration increased to 16%. At the end of the trial, no insects of any species tested survived in the treated big bag. Conversely many insects were alive in the samples of the control big bag (Table 3).

**Table 3** Cocoa beans in big bags. Number *T. confusum* and *E. kuehniella* adults after 13 d of exposure to CO<sub>2</sub>. Samples were distributed at three different levels (top, middle and bottom).

Big bag	Level	Sample	Number of <i>T. confusum</i>	Number of <i>E. kuehniella</i>
Treated (CO <sub>2</sub> )	Top	1.1	0	0
		1.2	0	0
		1.3	0	0
	Middle	2.1	0	0
		2.2	0	0
		2.3	0	0
	Bottom	3.1	0	0
		3.2	0	0
		3.3	0	0
Control	Top	1.1	255	39
		1.2	235	38
		1.3	290	24
	Middle	2.1	129	33
		2.2	84	34
		2.3	245	37
	Bottom	3.1	79	35
		3.2	49	37
		3.3	106	33

### 3.3. Chamomile in big bags

The CO<sub>2</sub> and O<sub>2</sub> contents in nine of the randomly selected big bags were in average 89% and 1.4%, respectively. Between 18 and 24 d after dosing, CO<sub>2</sub> content was still higher than 82% and O<sub>2</sub> content remained below 3%. At the end of treatment time, no adults emerged in the samples that were treated with CO<sub>2</sub>. On the contrary, 2 and 1 *L. serricornis* adults emerged in 2 samples of the control treatment without CO<sub>2</sub> (Table 4).

**Table 4** Chamomile in big bags. Number of *L. serricorne* adults in samples with a natural occurring infestation, after 21 d of treatment with CO<sub>2</sub>.

Big bag	Level	Sample	Number of <i>L. serricorne</i>
Treated (CO <sub>2</sub> )	Top	1	0
	Top	2	0
	Top	3	0
	Top	4	0
Control	Top	1	0
	Top	2	2
	Top	3	1

### 3.4. Dried herbs treatment in container

The initial CO<sub>2</sub> content inside the container reached 70%. However, the level of CO<sub>2</sub> declined during the exposure to 15% and the residual O<sub>2</sub> content increased to 18%. Therefore, it was necessary to add gas to maintain the CO<sub>2</sub> content inside the container. However, treated samples with *L. serricorne* and *P. interpunctella* distributed in the container did not contain any live insects at the end of the trial (Table 5).

**Table 5** Dried herbs in container. Number of *L. serricorne* and *P. interpunctella* adults after 18 d exposure to CO<sub>2</sub>.

Big bag	Level	Sample	Number of <i>L. serricorne</i>	Number of <i>P. interpunctella</i>
Treated (CO <sub>2</sub> )	Top	1	0	0
		2	0	0
		3	0	0
	Bottom	1	0	0
		2	0	0
		3	0	0
Control	-	1	98	72

The results confirmed that the use of high CO<sub>2</sub> MA in gastight big bags is a feasible alternative to control the occurrence of pests on rice, cocoa beans various dried herbs during their storage. Levels of CO<sub>2</sub> in the sealed big bags remained quite constant throughout the period of exposure and control efficacy was verified in all cases tested. The use of containers for the treatment of food commodities with high CO<sub>2</sub> MA should be implemented in order to keep gas contents above the required levels and to guarantee a high level of control.

### Acknowledgements

This research was financially supported by Carburos Metálicos-Air Products Group, under the Project CEN-2008-1027, a CENIT Project belonging to the Program Ingenio 2010, from the Spanish Government (CDTI). The authors would like to thank also A.G. Protectpack S.L. (Barcelona) Spain for supplying the big-bags.

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