UNIVERSITI PUTRA MALAYSIA

EFFECTS OF HIGH CARBON DIOXIDE CONCENTRATION ON STORED-PRODUCT INSECTS, AFLATOXIN PRODUCTION AND STORAGE QUALITY CHANGES IN COCOA BEANS

TEGUH WAHYUDI

FSMB 2003 3

EFFECTS OF HIGH CARBON DIOXIDE CONCENTRATION ON STORED-PRODUCT INSECTS, AFLATOXIN PRODUCTION AND STORAGE QUALITY CHANGES IN COCOA BEANS

By

TEGUH WAHYUDI

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of
Doctor of Philosophy

April 2003

Dedicated to my beloved
wife Jully Adriretnani
son Abesadi Wahyuaji
daughter Amrita Sulihkanti Nareswari

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

EFFECTS OF HIGH CARBON DIOXIDE CONCENTRATION ON STORED-PRODUCT INSECTS, AFLATOXIN PRODUCTION AND STORAGE QUALITY CHANGES IN COCOA BEANS

By

TEGUH WAHYUDI

April 2003

Chairman : Professor Jinap Selamat, Ph.D.

Faculty : Food Science and Biotechnology

The potency of CO_2 gas as an alternative fumigant in controlling stored-product insects, its inhibitory characteristics on the growth of molds and mycotoxins production and quality changes of the treated cocoa beans have been evaluated in four consecutive research experiments. These experiments were focused on the use of high CO_2 concentration during fumigation and followed by prolonged storage practices in laboratory hermetic room, under plastic enclosures or high-pressure chamber. As comparison, treatments using methyl bromide, phosphine and normal air atmosphere were also carried out.

Carbon dioxide concentration of 40, 60 and 80% were effective in controlling *Ephestia cautella* and *Araecerus fasciculatus* during 1 to 3 months of exposure, whereas the test insects in the control showed 20-27 survivors for *E. cautella* and 20

survivors for *A. fasciculatus* from the initial number of 30 and 20 insects, respectively. This study also found no significant effect of high CO_2 concentration treatments on cocoa beans quality during the exposure time.

The growth of *A. flavus* and aflatoxins production (except for aflatoxin B_2) was significantly inhibited in the presence of high concentration of CO_2 atmosphere during 1-month storage. Free fatty acids production in the treated cocoa beans was not inhibited by the treatments.

Methyl bromide concentration of 15 g/t or 0.66 g/t of phosphine were required to control *E. cautella, A. fasciculatus* and *Tribolium castaneum.* Levels of methyl bromide residues increased from 0.27 ppm to 1.27, 1.49 and 3.10 ppm with an increase in concentration of methyl bromide from 3 g/t to 5, 10 and 15 g/t, respectively. Phosphine residues increased from 0.07 ppm to 0.12, 0.11, 0.14 and 0.15 ppm with an increase in concentration of phosphine from 0.33 g/t to 0.66, 1, 2 and 3 g/t respectively. Methyl bromide fumigation resulted in methylation of proteins as indicated by the formation of 7-methylguanine in treated cocoa beans.

Complete elimination of *E. cautella* was achieved by using CO_2 under high-pressure at 4 bar within 4 hours of exposure. A longer exposure time of 6 hours was needed to control *A. fasciculatus* and *T. castaneum* at the same pressure. Complete elimination was also achieved at 7 bar within 4 hours of exposure or at 10 bar within 2 hours of exposure for controlling *A. fasciculatus* and *T. castaneum.* In the control treatments using normal air at 10 bar within 4 hours of exposure, the percent RIE (reduction in

emergence) for *E. cautella* was only 3.33% whereas no mortality were found for *T. castaneum* and *A. fasciculatus*. Carbon dioxide under high-pressure treatments exhibited low possibility to effect the quality of the treated cocoa beans.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi syarat keperluan untuk Ijazah Doktor Falsafah

KESAN DARIPADA KEPEKATAN KARBON DIOKSIDA YANG TINGGI TERHADAP SERANGGA GUDANG, PENGHASILAN AFLATOKSIN DAN PERUBAHAN KUALITI BIJI KOKO

By

TEGUH WAHYUDI

April 2003

Pengerusi : Profesor Jinap Selamat, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Kemampuan gas karbon dioksida sebagai suatu pengasap alternatif dalam pengawalan serangga gudang, sifat-sifat penghalangannya pada pertumbuhan fungi dan penghasilan mikotoksin, dan perubahan kualiti biji koko yang dirawat telah dinilai dalam empat eksperimen yang berturutan. Eksperimen-eksperimen tersebut difokuskan pada penggunaan kepekatan karbon dioksida tinggi semasa pengasapan dan praktik penyimpanan yang berikutannya, dalam ruangan hermetik, sungkup plastik dan ruangan tekanan tinggi. Sebagai perbandingan, rawatan menggunakan metil bromida, phosphine, dan udara biasa juga dilaksanakan.

Rawatan menggunakan kepekatan karbon dioksida 40, 60 dan 80% berjaya mencapai kadar kematian sempurna (tiada yang kekal) untuk *Ephestia cautella* dan *Araecerus fasciculatus* pada 1-3 bulan masa pendedahan, sedangkan serangga uji pada kawalan menunjukkan 20-27 ekor serangga *E. cautella* dan 20 ekor serangga *A. fasciculatus* yang kekal hidup. Kajian ini juga menemui bahawa rawatan kepekatan karbon

dioksida yang tinggi tidak memberi kesan yang nyata pada kualiti biji koko ketika masa pendedahan.

Pertumbuhan dari *A. flavus* dan penghasilan aflatoksin (kecuali aflatoksin B_2) dihalang secara nyata dalam atmosfera kepekatan karbon dioksida yang tinggi selama 1 bulan masa penyimpanan. Peenghasilan asid lemak bebas di dalam biji koko yang dirawat tidak dihalang dalam atmosfera tersebut.

Pengasapan menggunakan metil bromida dengan dosej 15 g/tan atau phosphine dengan dosej 0.66 g/tan berjaya mencapai kadar kematian sempurna (100% reduction in emergence) pada pengawalan *E. cautella, A. fasciculatus* dan *Tribolium castaneum.* Saki-baki metil bromida meningkat dari 0.27 ppm ke 1.27, 1.49 dan 3.10 ppm dengan kenaikan dosej metil bromida dari 3 g/tan ke 5, 10, dan 15 g/tan. Saki-baki phosphine meningkat dari 0.07 ppm ke 0.12, 0.11, 0.14, dan 0.15 ppm dengan kenaikan dosej phosphine dari 0.33 g/tan ke 0.66, 1, 2, dan 3 g/tan. Pengasapan menggunakan metil bromida menyebabkan metilasi protein biji koko seperti ditunjukkan oleh pembentukan 7-methylguanine di dalam biji koko yang dirawat.

Pengawalan sempurna untuk *E. cautella* dicapai dalam rawatan menggunakan gas karbon dioksida tekanan tinggi iaitu 4 bar dalam 4 jam masa pendedahan. Masa pendedahan 6 jam diperlukan untuk pengawalan *A. fasciculatus* dan *T. castaneum* pada tekanan yang sama. Pengawalan sempurna juga dicapai pada tekanan 7 bar dalam 4 jam masa pendedahan atau tekanan 10 bar dalam 2 jam masa pendedahan untuk mengawal *T. castaneum* dan *A. fasciculatus*. Perawatan kawalan menggunakan udara biasa pada tekanan 10 bar dalam 4 jam masa pendedahan mengakibatkan kadar

kematian hanya 3.33% RIE (reduction in emergence) untuk *E. cautella*, sedangkan tiada kematian didapati pada *T. castaneum* dan *A. fasciculatus*. Rawatan gas karbon dioksida pada tekanan tinggi mempertunjukkan kemungkinan yang rendah dalam mempengaruhi kualiti biji koko yang dirawat.

ACKNOWLEDGEMENTS

The author wishes to express his indebtedness and gratitude to his supervisors Prof. Dr. Jinap Selamat, Dr. Zaiton Hasan, and Assoc. Prof. Dr. Russly Abd. Rahman for their continued encouragement and support, suggestions, and advices from the beginning to the completion of his doctoral study in Universiti Putra Malaysia (UPM). A special tribute goes to Assoc. Prof. Dr. Azizah Osman and Prof. Dr. Athapol Noomhorm for their thoughtful comments and review on the author's thesis.

Sincere appreciation is expressed to Government of Indonesia and Director of Indonesian Coffee and Cocoa Research Institute (ICCRI) for the financial and other support, to many other acquaintances especially colleagues in Cocoa Laboratory of Faculty of Food Science and Biotechnology and ICCRI for their helpfulness during research work in Malaysia and Indonesia.

Special thanks and appreciations are also due to his wife Jully, his son Abe, and his daughter Amrita for their invaluable, nay indispensable support, love and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

kg	: kilogram
g	: gram
mg	: milligram
μg	: microgram
ng	: nanogram
m	: metre
cm	: centimetre
mm	: millimetre
μm	: micrometre
nm	: nanometre
mL	: millilitre
μL	: microlitre
mM	: millimole
hr	: hour
min	: minute
sec	: second
L	: litre
lb	: pound
M	: molarity
N	: normality
ppb	: part per billion
ppm	: parts per million
t	: metric tonne
v	: volume
bp	: boiling point
RH	: relative humidity
rpm	: rotation per minute
eq	: equivalent

CHAPTER I

GENERAL INTRODUCTION

Protection of already produced grains is critical to human survival. Storage and warehousing have an important role to protect inherent natural nutritional values of grains and provide wholesome food, free of insect damage, live insects and mites, insects' fragments, molds, mycotoxins, and residues of pesticides.

The storage of cocoa beans in the tropics presents two potential problems: the spread of the storage product pest and the development of molds. The common storage insect, which attacks cocoa beans, is *Ephestia cautella.* Apart from the tropical warehouse moth, there are two other pests, *Lasioderma serricorne* (the tobacco beetle) and *Araecerus fasciculatus* (the coffee weevil) (Wood, 1985a). *Aspergillus* and *Penicillium* are the two main types of mold isolated from cocoa beans (Hansen and Keeney, 1970).

Currently, fumigation is still widely used as a rapid method to control storage pest of cocoa beans. Methyl bromide and phosphine are the most commonly used fumigant for treatment prior to storage and export of cocoa beans.

The current concern about the possible withdrawal of methyl bromide as a plant quarantine treatment stems from identification of the fumigant as an ozone depletant in the atmosphere (Catley, 1992). Methyl bromide is being phased out internationally under the Montreal Protocol in 1992. The Montreal Protocol is a treaty signed by over 160 countries to protect the stratospheric ozone layer, which protects the earth