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Effects of pressurized carbon dioxide on controlling *Sitophilus zeamais* (Coleoptera: Curculionidae) and the quality of milled rice

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ABSTRACT

Carbon dioxide was applied at atmospheric pressure (1 bar) and under higher pressures (4, 6 and 8 bars) using a specially designed pressure chamber with the intention of killing *Sitophilus zeamais* in milled rice. Pressures of 4, 6 and 8 bars shortened the exposure time required to obtain 99% mortality of all life stages of *S. zeamais* from 148 h at atmospheric pressure to 29, 9.0 and 4.8 h respectively. Adults were the most susceptible stage in all the treatments. Pupae were the most tolerant stage at atmospheric and low pressure (4 bars) but at higher pressure (6 and 8 bars) no difference among immature stages was found. After application of carbon dioxide under pressure, a significant decreasing trend ($P < 0.05$) was observed in water absorption, cooked rice hardness, final viscosity, setback and consistency with prolonged exposure time. High pressure produced more distinctive changes than low pressure. However, panelists could not detect any differences between non-treated and treated rice when sensory qualities were evaluated.

1. Introduction

Insect damage is known as one of the most important problems faced during storage of milled rice. In Thailand, the maize weevil *Sitophilus zeamais* Motchulsky is the most abundant of several species found in rice mills. Hidden infestation of eggs inside and outside rice kernels results in significant quality loss when insects hatch and develop inside rice kernels, even inside sealed plastic packages. A common practice with rice is to use chemical fumigants to prevent insect development. There have been a number of attempts to introduce other insect control methods to replace fumigation. Controlled atmosphere systems are not applicable in the developing countries due to the high cost involved in producing sealed enclosures. The initial investment and the operating costs of cooling systems are high, moreover, low temperature inhibits insect growth but may not kill insects (Donahaye, 2000). Heat treatment effectively kills insects but it also causes thermal-stress resulting in grain cracking (Nelson, 1996). Irradiation seems to have a promising future due to its ability to penetrate grain and kill and sterilize insects inside kernels. Although Crawford and Ruff (1996) concluded that food irradiation, up to 10 kGy, is a safe and effective method to improve the integrity and security of the food supply, irradiation is not used widely.

Carbon dioxide could be an alternative to chemical fumigation due to its safety to the environment and its toxicity to insects. Most stored-product insects are killed under atmospheres of <3% O_2 or >40% CO_2 (Bailey, 1965). Carbon dioxide fumigation required several days exposure for different species of insects at different growth stages. Annis (1987) identified that the rice weevil *Sitophilus oryzae* (L.) was amongst the most tolerant stored-product insects to a carbon dioxide rich atmosphere. He proposed that the critical exposure time for *S. oryzae* using >40% carbon dioxide at 25 °C was 15 days, and suggested that this would also be adequate for all other species. However, to obtain high levels of acute mortality for this kind of insect, high concentrations of carbon dioxide were more effective (Annis and Morton, 1997). Pupae were the most tolerant stage of *S. oryzae* and adults were the most susceptible stage. Adults of other insects have also been confirmed to be the most susceptible stage to carbon dioxide, including the granary weevil *Sitophilus granarius* (L.), *S. oryzae* (Lindgren and Vincent, 1970) and the rust red flour beetle *Tribolium castaneum* (Herbst) (AliNiazee, 1971). Even at high concentrations of carbon dioxide, however, longer exposure times are required than are normally required with chemical fumigations such as with methyl bromide or phosphine (Annis and Morton, 1997). To reduce exposure time, Locatelli and Daolio (1993) reported the effective use of

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carbon dioxide gas treatment under reduced pressure. Also Nakakita and Kawashima (1994) introduced the use of carbon dioxide under pressure (5–30 bars) followed by a sudden pressure drop. Complete kill of all life stages of *S. zeamais* was obtained when treated with 30 bars for 5 min. However, from a commercial point of view, application of carbon dioxide gas at high pressure involves use of costly equipment and technical expertise.

As well as causing insect mortality, any treatments applied to stored grains should not have adverse effects on grain quality. Due to carbon dioxide's highly sorptive nature in grain (Cofie-Agblor et al., 1995), many researchers have tried to determine its effect on the quality of grain and grain products stored under this gas (Fleurat-Lessard et al., 1988; Avital et al., 1990; Gras et al., 1990). For rice, there are several reports on better retention when rice was stored under carbon dioxide rather than air in terms of retarding stale flavor, preventing lipid hydrolysis and lipid oxidation and slowing down loss of rice aroma and flavor (Mitsuda et al., 1972; Ory et al., 1980). However, these studies were conducted in long-term storage of rice and thus the effects of high pressure carbon dioxide gas treatment on the quality of rice remain to be determined. The current study was designed to investigate the effects of carbon dioxide at high pressure (up to 8 bars) against all developmental stages of *S. zeamais* (i.e. eggs, larvae, pupae and adults), and determine whether high pressure carbon dioxide treatment affects the physical, cooking and pasting properties of milled rice.

2. Materials and methods

2.1. Preparation of insect samples

The aromatic rice cultivar, Kao Dawk Mali 105 (KDML 105), was obtained from Nakorn Sawan province, Thailand. The paddy was dried and milled using horizontal rubber rollers and vertical abrasive rollers in a double-pass-whitening operation. The moisture content of fully milled rice was $13.4 \pm 0.1\%$ (wb). Adults of *S. zeamais* were collected from rice mills located in different locations in Thailand and cultured using milled rice and coarse rice bran. For tests requiring eggs, samples of milled rice (250 g) were infested with 50 adults 2 days before treatment. Samples of milled rice (250 g) in which adults had laid eggs 18–20 or 25–27 days previously were used for tests on larvae, and pupae respectively. Infested samples were placed individually in plastic cylindrical containers (8 cm diameter $\times$ 9 cm high) each of which was covered with muslin cloth before being subjected to the treatment. Batches of insect samples were prepared in triplicate with an untreated control sample for each insect life stage and each carbon dioxide treatment.

2.2. Carbon dioxide fumigation under pressure

Three stainless steel cylindrical chambers (25 cm diameter $\times$ 50 cm high) were locally designed and fabricated. Sensors measured the pressure inside the chambers using programmable logic control (PLC) connected to solenoid valves through which carbon dioxide gas was supplied into the chambers. The experimental set up is shown in Fig. 1. Boxes of infested rice samples were placed in the chambers and air was removed using a negative pressure pump ($<$1 bar). The chambers were filled with carbon dioxide (100% purity) to the required pre-set pressure (4, 6 and 8 bars). Carbon dioxide at 1 bar represented atmospheric pressure. For adults, the exposure times were 3, 6, 9, 12, 15, 18 and 21 h at 1 bar; 1, 2, 4, 6 and 8 h at 4 bars; and 0.5, 1, 1.5, 2, 2.5 and 3 h at 6 and 8 bars. For the immature stages, the exposure times were 10, 20, 30, 40, 50, 60 and 70 h at 1 bar; 4, 6, 8, 10, 12 and 14 h at 4 bars; and 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6.5 h at 6 and 8 bars. Fumigation treatment was repeated three times by using similar fumigation chambers for each pressure level and each life stage of the insect. Approximately 20 sample boxes containing a total of 5 kg of milled rice, which had been infested with various life stages of the insect, were used in each trial. Mortality of *S. zeamais* adults was assessed 1 day after treatment. Samples containing eggs, larvae and pupae were kept at laboratory temperature (30 ± 2 °C) for the required time for adult emergence. For samples originally containing eggs, larvae and pupae, adult emergence was assessed after 45, 25 and 15 days respectively. Physical, cooking and sensory qualities of the treated milled rice were also evaluated.

2.3. Determination of milled rice quality

The color of milled rice was measured in terms of b value (yellowness) using a color-difference meter (Model TC-P III A, Tokyo Denshoku Co., Ltd, Tokyo, Japan).

Water absorption of rice during cooking was determined by the method of Sabularse et al. (1991) with some modifications. Distilled water (20 mL) and milled rice (2 g) were placed in a test tube, which was covered with a cotton plug and heated at 80 °C in a covered thermostatically-controlled water bath. The milled rice samples were allowed to cook for 30 min, cooled, drained and placed upside down for 1 h and then carefully weighed. The increase in weight was calculated and the water absorption was reported as g of water per g of milled rice.

The hardness of cooked rice was measured after cooking the rice by the calculated water method, using back extrusion tests with an Instron Universal Tester (Model LRX 5 k, Lloyd Instruments, Hampshire, UK) equipped with a 50 kg-capacity compression load cell (Banjong, 1986). The crosshead speed was 50 mm/min. The back extrusion test cell consisted of a stainless steel cylinder with a 15.5 mm inner diameter, 1.55 cm^2 cross-sectional area and was 4.91 cm in length, with a spherical-shaped stainless steel plunger of 12.4 mm diameter. About 4 g cooked rice sample was placed in the cylinder. The plunger was allowed to move downward until it stopped 1 mm above the cell base. Hardness of cooked rice was determined from the maximum extrusion force in terms of kg force (kgf).

Paste viscosity of rice flours was determined with a Rapid Visco Analyser (RVA, Model 4D, Newport Scientific, Australia) using the AACC Approved Method (AACC, 2000). The parameters determined were pasting temperature, peak viscosity, breakdown, final viscosity, setback, and consistency.

For sensory analysis, rice was cooked by the calculated water method. Sensory evaluation was performed by 15 trained panelists. A hedonic scale of 1–9, equating to extreme dislike to extreme like, was applied to determine consumer preference for cooked rice samples. Sensory attributes of cooked rice evaluated were color, odor, texture, taste and overall acceptability.

2.4. Data analysis

Insect mortality data were analyzed with probit analysis using SPSS v. 11.0 software (SPSS, 2001). Data for each life stage at each pressure level were analyzed separately. Inputs to the probit analysis were dose (exposure time, h), number of insects treated and number of killed insects. However as the number of eggs, larvae and pupae of *S. zeamais* were not known, the numbers of treated eggs, larvae and pupae were assumed to be equal to the highest number of insects which emerged from a control sample. Estimated exposure times to achieve 50, 90 and 99% mortality were compared (Finney, 1964) among given life stages and pressure levels. Batches of rice were fumigated separately, three times for each pressure level, and the whole analysis for rice properties was performed in