DISINFECTION AND DISINSECTION EFFECT OF CO_2 UNDER PRESSURE ON FOOD MATRIX

Capilla, V. [1*]**; Mañez, M.** [1] **; Moreno Marí, J.** [2] **; Jiménez, R.**[2]

1. AINIA, Agrofood Technological Institute, Valencia Parc Tecnologic, 46890 Paterna, Valencia, Spain. E-mail: vcapilla@ainia.es. Fax: +34 96 131 80 08.
2. Instituto Cavanilles de Biodiversidad y Biología Evolutiva. Universitat de València. Apartado Oficial 2085. 46071 Valencia (Spain).

Cereals are one of the most important food resources for human nutrition. During storage, grains are frequently attacked by microorganism and arthropods that damaged them. Legal and environmental pressure on chemical treatments for sterilisation and insect control (e.g. methyl bromide fumigation) reinforce the research on new and alternative treatments to ensure product quality.

One of the major primary pests in rice is *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae). Control of larvae as well as of adult forms of this specie is difficult as long as their vital circle occurs inside the grain. Moreover, different bacteria and fungus represent microorganism population in this cereal, primarily due to wet and hot conditions experienced during harvesting.

In this work, several experiments have been carried out at different conditions, varying pressure, temperature and exposure time to evaluate the disinfection and disinsection effect of CO_2 under pressure. All the experiences have been carried out with rice samples previously infected, analysing the lethal effect on adults and larvae.

For microbiological monitoring rice samples were contaminated with *Escherichia coli* and *Penicillium* sp. For entomological monitoring rice samples were infested with *Sitophilus oryzae* according to the infestion methodology developed at laboratory scale.

The results obtained show that is possible to achieve 100% mortality in insects without loss of product quality, while the effectiveness on microorganism inactivation has not been so successful.

INTRODUCTION

Rice (*Oryza Sativa*) is an annual herbaceous crop that represents the basic food for more than one third of humanity. Its composition and attribute variability is wide and it depends on cultivars and crop harvest conditions.

From harvest to consumption, cereals go through different stages: crop, threshing and ventilation, drying, storage, preliminary methods of processing and final processing. During storage, rice can be subjected to divers alterations due to physic-chemical causes or parasite invasions, and the causes can be excess room humidity or destructive action of pests.

To ensure the correct state of final product, different chemical treatments are applied. Although these treatments (methyl bromide, cianidric acid, fosfines, etc) are highly effective, with low risk of toxic residues permanency, they must be applied by qualified staff and their legal requirements for use are becoming more restrictive due to its high toxicity and high environmental impact.

Nowadays, alternative methods are been studied as irradiation or microwaves [1]. However these technologies are expensive, lack total security guaranty or can affect product quality. Parallel to these technologies, some authors indicate the use of carbon dioxide under temperature and pressure near or upper critical conditions for inactivation of microorganims [2, 3, 4, 5]. In fact, there are literature references about the possibility of destroying yeast, *Escherichia coli* and *Staphylococcus aureus* with supercritical CO_2 at 200 atm and temperature of 35ºC [2]. At these conditions, if microorganims are in a high humidity content medium (70-90%), the treatment if effective. If the humidity content is lower, sterilisation effect is achieved adding minimum amounts of ethanol (2%) or acetic acid (0,5%).

The principal factors that influence antimicrobial effectiveness of treatment applied are: the type of microorganims, treatment conditions (pressure, temperature and exposure time), the nature of matrix (water content), and the use of modifiers as ethanol or acetic acid.

The microorganims inactivation was suggested come from the inactivation of some enzymes, from the lowered intracellular pH due to penetration of CO_2, or the extraction of intracellular components as phospholipids [6].

CO_2 treatment under pressure can also produce selective enzymatic inactivation. This inactivation effect could result from a drop of internal pH of the microorganims during the applied treatment, that tend to precipitate those enzymes which have an acid isoelectric point as β-galactosidase or alkaline phosphatase, without effect on the solubility of enzymes with a basic isoelectric point such as acid phosphatase [2]. The sorption of CO_2 into enzyme molecules would cause conformational changes and then giving rise to the loss of activity. The decomposition of the secondary structure in enzyme molecules after treatment could cause the loss of activity. The α-helix structure undergoes a conformational change in acidic solution, and could decompose in a carbonated aqueous solution containing microbubbles of SC-CO_2. This decomposition of the α-helix structure would result in the loss of activity [4].

Anyway, all the above referenced works have been developed in cellular culture or microorganims previously isolated and stabilised by dehydration. In any case, experiences have been developed with food or vegetal material matrix.

In the same way, there are several references describing process using CO_2 under pressure for pest control [7, 8]. Its lethal effect on the insects seems to be due, on the one hand, to the physiological stress on their cells (owing to the quick pressure build up and subsequent decrease of the pressure). As a result of the lower viscosities of the SC-CO_2, this invades the inner part of the cells giving rise, in the phase of depressurisation, to the split of the cells causing the death of insects and microorganisms. On the other hand, the lethal effect could be due to the lack of O_2 in the environment which becomes anaerobic, and also to the changes in the pH of cells caused by absorption of CO_2 [9].

Furthermore, using CO_2 under high pressure has the added advantage of requiring short lethal exposure times, comparing with fumigation exposure times, ranging from minutes to only few hours [10, 11].

I - MATERIALS AND METHODS

Bahia rice cultivar has been selected as raw material because of its wide extension and acceptability. Samples of this cultivar were obtained from a local producer. Experiments were carried out in a pilot plant described previously [12]. Dry CO_2 was supplied by Abello-Linde (Valencia, Spain), with purity higher than 99 % w. Approximately 200g. of rice were used in each experiment.

In order to study the effect of the treatment with CO_2 under pressure on the inactivation of microorganims and insects mortality, a series of experiments was designed, using rice previously infected as raw material, and varying the operation conditions. The operative variables evaluated were: pressure, temperature and exposure time.

During experimental procedure rice samples were located in the autoclave, and CO_2 was pumped until working pressure. After the required exposure time, the autoclave was depressurised and atmospheric pressure was reached.

For entomological control, experiments were undertaken with adults and eggs of *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera, Sylvanidae). Both species came from real samples of contaminated rice and they were raised in rearing chamber at 26,5 ºC and 60% HR.

The methodology of infestation for the two species is the same: Each sample of 200 g of rice is infested with 50 adults, is kept 5 days in the chamber to allow the oviposition before submitting it to the treatment with CO_2. We will evaluate the efficacy of the processing upon adults and eggs of each of the species.

A large number of tests have been made for each species. They consist on three repetitions of 36 tests each one, combining pressures ranged from 1 to 100 bar; temperature ranged from 20 to 60 ºC and times exposure ranged from 5 to 60 min. and control samples.

In the case of microorganism inactivation, experiments were undertaken with *Escherichia coli*, and *Penicillium* sp. The specie comes from CECT (Spanish Type Culture Collection, Nº 471), while the mould was a wild strain. In this case the exposure time was 30 min., pressure ranged from 1 to 100 bar and temperature ranged from 40 to 60ºC.

Treated samples are then packaged in sterilised bags and stored at refrigerated conditions (3ºC) until analysis.

II.- RESULTS AND DISCUSSION

Figure 1 shows results for rice samples inoculated with *Penicillium* sp. As it is observed, the logarithmic reduction grew when pressure increased, being this effect higher at higher temperature.

The treatment at 40ºC requires pressure range of 100 bar to achieve a proper population reduction (3 un. log.). However, temperature range from 50ºC to 60ºC is more effective even being applied at low pressure (20 bar), producing a decrease in population of 99,9%.

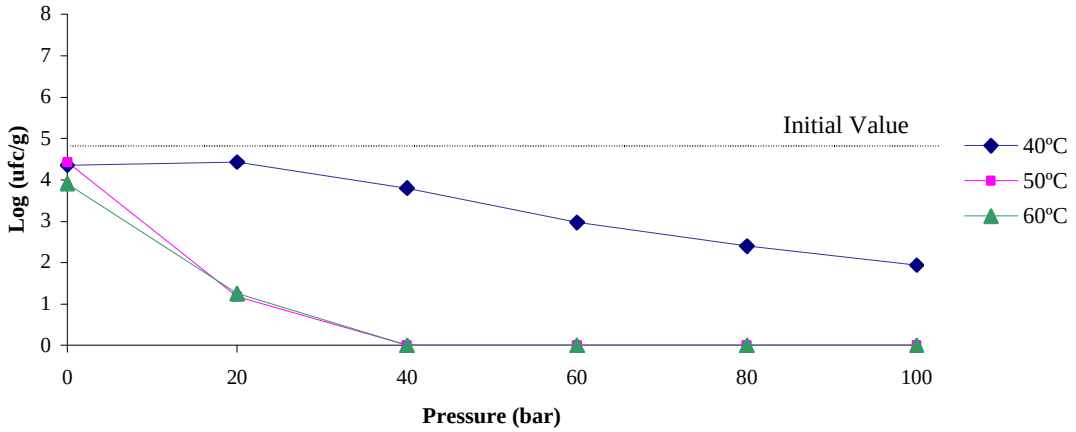

Figure 1.- Inactivation of *Penicillium* sp. at different temperatures and pressure treatments.

Figure 2 shows results for rice samples inoculated with *Escherichia coli*. In this case, temperatures near 40ºC do not produce any antimicrobial effect. Indeed at temperatures of 40ºC and 50ºC, *Escherichia coli* population decrease by pressure effect is much lower than at 60ºC. On the other hand, at 60ºC, pressure range of 20 bar, will produce a decrease in Enterobacteria population.

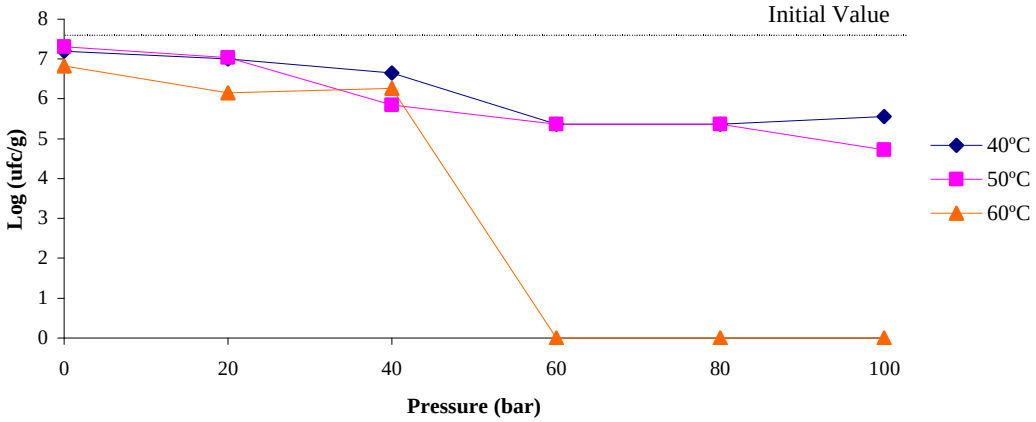

Figure 2.- Inactivation of Enterobacteria at different temperatures and pressure treatments.

For entomological control, the efficacy of the treatment on adults and eggs of the two species is established from the results obtained and showed in Table 1 and 2.

T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h
20	1	5	0	283	40	1	5	6	56	60	1	5	4	175
			2	171				4	238				4	248
			0	189				0	138				0	262
		30	2	396			30	4	259			30	16	39
			6	224				8	125				2	150
			0	152				0	144				13	89
		60	4	256		25	5	100	18	Control			0	103
			8	248				100	18				0	250
			0	186				100	21				0	262
	25	5	100	111	Control			0	184				0	194
			100	143				0	292				0	163
			100	41				0	108				0	279
Control			0	180				0	123				0	93
			0	194				0	110				0	195
			0	277				0	177				0	157
Other combinations of the parameters tested					100% mortality for adult and eggs									

(% M= % adult mortality; nº h= survival eggs)

Table 1.- Results of the preliminary tests to *S. oryzae.*

T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h
			4	46				0	41				0	73
		5	8	27			5	0	27			5	0	72
			6	36				1	76				0	94
			0	38				0	30				0	12
	1	30	18	34		1	30	4	34		1	30	0	64
			4	47				2	173				0	1
			0	3				0	60				100	1
		60	6	34			60	34	59			60	100	0
			0	68				28	69				100	0
			100	12				100	9				100	2
		30	100	9			5	100	13			30	100	5
	25		100	0				100	0		25		100	0
			100	4				100	1				100	2
		60	100	6		25	30	100	28			60	100	5
			100	0				100	0				100	0
			100	10				100	1				100	1
		5	100	7			60	100	0		60	30	100	1
20			100	0	40			100	0	60			100	0
			100	5				100	0				100	5
	60	30	100	3			5	100	0		100	5	100	1
			100	0				100	0				100	0
			100	1				100	0				100	0
		60	100	7		60	30	100	33			30	100	0
			100	0				100	0				100	0
			100	9				100	3				0	52
		5	100	0			60	100	3				0	30
			100	0				100	0				0	41
	100		100	24				100	9				0	61
		30	100	2		100	30	100	8		Control		0	11
			100	0				100	0				0	30
			0	11				0	17				0	57
	Control		0	38		Control		0	40				0	17
			0	24				0	28				0	37
			0	17				0	106				0	60
Other combinations of the parameters tested					100% mortality for adults and eggs									

(% M= % adult mortality; nº h= survival eggs)

Table 2.- Results of the tests to *O. surinamensis*

The treatment is effective for adults of *Sitophilus oryzae* and *Oryzaephilus surinamensis* at 60ºC, 1 bar and 60 minutes, as well as 25 bar of pressure and 5 minutes for the three temperatures tested. At 20ºC and 40º C, 25 bar and 30 minutes, 100% mortality of adults and eggs of *S. oryzae* was obtained as well as at 60ºC, 1 bar and 60 minutes.

CONCLUSION

In this work, effect of CO_2 under pressure as disinfection and disinsection process on contaminated stored rice by different microorganims and insects was studied. The experiments confirm that treatment applied with CO_2 is effective depending on operative conditions, and microorganims and pest type. Moreover a synergist effect is observed between pressure and temperature.

The results obtained show that is possible to achieve 100% mortality in insects (adults and eggs) without loss of product quality, while the effectiveness on microorganism inactivation is less successful as it is required higher conditions.

Acknowledgement

This work was funded through grant Project 1FD97-0996-CO2, from the Comisión Interministerial de Ciencia y Tecnología (CICYT). Their contribution is greatly appreciated.

REFERENCES :

[1] JUNG-KUE S. and YU-RYANG P. Journal of Food Science. Vol. 62, Nº 1, **1997**, p. 163.

[2] BALLESTRA P., ABREU DA SILVA A. and CUQ J.L. Journal of Food Science. Vol. 61, Nº 4, **1996**, p. 829.

[3] ISENSCHMID A., MARISON I.W., VON STOCKAR U. Journal of Biotechnology. Vol. 39, **1995**, p. 229.

[4] ISHIKAWA H., SHIMODA M., YONEKURA A. and OSAJIMA Y. J. Agric. Food Chem. Vol. 44, **1996**, p. 2646.

[5] LIN H., YANG Z. and CHEN L. Biotechnology. **1992**, p. 458.

[6] KAMIHIRA M., TANIGUCHI M., KOBAYASHI T. Agriculture Biologycal and Chemistry. Vol. 51(2), **1987**, p. 407.

[7] STAHL, E., RAU, G. and ADOLPHI, H. Pharm. Ind., Vol. 47, **1985**, p. 528.

[8] STAHL, E. and RAU, G. Anz. Schädlingskde., Pflanzenschutz, Umweltschutz. Vol. 58, **1985**, p. 133.

[9] ULRICHS, C., REICHMUTH, C. and RASSMANN, W. Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products. **1996**, p. 335.

[10] REICHMUTH, C. and WOHLGEMUTH, R. Proceedings of the 6th International Working Conference on Stored-product Protection. **1994**, p. 163.

[11] PROZELL, S., REICHMUTH, C., ZIEGLEDER, G., SCHARTMANN, B., MATISSEK, R., KRAUS, J., GERARD, D.and ROGG, S. Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products. **1996**, p. 325.

[12] MIRA, B., BLASCO, M., SUBIRATS, S., BERNA, A. Journal of Supercritical Fluids, Vol. 9, **1996**, p. 238.