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Industrial Application of Carbon Dioxide and High Pressure as a Method to Perform Pest Control on Foodstuff Raw Materials

Leonardo Serrani[1*], Luciano Süss[2], Alessio Miliani[3], Guglielmo Cassani[4]

[1]*Fertitecnica Colfiorito S.R.L, via Adriatica 1, 06034, Colfiorito di Foligno (PG), Italy*
[2]*Università degli Studi di Milano, via G. Celoria 2, 20133, Milano (MI), Italy*
[3]*Fertitecnica Colfiorito S.R.L, via Adriatica 1, 06034, Colfiorito di Foligno (PG), Italy*
[4]*Agroblu S.R.L, via San Bernardo 35, 20017, Rho (MI), Italy*

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[*]**Corresponding author:** Leonardo Serrani, Fertitecnica Colfiorito S.R.L, via adriatica 1, 06034, Colfiorito di Foligno (PG), Italy. Tel: +39 366 634 12 37; Email: info@tenutamattioni.it

Abstract

The current request for the reduction of chemical inputs in agri-food processes has led to rethink the disinfestation methods of raw materials. The need for rapid turnover in the industrial warehouses determines the request of disinfestation processes capable to shortening the execution time. This paper illustrates the mortality on eight species of coleoptera and lepidoptera due to a treatment with carbon dioxide in an industrial autoclave, pressurized at 1.8 MPa, for 90 minutes. In the case of *Callosobruchus maculatus* (F.), 120 minutes of treatment proved necessary. The method tested allows to obtain the total mortality of the tested species.

Keywords: Carbon Dioxide; High Pressure; Industrial Autoclave; Insects of Storage Products; Seed Weevils

Introduction

There are growing consumers, retailers and public institutions requests to reduce or have no presence of insecticidal residues in food, these needs ask to rethink the post-harvest pest management programs, these necessities are even stronger in the organic food branch where synthetic pesticides are banned.

To go forward with chemicals (e.g. phosphine or sulfuryl fluoride) the alternative treatments searched should provide same or better efficacy and application times besides an easy performing of the technique.

The possibility of obtaining the eradication of seed weevils and insects of storage products in a short time, without pesticides, has long been investigated **[1-9]**.

The published data mainly refer to laboratory experiments, using insects bred with specific food substrates. Riudiavets et al. **[7]** report of a wide experimentation carried out on eight species of insects in the various stages of development, as well as on one species of mite, with treatments at 1.5 and 2.0 MPa. It was thus possible to highlight that a 60 minutes application, with carbon dioxide, at 2.0 MPa determines the total mortality even on the eggs of *Liposcelis bostrychophila* Badonnel and *Lasioderma serricorne* (F.); overall, the most susceptible species were *Oryzaephilus surinamensis* (L.), *Ephestia kuehniella* (Zeller) and *Tyrophagus putrescentiae* (Schrk.).

Starting from these data, Süss et al. **[10]** reported the results obtained using an experimental autoclave capable of reproducing the same process conditions of an industrial plant on a small scale. Six species of Coleoptera and two species of Lepidoptera were used, enclosed in small packing, of material corresponding to those generally used in commerce. It was therefore observed that a treatment at 2.0 MPa per 90 minutes was necessary to obtain complete mortality of *Bruchus lentis* Frölich, while 120 minutes treatment was required in the case of *Callosobruchus maculatus* (F.).

For all the other species used (i.e.: *Rhyzopertha dominica* (F.); *Stegobium paniceum* (L.); *Oryzaephilus surinamensis* (L.); *Tribolium confusum* Jacquelin du Val; *Plodia interpunctella* (Hübner); *Corcyra cephalonica* (Stainton) was confirmed that a treatment at 2.0 MPa, prolonged for 60 minutes, determines total mortality.

Following this knowledge, the present study aims to investigate a real-life working scenario: a real scale autoclave installed in a food industry that need to perform pest-control on various foodstuff coming from different geographical areas of the world.

Material and Methods

Autoclave Technical Specifications

The industrial autoclave **(Figure 1)** has the following technical specifications:

- Design pressure is 2.7 MPa; maximum operating pressure is 2.5 MPa; body diameter is 2400 mm; empty weight is 28500 kg; internal volume is 57900 l; operating temperatures vary in a range between 1-50 °C;
- The carbon dioxide load curve has increments of 0.1 MPa/min^{-1}, this number was found with tests focused on the effects of high pressure on the mechanical properties of the raw materials commercialized at Fertitecnica Colfiorito S.R.L., the target is to avoid abnormal splitting and cracking of the products after the treatment.
- The standard treatment was 1.8 MPa continued for 90 minutes. In the case of ascertained presence of *Callosobruchus maculatus* (F.), based on the already known data, the treatment was lasted 120 minutes.
- The carbon dioxide for the treatment is validated for food use (E290). In this specific case, is used a carbon dioxide derived from natural subsoil deposits in central-east Tuscany, also used in the soft drink industry.
- The expulsion of carbon dioxide follows the same curve adopted for the load 0.1 MPa/min^{-1}

Figure 1: Industrial autoclave seen from outside and inside.

BioTest Used

The term biotest refers to containers of small size and variable shape according to the different needs, in which pests tested are placed with the specific food substrate. Biotests are used to verify the effectiveness of any pesticide treatment **[11]**.

The species tested were: *Rhyzopertha dominica* (F.); *Stegobium paniceum* (L.); *Oryzaephilus surinamensis* (L.); *Tribolium confusum* Jacquelin du Val; *Plodia interpunctella* (Hübner); *Corcyra cephalonica* (Stainton); *Bruchus lentis* Frölich; *Callosobruchus maculatus* (F.).

The species coming from the Laboratory of Applied Entomology AGROBLU located in Rho, Italy. Insects were reared in a climatic chamber at 25 °C, with 70% relative humidity. All the insects are in mixed population (eggs, larvae, pupae, adults) but in the case of *Bruchus lentis* Frölich were used only lentils with not less than 2 eggs on each seed. In the case of *Callosobruchus maculatus*

(F.) were used Cannellini beans with not less than 10 eggs per bean. A similar number of untreated Biotests were held in the AGROBLU Applied Entomology Laboratory, to check for any natural mortality.

Testing Operating Procedure

The procedure is similar to what was described in Süss et al. **[10]**. A first phase the autoclave is pressurized to 0.2 MPa in 2-3 minutes, with the charge of carbon dioxide performed from the bottom of the autoclave, upon which is depressurized, from drain valves located at the top of the autoclave, at 0.15 MPa to eliminate oxygen and other atmospheric gases; the operation is allowed by the molecular mass of carbon dioxide which is the heaviest atmospheric gas and naturally tends to stratify at the bottom in the presence of the other gases that make up the air and by the pressurization (from the bottom) and depressurization (from the top) techniques adopted in the design of the autoclave; after this operation the pressure can be increased up the treatment operating pressure with a gradient of 0.1 MPa/min^{-1}. As soon the time of the treatment expired, the pressure is released with the same gradient. The duration of the treatment was approximately 90 minutes.

Products Generally Introduced Into the Autoclave

Various types of beans are industrially processed in the autoclave (from the smallest ones, such as Mung beans, to the largest ones, such as Corona) as well as Quinoa seeds. Pulses flour, powdered spices (curry and turmeric), oilseeds (such as flax, pumpkin, hulled hemp, sunflower, soybean), lentils, dried peas, various hulled or whole grains (spelt, hulled millet), corn, whole or broken fava beans. All the products treated are therefore raw materials that can be attacked by pests belonging to foodstuffs.

Packaging

Considering the large number of products marketed, different packages and materials were used. However, the most widespread are made by polypropylene raffia fabric certified for use with food products.

Trial methods

The experimental design includes three replicates for every species for each treatment, carbon dioxide treated (1.8 MPa, 90 minutes) vs. untreated control, for a total of forty-eight biotests. The tests were carried out using a polypropylene raffia big bag, after having fully loaded the autoclave. To get closer to a real operating situation the trial was carried out with the biotests containing *Plodia interpunctella* (Indian Meal Moth) in mixed populations placed on top of the big bags in the center of the autoclave; all the other bio-tests, containing the various species and stages of development mentioned above, were placed in the big-bags covered by raw material, and carefully closed. To uniform the treatment all the big-bags were filled with the same raw material: lentils.

Results and Discussion

At the end of the treatment all the biotests were taken back to the laboratory and maintained at 25 °C, 60-65% relative humidity for 15-20 days, to verify the results obtained. Total mortality of all the species considered, including all eggs, was found in all stages of development (Except in the case of *Callosobruchus maculatus* (F.), as previously verified and reported). Consequently, while a standard treatment of 1.8 MPa, prolonged for 90 minutes, is certainly effective, in the case of ascertained presence of *Callosobruchus maculatus* (F.) the treatment itself must be of 120 minutes to obtain 100% of the mortality of the insect. All insects in the untreated control bio-tests developed normally.

The total effectiveness of the method should be attributed to carbon dioxide and its action on respiratory enzymes at concentrations above 20% **[12]**.

The rapid efficacy times of the process should have been achieved thanks to the applied pressure, which helped the properties of carbon dioxide, leading to an environment totally saturated with carbon dioxide. The high pressure, moreover, should have ensured a better control of the eggs by performing a mechanical as well as toxic action.

Considering that several papers report that the use of carbon dioxide and high pressure is also effective on microorganisms sometimes present on cereals **[13, 14]** it will be necessary to investigate that this positive effect can also be found in the case of the use of an industrial autoclave, with the application times and the carbon dioxide concentration reported in this paper. It should be noted that the application of this technique leads to a greater timeliness in the execution of the industrials processes, with the possibility to increase the warehouse turn-over of foodstuff raw-materials, reducing the time due to supply-chain operations and providing to the final customers packaged products with greater freshness of the raw-materials contained. Finally, it should be noted that this method ensuring eradication of insects bring to strong reduction in packages complaints, leading to improve the reliability of the company towards the retail companies and the consumers.

Conclusion

In the case of the practical experience that has been reported, it is therefore possible to confirm that the use of carbon dioxide and high pressure ensures in a short time the total mortality of many species of insects infesting foodstuff raw-materials, even if the same insects are present in the packaging. Even in the presence of more resistant species such as *Callosobruchus maculatus* (F.) the time required for disinfestation is however short.

Author Contributions

Name	Contribution
Leonardo Serrani	Substantial contributions to the acquisition of data for the work and drafting the work.
Luciano Süss	Substantial contributions to the design of the work, interpretation of data for the work, drafting the work and revising it critically for important intellectual content.
Alessio Miliani	Substantial contributions to the conception of the work and final approval of the version to be published.
Guglielmo Cassani	Substantial contributions to the analysis of data for the work and drafting the work.

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