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CARBON DIOXIDE UNDER HIGH PRESSURE FOR STORED-PRODUCT PROTECTION IN TEMPERATE CLIMATES

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ABSTRACT

The exposure to carbon dioxide (CO_2) under high pressure is a new control method in the food processing industry. Agricultural raw products, such as cereals, nuts or dried fruits require rapid disinfestation prior to storage. The organic food branch in particular needs a preventive method that does not leave chemical residues or lead to any reduction in quality. High-pressure industrial plants work with CO_2 under pressure of 10 bar - 40 bar for a few hours. Under practical conditions, the problem of incomplete control of insect pests, occurs mostly at low temperature regimes and pressures below 15 bar.

Our experiments were conducted with the following caged stored-product insects at some of their developmental stages in different products and packages in 10 m^3 chambers: *Plodia interpunctella, Stegobium paniceum, Tribolium confusum, Sitophilus granarius, Ephestia kuehniella, Cryptolestes ferrugineus, Cryptolestes turcicus, Trogoderma granarium*, as well as the parasitic wasp *Lariophagus distinguendus*. The results showed that at low temperatures of about 10ºC at 10 bar to 15 bar of CO_2 the exposure period required for complete control varied with the product surface and the packaging material. At 15 bar and 8 to 10ºC, 100% mortality was not achieved within ten hours for some of the species tested.

The rapidity at which the gas reached even distribution was found to depend on the type and mass of the product. The experiments showed that to ensure complete control it is necessary to identify the pest and classify its susceptibility prior to CO_2/high pressure treatment. The practical exposure time must also take into account the type and temperature of the product.

INTRODUCTION

Stahl and Rau (1985) and Stahl *et al.*, (1985) described a new process for residue free insect pest control by using CO_2 under high pressure. Mitsura *et al.*, (1973) were the first to report on the effects of this treatment against stored product mites. It has been found that the quality of the treated products is not disadvantageously influenced when the depressurization time is appropriately adjusted (Gerard *et al.*, 1988; Pohlen *et al.*, 1989). The growing public pressure

against the presence of insecticidal residues in food and the impending ban on the use of methyl bromide has led to investigations on alternatives for pest control. The acceptance of this new high pressure/CO_2 approach is supported by the extremely short lethal exposure time in the range of minutes or a few hours (Prozell and Reichmuth 1990 and 1991; Nakakita and Kawashima 1994; Reichmuth and Wohlgemuth 1994; Prozell *et al*. 1997). In Germany the organic food branch in particular, uses this rapid method for disinfestations of their products as a standard procedure. The objective of these experiments was to examine the efficacy of this technology during exposures at a relatively low temperature of 10ºC.

MATERIAL AND METHODS

High pressure facility

All experiments were conducted in high-pressure installations of the CARVEX company (Fig. 1). The volume of the pressure chamber was 9 m^3. Carbon dioxide in a pressurized tank connected to the exposure chamber, provided the gas supply. Before initiating treatment, the CO_2 was warmed and than introduced into the pressure-chamber. Different pressure regimes were made available by adjustment of the regulator (Table 1).

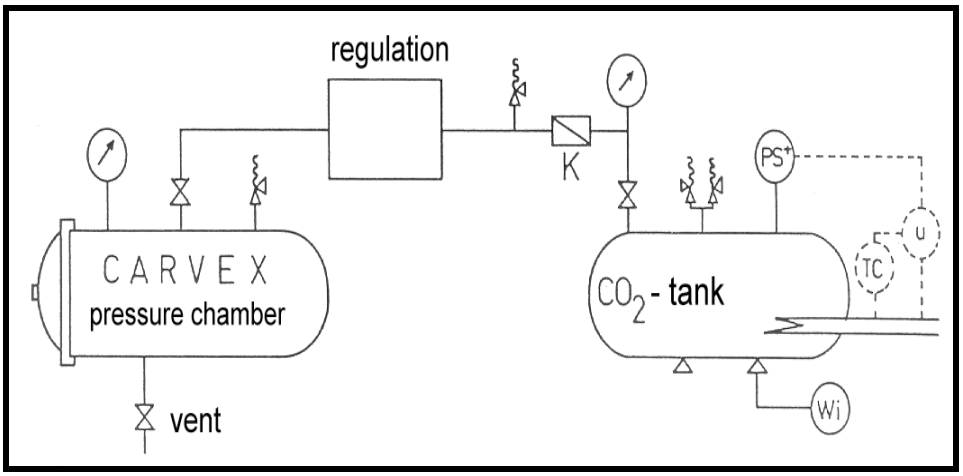

Fig. 1: Pressure chamber to hold carbon dioxide, carbon dioxide supply tank and regulation unit (Wi: balance, PS+, u, TC: supply gear to adjust temperature and pressure in the tank, K: valve) (after Gerard *et al.,* 1990).

At the end of the required exposure-period the pressure was released. The exposure time included the time to build up the targeted pressure. The time for depressurization ranged from 14 to 25 min.

TABLE 1
Pest species, carbon dioxide pressure, temperature and exposure period of the experiments

Insect species	Pressure in bar	Temp °C	Exposure time in h
	15	0 to 2	11.5
Sitophilus granarius	20	4	8
	15	5	15
	15	0 to 2	11.5
Lariophagus distinguendus	20	4	8
	15	5	15
	15	0 to 2	11.5
Tribolium confusum	20	4	8
	15	/5	15
	15	0 to 2	11.5
Ephestia kuehniella	20	4	8
	15	5	15
	15	0 to 2	11.5
Cryptolestes ferrugineus	20	4	8
	15	5	15
	15	0 to 2	11.5
Cryptolestes turcicus	15	0 to 5	15
	20	/0 to 4	8
	15	0 to 2	11.5
Stegobium paniceum	15	0 to 5	15
	20	0 to 4	8
	15	0 to 2	11.5
Trogoderma granarium	15	0 to 5	15
	20	0 to 4	8
	15	0 to 2	11.5
Plodia interpunctella	15	0 to 5	15
	20	0 to 4	8

Insects

Experiments were performed using all developmental stages of a mixture of several pest species (Table 1). The insects were introduced inside stainless steel wire mesh cages (10 cm length, 1 cm diameter) fitted with rubber stoppers. Prior to treatment, separate cages were distributed to different positions inside the chamber. Two exposure profiles were examined: in one, the cages were placed at the centre of a metal bucket containing flour; in the other, the cages were placed at the centre of a 'big-bag' of 1 m^3 capacity, made from webbed PP mesh and containing herbal tea. The chamber was then closed and pressurized with CO_2. At the end of the treatment the pressure was released, the cages removed, and the samples were transferred to an incubator at 26°C and 75% r.h., and observed weekly for detection of survivors during the following 14 weeks. Control samples of insects were prepared similarly but not subjected to treatment.

RESULTS

High-pressure treatment in big-bags
High pressure treatments in 'big-bags' caused 100% mortality of *Plodia interpunctella, Stegobium paniceum* and *Lariophagus distinguendus*. (See Table 2 for comprehensive details of results).

High-pressure treatment in flour
More survivors were found in test cages placed in the flour. Only *Sitophilus granarius*, *Tribolium confusum* and *Stegobium paniceum* were completely controlled in all experiments. (See Table 2 for all results)

Control insects
All insects in the untreated control samples developed normally.

DISCUSSION AND CONCLUSIONS

The results of insect mortality presented in these experiments are similar to those with *S. granarius* described by Prozell and Reichmuth (1990 and 1991). The toxic action of inert gases under increased pressure was first described by Ferguson and Hawkins (1949), and later by Johnson and Quastel (1953), and Carpenter (1954). They mentioned narcotic effects after treatment with these gases. Insect death presumably occurs during treatment under high pressure as a consequence of prolonged and intense narcosis. Destruction of cell membranes during decompression also causes severe damage (Ulrichs 1994). Prozell *et al.*, (1997) stated that the speed of distribution of the CO_2 under pressure seems to depend on the type and density of the treated product.

The presented investigation into the rapidity at which CO_2 distributes itself through the product revealed similar results. Previous work showed that at first, compressed air remained in the centre of the product, surrounded by CO_2 under pressure. The initial difference in pressure is not sufficient to quickly remove all the residual air from the interstitial space within the product. On the other hand, pressurization of the air alone does not control insect pests in a short time (Prozell and Reichmuth 1991). Later during exposure, the CO_2 content increased also in the centre of the product mainly due to relatively slow diffusion. Four time phases of penetration can be discussed which follow the classical transport phenomena (Bird *et al*. 1960). The time required to obtain the necessary CO_2 content to control insect pest can be delayed inside compressed products, because a longer time for uniform distribution will be required (Prozell *et al*. 1997). The results presented here show that it is advantageous and even necessary to identify and classify the sensitivity of the pest to treatment, the nature of the product to be treated, the exposure temperature and possibly the existence of developmental stages prior to undertaking a high-pressure treatment. Complete mortality can be achieved more slowly in 'big-bags' than in small containers with flour. Mortality rates depend also on the size of the insects and their developmental stages and whether infestation occurs inside or outside the particles of a treated commodity (Ulrichs *et al*., 1997a and 1997b). With all this information at hand, the required exposure time can be adjusted accordingly. In contrast to the conventional insecticides and toxic fumigants this treatment can be used as a preventive method to ensure pest free food and feed, without leaving chemical residues.

TABLE 2

Results of carbon dioxide/high pressure treatment of various developing stages of various stored product pest insects in flour and big bags, (x = survivors, 0 = no survivors)

Insect species	Product/ container	Exposure time in h	Temp ºC	Survivors
Sitophilus granarius	flour	11.5	0 to 2	0
	flour	11.5	0 to 2	0
	flour	15	5	0
	big bag	15	5	0
	big bag	8	4	0
	flour	6	12 to 15	0
	big bag	6	12 to 15	0
	flour	7	12	0
	big bag	7	12	X
Lariophagus distinguendus	flour	11.5	0 to 2	X
	big bag	11.5	0 to 2	0
	flour	8	4	X
	big bag	8	4	0
	flour	15	5	X
	big bag	15	5	X
Tribolium confusum	flour	11.5	0 to 2	X
	flour	8	4	0
	flour	15	5	0
Ephestia kuehniella	Flour	11.5	0 to 2	X
	flour	8	4	0
	flour	15	5	0
Cryptolestes ferrugineus	big bag	11.5	0 to 2	0
	big bag	8	4	0
	flour	8	4	X
	flour	15	5	X
	big bag	15	5	X
Cryptolestes turcicus	big bag	6	12 to 15	X
	flour	6	12 to 15	X
	big bag	7	12	X
	flour	7	12	X
	big bag	10	8 to 12	X
	flour	10	8 to 12	X
Stegobium paniceum	big bag	6	12 to 15	0
	flour	6	12 to 15	0
	big bag	7	12	0
	flour	7	12	0
	big bag	10	8 to 12	0
	flour	10	8 to 12	0
Trogoderma granarium	big bag	6	12 to 15	X
	flour	6	12 to 15	X
	big bag	7	12	X
	flour	7	12	X
	big bag	10	8 to 12	0
	flour	10	8 to 12	X
Plodia interpunctella	big bag	6	12 to 15	0
	flour	6	12 to 15	X
	big bag	7	12	0
	flour	7	12	X
	big bag	10	8 to 12	0
	flour	10	8 to 12	X

REFERENCES

Bird, R.S., Stewart, W.E. and Lightfood, E.N. (1960) Transport Phenomena. Wiley, New York.

Carpenter, F.G. (1954) Anaesthetic action of inert and inreactive gases on intact animals and isolated tissues. *Am. J. Physiol.*, **178**, 505-509.

Ferguson, J. and Hawkins, S.W. (1949) Toxic action of some simple gases at high pressure. *Nature,* **164**, 963-964.

Gerard, D., Kraus, J. and Quirin, K.W. (1988) Rückstandsfreie Druckentwesung mit natürlicher Kohlensäure [Residue free pressure disinfestation with natural carbon dioxide]. *Gordian,* **88**, 90-94.

Gerard, D., Kraus, J., Quirin, K.W. and Wohlgemuth, R. (1988) Anwendungen von Kohlendioxid unter Druck zur Bekämpfung vorratsschädlicher Insekten und Milben [Carbon dioxide under pressure to control stored product pest insects and mites]. *Pharm. Ind.*, **50**, 1298-1300.

Gerard, D., Kraus, J., Fröhlingsdorf, B., Jerga, C. and Dallüge, A. (1990) Rückstandsfreier Vorratsschutz für Arznei- und Teedrogen [Residue free stored product protection of medical and tea drugs]. *Deutsche Apotheker Zeitung,* **130**, 2014-2018.

Johnson, W.J. and Quastel, J.H. (1953) Narcotics and biological acetylations. *Nature,* **171**, 602-603.

Mitsura, A., Amano, R. and Tanabe, H. (1973) The acaricidal effects of compressed gas treatments on the grain mite, *Tyrophagus putrescentiae*. *Shokuhin Eiseigaku Zashi,* **14**, 511-515.

Nakakita, H. and Kawashima, K. (1994) A new method to control stored product insects using carbon dioxide with high pressure followed by sudden pressure loss. In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.) Canberra, Australia, 17-23 April 1994, CAB International, Wallingford, Oxon, UK, **1**, 126-129.

Pohlen, W., Rau G. and Finkenzeller, E. (1989) Erste praktische Erfahrungen mit einem Verfahren zur Druckentwesung mit Kohlendioxid [First practical experiences with carbon dioxide under pressure for pest control]. *Pharm. Ind.*, **8**, 917-918.

Prozell, S. and Reichmuth, Ch. (1990) Wirkung von Kohlendioxid unter Hochdruck auf den Kornkäfer *Sitophilus granarius* (L.) [Effect of carbon dioxide under high pressure on the granary weevil *Sitophilus granarius* (L.). *Mitteilungen der Deutschen Phytomedizinischen Gesellschaft,* **20**, 14.

Prozell, S. and Reichmuth, Ch. (1991) Response of the granary weevil *Sitophilus granarius* (L.) (Col.: Curculionidae) to controlled atmospheres under high pressure. In: *Proc. 5th Int. Working Conf. on Stored-product Protection,* (Edited by Fleurat-Lessard, F. and Ducom, P.), Bordeaux, France, 1990, **2**, 911-918.

Prozell, S., Reichmuth, Ch., Ziegleder, G., Schartmann, B., Matissek, R., Kraus, J., Gerard, D. and Rogg, S. (1997) Control of pests and quality aspects in cocoa beans and hazelnuts and diffusion experiments in compressed tobacco with carbon dioxide under high pressure. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products*, (Edited by Donahaye, E.J., Navarro, S. and Varnava, A.), 21-26 April 1996, Printco Ltd., Nicosia, Cyprus, 325-333.

Reichmuth, Ch. and Wohlgemuth, R. (1994) Carbon dioxide under high pressure of 15 bar and 20 bar to control the eggs of the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) as the most tolerant stage at 25°C. In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.) Canberra, Australia, 17-23 April 1994, CAB International, Wallingford, Oxon, UK, **1**, 163-172.

Stahl, E. and Rau, G. (1985) Neues Verfahren zur Entwesung [A new method for disinfestation]. *Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz,* **58**, 133 -136.

Stahl, E., Rau, G. and Adolphi, H. (1985) Entwesung von Drogen durch Kohlendioxid-Druckbehandlung (PEX-Verfahren) [Disinfestation of drugs with carbon dioxide treatment under pressure (PEX-Method]. *Pharm. Ind.,* **47**, 528-530.

Ulrichs, C. (1994) Effects of different speed of build up and decrease of pressure with carbon dioxide on the adults of the tobacco beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.) Canberra, Australia, 17-23 April 1994, CAB International, Wallingford, Oxon, UK, **1**, 214 - 216.

Ulrichs, C., Reichmuth, Ch. and Rassmann, W. (1997a) Carbon dioxide under high pressure to control the tobacco beetle *Lasioderma serricorne*. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products*, (Edited by Donahaye, E.J., Navarro, S. and Varnava, A.), 21-26 April 1996, Printco Ltd., Nicosia, Cyprus, 335-341.

Ulrichs, C., Reichmuth, Ch., Tauscher, R. and Westphal, K. (1997b) Rate of gas exchange during treatment of compressed tobacco with nitrogen or carbon dioxide for pest control. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products*, (Edited by Donahaye, E.J., Navarro, S. and Varnava, A.), 21-26 April 1996, Printco Ltd., Nicosia, Cyprus, 343-347.