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Toxicity Effects of High Carbon Dioxide Modified Atmospheres in Combination with Sulphur Dioxide on the Rice Weevil *Sitophilus oryzae*

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Abstract: Control of insects in processed food by means of modified atmospheres (MA) is sometimes a relatively long treatment to accommodate the requirements for marketing in the food industry. Therefore, it is necessary to find alternative methods or additives that permit the reduction the length of the treatments and to achieve a high control efficacy. The objective of this laboratory study was to evaluate the feasibility of combining high carbon dioxide (CO_2) MA and low concentrations of a food additive, sulphur dioxide (E-220) (SO_2), to control adults and eggs of the rice weevil *Sitophilus oryzae*. The combination of two different CO_2 concentrations (70% and 95%) and four SO_2 concentrations (35 ppm, 50 ppm, 150 ppm and 3%) were tested for a range of exposures times. The addition of SO_2 to a MA containing 70% CO_2 significantly increased the mortality of both eggs and adults of *S. oryzae*. In comparison, when increasing the CO_2 concentration form 70% to 95%, adult mortality and emergence reduction rates of *S. oryzae* were much less influenced by the addition of SO_2. Different SO_2 sorption and desorption curves were observed in flour wheat, rice and almonds. Residual contents of SO_2 after the treatment were low except for the 3% SO_2 concentration treatment. Seven days after the treatment, residual levels in all treatments were very low or undetectable. Therefore, SO_2 could be effective in reducing the exposure times needed to control this pest species with high CO_2 MA.

Key words: modified atmospheres, food additives, carbon dioxide, sulphur dioxide, *Sitophilus oryzae*, pest control, stored products.

Introduction

Modified atmospheres (MA) with a high carbon dioxide (CO_2) content are safe and environmentally friendly pest control methods for raw and manufactured food products[1,2]. The main advantages of using high CO_2 MA are that they are effective for the control of a wide range of pest species, and they can be used for the treatment of different food products without an accumulation of toxic residues after the treatment. However, control of insects in processed food by means of MA is sometimes too long for the marketing requirements of the food industry. Data on the effects of different CO_2 types of treatments and dosages are available for many species and stages of stored product pests under many particular sets of conditions[3,4]. The recommended exposure time to achieve complete pest control at the most appropriate gas concentration may take from several days to weeks, according to CO_2 concentration, developmental stage and species. For example, to control one of the most tolerant species to MA, the rice weevil *Sitophilus oryzae*, the estimated LT_{95} values using 40% to 100% CO_2 MA range between 1 and 4 days for adults and between 3 and more than 5 days for eggs[5]. However, 12 days at 90% CO_2 and more than 12 days at 50% CO_2 are needed to achieve full mortality for eggs and pupae of this species[6].

Since MA with high concentrations of CO_2 causes permanent opening of the spiracles of insects, a synergistic toxic effect of CO_2 when combined with other compounds has been sought[7]. For example, adding CO_2 to certain fumigants such as acrylonitrile, methyl bromide, phosphine, and hydrogen cyanide increases their toxicity and permits the reduction of exposure times[8].

Sulphur dioxide (SO_2) is a gas accepted as a food additive (E–220). Although it has a large history as a food preservative due to its antimicrobial properties in a range of food products and beverages, it has also been used for the control of some insect pest of grapes during storage[9,10,11]. Official dosages approved for the treatment of food products range from 50ppm in

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cereal by-products, to 500ppm in nuts and 2000ppm in dried fruits.

The objective of this paper is to present preliminary results of on-going research on the evaluation of the effectiveness of combining high CO_2 MA with low concentrations of SO_2, for the control of the rice weevil *S. oryzae*. A second objective was to determine the residual SO_2 levels in samples of rice, flour and almonds treated with this gas mixture.

Materials and Method

Insect mortality

Sitophilus oryzae used for the experiments came from colonies maintained on brown rice at IRTA (Cabrils, Barcelona). Experiments were conducted with adults and eggs. All laboratory studies were conducted in a climatic chamber at 25 ± 1℃ and 70% ± 10% RH.

The experimental arenas were ventilated plastic cages (7.5 cm diameter and 3 cm high) containing 50g of brown rice. The sides of the arenas were painted with *Fluon* in order to avoid the escape of insects during the treatment.

Fifty adults were added to arenas. For obtaining eggs, adult weevils (1280 adults/kg of brown rice) were added and allowed to oviposit for 7 days. Afterwards, adults were removed with a 2.0 mm mesh sieve and rice with eggs was used for the experiments.

Two different experiments were carried out:

1. To evaluate the effectiveness of two high CO_2 modified atmospheres, 70% CO_2 and 95% CO_2, combined with SO_2 (0%, 35 ppm, 50 ppm, 150ppm and 3%) against adults and eggs of *S. oryzae*.

2. To evaluate the mortality responses of *S. oryzae* adults exposed to a modified atmosphere of 70% CO_2 with and without the addition of 35 ppm of SO_2 for a range of exposure times.

Insects were exposed to each gas mixture in sealed glass desiccators (200 mm diameter). Three cages of each stage were exposed in the same desiccators and five replications were made for each gas mixture and exposure time according to each experiment.

Gas mixtures were previously prepared in cylinders and gas concentration verified by the company that made up the gases mixtures (Carburos Met licos S. A.). Modified atmospheres were balanced with N_2 and 5% O_2. Gas mixtures from the cylinders were continuously flushed into the desiccators at a pressure of 2 bars until the desired gas concentrations were reached. A gas analyzer (Abiss model TOM 12) was used to check the concentrations of CO_2 and O_2 inside the desiccators during the experiment.

After the treatment, desiccators were opened and cages were kept in a climatic chamber at 25 ± 2℃ of temperature and 70 10% r. h. To evaluate mortality on adults, the number of living adults in each cage was counted after 24h. The percentage of mortality was calculated using the initial number of individuals placed in each cage. Sets of control cages were used to determine the percentage of natural mortality. For eggs, emerging weevil progeny was counted after 9 weeks. The number of living adults in each arena was counted and the percentage of mortality was calculated using the number of individuals emerged in the control cages. Results were subjected to one-way analysis of variance (ANOVA) (SAS System for Windows 8.02. SAS Institute, Cary, NC. 2001). For the second experiment, time-mortality data for each treatment were subjected to Probit analysis by the POLO-PC computer program (LeOra Software Polo Plus 1.0 2002 – 2007) to determine LT_{50}, and their respective 95% confident intervals.

SO_2 sorption and desorption in samples of rice, wheat flour and almonds

The experimental arenas were glass Petri dishes (9 cm diameter and 2 cm high) containing 25 g of three different food products: polished rice, wheat flour, and almonds. Three different gas mixtures were used: 70% CO_2 with 50ppm SO_2, 90% CO_2 with 150ppm SO_2, and 70% CO_2 with 3% SO_2. Gas mixtures were balanced with N_2 with 5% O_2.

Products were exposed to the modified atmosphere in sealed crystal desiccators (200 mm diameter). Three Petri dishes were exposed in the same desiccator to each gas mixture, except in the case of the mixtures containing 3% SO_2 where only one Petri dish was exposed in each desiccator. Four different exposure times were tested: 1, 2, 3 and 7 days. Three desiccators were used for each product and exposure time. Gas mixtures were previously prepared in cylinders and gas concentration verified as in the previous experiments. For the treatment, gas mixtures were continuously flushed through the desiccators for 2 minutes at a pressure of 2 bars. Desiccators where then kept in a climatic chamber at 25 ± 1℃. After the treatment, a gas analyzer (Abiss model TOM 12) was used to check CO_2 and O_2 levels inside desiccators. SO_2 content in the food product was determined by the method of *Monier-Williams*[12]. Analysis were conducted immediately after opening desiccators, and 3 h, 24 h and 7 days after the end of the treatment. Treated samples were held in the laboratory at room temperature. SO_2 levels in untreated control samples were also analyzed in three samples of rice and wheat flour, and six samples of almonds.

Results and Discussion

Insect mortality

When exposed to a MA of 70% CO_2, the mortality rates of *S. oryzae* adults were strongly influenced by the addition of SO_2 (Table 1). Adding SO_2 significantly increased the percentage mortality from 7.8%, without SO_2, to 28% with 35ppm, to 62.7% with 50ppm and to total mortality when combined with 3% SO_2.

When increasing the CO_2 concentration from 70% to 95% and the exposure time from 20 h to 24 h, adult mortality in the treatments without SO_2 increased from 7.8% to 54.4%. However, the addition of 50ppm SO_2 to the 95% CO_2 MA did not increase the mortality when compared to the treatment without SO_2, as had been found with 70% CO_2 (Table 1). Only the addition of 150ppm of SO_2 resulted in a significant increase in mortality compared to the treatment without SO_2.

The mortality of adults exposed to a MA of 70% CO_2 with and without the 35ppm of SO_2 for a range of exposure times are given in Fig. 1. Time-to-death curves show that mortality of adults was higher for the range of 20 h to 48 – h in presence of SO_2, although variability observed was high, mainly after 20 hours exposure. At the LT_{50} level, adults exposed to the combination of SO_2 and CO_2 were more susceptible by approximately 6 h, than adults exposed to CO_2 alone (Table 2).

The reduction of adult emergence was significantly higher when exposed eggs to 70% CO_2 in combination with 35ppm of SO_2 than without SO_2 (Table 3). After 120 hours treatment, the number of emerging adults in each control cage averaged 237. When treated with 70% CO_2 the number of emerging adults was reduced to an average of 6.6 per cage, ranging from 6 to 10 in all cages. Only one adult in one cage emerged when treated with the same CO_2 modified atmosphere in combination with 35ppm of SO_2. No adult emergence was recorded at 70% CO_2 and 3% SO_2.

With the emergence of adults from egg exposure, the same trend was observed as with adult mortality, when increasing the CO_2 concentration form 70% to 95%, the addition of SO_2 did not reduce the emergence of *S. oryzae* in comparison to the non-SO_2 treatment (Table 3). Although nearly a reduction of 10% in emergence was observed in average between the highest SO_2 content (150ppm) and the treatment without SO_2, no significant differences were found among treatments according to the ANOVA.

Analysis of sulfur dioxide residues

The initial residues of SO_2 detected in almonds, wheat flour and rice before the treatments were 3.6 ± 1.85ppm, 23.6 ± 12.80ppm, and 0ppm, respectively.

When treated with a MA of 3% SO_2, different sorption curves were observed for the three food products during the study. Wheat flour reached the highest values (aprox. 3500ppm), and remained more or less stable from the third day of analysis onwards (Fig. 2). SO_2 adsorption of both almonds and rice steadily increased from the first day until the end of the study (to aprox. 2300ppm). After aeration, a high degree of desorption was observed for wheat flour treated with 3% SO_2 (Fig 3). Values dropped from more than 2500ppm just after the treatment to around 400ppm after 7 days ventilation.

When treated with a low SO_2 content (150ppm and 50ppm), initial residual contents of SO_2 after the treatment were low. After 3 h and 24 h ventilation, the residual limits also decreased quickly even to the minimum detection limit of the technique.

In conclusion, the addition of a low concentration of SO_2 (from 35 ppm to 3%) in a MA of 70% CO_2 increased the mortality of *S. oryzae* adults and eggs compared to the mortality obtained with the same MA without SO_2. The highest level of SO_2 tested (3%) had a strong effect on mortality, while concentrations of 150ppm SO_2 or lower had less impact on mortality. In comparison, when increasing the CO_2 concentration form 70% to 95%, adult mortality and the reduction of egg emergence of *S. oryzae were much less influenced by the addition of SO_2*. Residual concentrations of SO_2 in wheat flour were low after the 150ppm and 50ppm SO_2 treatments. After 7 days aeration residual levels in all treatments were low or undetectable.

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Table 1. Total mortality (mean ± SEM) of *S. oryzae* adults exposed to 70% and 95% CO_2 (+5% O_2) alone or in combination with different SO_2 concentrations (35ppm, 50ppm, 150ppm and 3%) during 20 h and 24 h, respectively

Content of SO_2	Mortality (%) 70% CO_2	Mortality (%) 95% CO_2
0ppm	7.8 ± 1.22 d	54.4 ± 3.71 b
35ppm	28.1 ± 5.93 c	–
50ppm	62.7 ± 11.35 b	52.0 ± 3.13 b
150ppm	–	66.5 ± 3.04 a
3%	100 ± 0 a	–

Means followed by the same letter in a given column indicate no significant difference between treatments (Tukey, n = 5, a = 0.05)

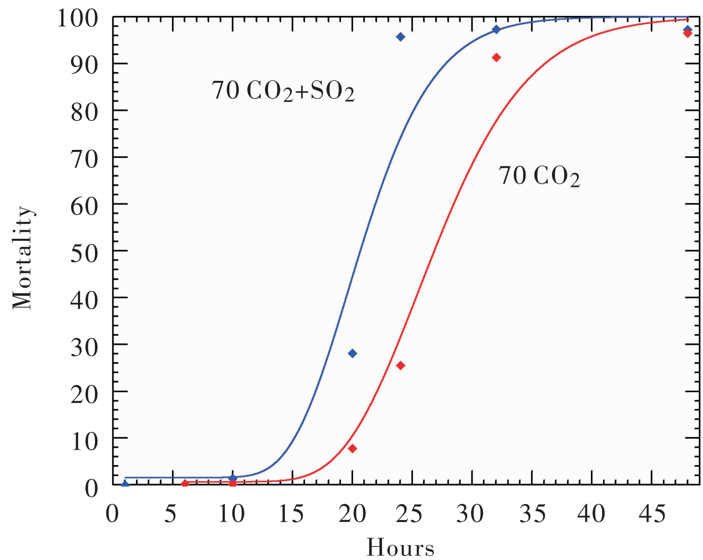

Fig. 1 Time-to-death curves for 70% CO_2 (70 CO_2), and 70% CO_2 and 35 ppm SO_2 (70 CO_2 + SO_2) applied to adults of *S. oryzae*.

Table 2. Mortality responses (LT_{50}) of *S. oryzae* adults to 70% CO_2 (+5% O_2) and 70% CO_2 and 35 ppm SO_2 (+5% O_2) resulting from laboratory fumigations at 25℃

Treatment	χ^2 (d. f.)	Slope ± SE	LT50 (hours)[a]
70% CO_2	805.93 (88)	10.71 ±0.33	26.8 (25.7 – 28.0)
70% CO_2 + 35ppm SO_2	3202.3 (103)	8.99 ±0.36	20.4 (17.5 – 22.4)

a: numbers in brackets give the 95% confidence range

Table 3. Adult emergence reduction (means ± SEM) of *S. oryzae* eggs exposed to 70% and 95 % CO_2 (+5% O_2) alone or in combination with different SO_2 concentrations (35ppm, 50ppm, 150ppm and 3%) during 5 days and 3 days, respectively.

Content of SO_2	Emergence reduction (%) 70% CO_2	Emergence reduction (%) 95% CO_2
0ppm	97.2 ±0.22 b	56.8 ±3.10 a
35ppm	99.9 ±0.03 a	–

Content of SO_2	Emergence reduction (%) 70% CO_2	Emergence reduction (%) 95% CO_2
50ppm	–	57.2 ± 1.36 a
150ppm	–	64.8 ± 2.87 a
3%	100 ± 0 a	–

Means followed by the same letter in a given column indicate no significant difference between treatments (Tukey, n = 5, α = 0.05)

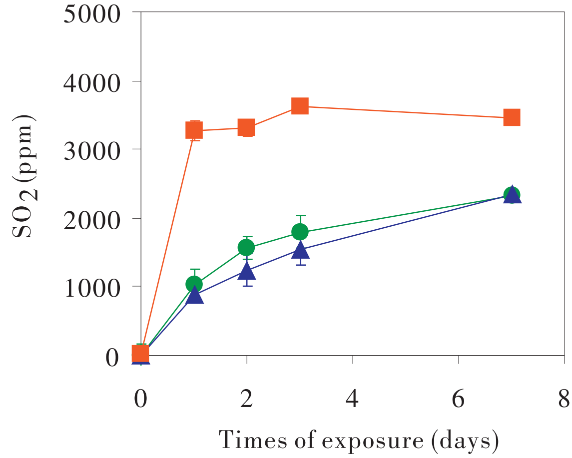

Fig. 2 SO_2 residual content (mean ± SEM; n = 3) in polished rice (circle), almonds (triangle) and wheatflour (square), after fumigations in desiccators (200 mm diameter) with 70% CO_2, 5% O_2 and 3% SO_2 at 25℃.

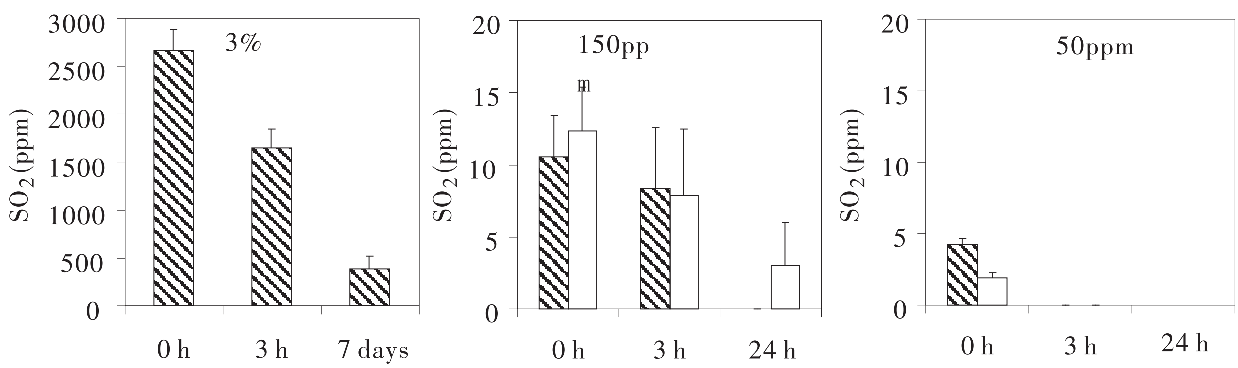

Fig. 3 SO_2 residual content (mean ± SEM; n = 3) from wheat flour fumigated in desiccators (200 mm diameter) with 70% CO_2, 5% O_2 and 3%, 150ppm and 50ppm SO_2 during 24 h (striped bars) and 7 days (white bars) at 25℃. Measurements were made at after the treatment (0 h), and after 3 h, 24 h and 7 days aeration.

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