UNIVERSITI PUTRA MALAYSIA

AN INTEGRATED MODELLING APPROACH TO POSTHARVEST CONTROL OF Aspergillus flavus GROWTH AND AFLATOXIN PRODUCTION IN PADDY GRAINS AND RICE

WAEL MOUSA

FSTM 2012 15

AN INTEGRATED MODELLING APPROACH TO POSTHARVEST CONTROL OF *Aspergillus flavus* GROWTH AND AFLATOXIN PRODUCTION IN PADDY GRAINS AND RICE

By

WAEL MOUSA

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

May 2012

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

AN INTEGRATED MODELLING APPROACH TO POSTHARVEST CONTROL OF *Aspergillus flavus* GROWTH AND AFLATOXIN PRODUCTION IN PADDY GRAINS AND RICE

By

WAEL MOUSA

May 2012

Chairman: Farinazleen Mohammed Ghazali, PhD

Faculty: Food Science and Technology

The main aim of the present study was to develop an integrated postharvest strategy for controlling the growth of and aflatoxin production by *Aspergillus flavus* on paddy and rice. Initially, equilibrium moisture content of paddy was studied at 20, 30, 40 and 50°C at relative humidity (RH) between 0.113 and 0.976 using gravimetric technique. The isotherms displayed the general sigmoidal curve Type II and exhibited hysteresis where it was more pronounced at lower temperatures. At fixed RH, the sorption characteristics were temperature-dependent where the sorption capacity of the paddy increased as the temperature was decreased. Among the models assessed for their ability to fit the sorption data, the Oswin equation was the best followed by the third order polynomial, GAB, Smith, Chung-Pfost, and Henderson models. Therefore, the Oswin model was chosen to estimate the amount of water required to rehydrate dried paddy to the desired water activity (a_w) in subsequent studies. Thereafter, the growth of two aflatoxigenic *A. flavus* on paddy and aflatoxin production were studied following a full factorial design with seven a_w levels between 0.82 and 0.99 and seven temperatures between 10 and 43°C. The growth of the fungi, expressed as colony diameter (mm), was measured daily and aflatoxin production was analyzed using isocratic HPLC with a fluorescence detector. The maximum colony growth rates of both isolates were estimated by fitting the primary model of Baranyi to growth data. Three potentially suitable secondary models; Rosso, polynomial, and Davey, were assessed for their ability to describe the radial growth rate as a function of temperature and a_w. Both strains failed to grow at the marginal temperatures (10 and 43°C) regardless of the a_w studied, and at the a_w level of 0.82, regardless of the temperature. Despite that the predictions of all studied models showed good agreement with the observed growth rates, the Davey model proved to be the best predictor of the experimental data. Aflatoxins were detected at a_w between 0.86-0.99 with an optimal a_w of 0.98 and the optimal temperature was in the range of 25-30°C. Then, the effect of a_w (0.82-0.92) and temperature (15-42°C) on the growth and aflatoxin production by *A. flavus* on polished and brown rice was also studied. Four secondary models were used to implicit the combined effects of a_w and temperature on the growth rates. All models were validated using independent experimental data. According to the assessment indices, the performance of the Davey model in describing the experimental data was the highest, followed in decreasing order by the polynomial, Gaussian and Rosso models. The estimated optimal growth temperature was between 30-34°C. Neither growth nor aflatoxins were detected at a_w 0.82 on polished rice while growth and aflatoxins were detected at this a_w between 25-35°C on brown rice. The highest amounts of aflatoxins were formed at the higher a_w values (0.90-0.92) and a temperature of 20°C after 21 days of incubation for both types of rice where the consistency of aflatoxin production within a wider range of a_w values occurred between 25-30°C. The results also showed that brown rice tended to support higher

A. flavus growth and aflatoxin production than the polished rice. Logistic models describing the growth and aflatoxin production boundaries of *A. flavus* were also developed. Experiments were conducted at a_w between 0.80 and 0.99 and temperature between 10 °C and 45°C on rice meal agar (RA) for duration of four weeks. The degree of agreement between the predicted and observed data in terms of concordance was > 97% and > 98% for growth and aflatoxin production, respectively. Probabilities of growth and aflatoxin production at 21 days were almost equal to those at 28 days. The polynomial logistic models that were developed were validated with data obtained from repeated experiments on paddy. The models were successfully able to predict the probabilities with concordance rates of 85.2% and 88.9% for growth and aflatoxin production, respectively, whereas all the misidentified cases were found to be false positive. Then, the potential of modified atmosphere packaging with 20-80% CO_2 (balanced with nitrogen) in controlling the growth and aflatoxin production on paddy at different a_w (0.92-0.98) relative to the control (0% CO_2) was examined using the two above-mentioned fungal isolates. Except at 0.92 a_w, as much as 80% CO_2 failed to inhibit the growth of the fungi completely. However, at all a_w levels studied, the growth parameters as estimated by Baranyi function and aflatoxin production were affected by the increment in CO_2 where growth rate and aflatoxin production were negatively correlated with CO_2 while the lag phase correlated positively with CO_2. At 0.98 a_w, atmosphere enriched with 20% and 80% CO_2 led to at least 59% and 88% reduction in growth and 47% and 97% in aflatoxin production, respectively. At 0.95 a_w, the lag phases of both isolates on average increased by a factor of 1.7-12.0 when the CO_2 levels in the headspace were between 20-80% compared to the control. Finally, the effectiveness of three essential oils (cinnamon, glove and thyme) and three antioxidants [butylated hydroxyanisole (BHA), propyl paraben (PP) and butylated hydroxytoluene (BHT)] on controlling the growth of and aflatoxin formation by the *A. flavus* grown on rice meal agar and paddy grains at different a_w (0.92, 0.95, and 0.98) were evaluated. Two of the antioxidants (BHA and PP) and the three essential oils displayed significant inhibitory effect on the growth and aflatoxin formation on *in vitro* and on the paddy grains. Regardless of a_w of the paddy, the application of essential oils at 500 µg g^{-1} reduced the growth and aflatoxin formation by > 55% and >80%, compared with >75% and > 82% with usage of antioxidants (BHA, PP), respectively. The estimated effective doses 50% (ED_{50}) required to reduce growth and aflatoxin formation on rice meal agar were lower than their counterpart on paddy and those required to inhibit aflatoxin formation were lower those of growth.

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

PENDEKATAN MODEL BERSEPADU KAWALAN LEPAS TUAI PADI DAN BERAS DARI PERTUMBUHAN *Aspergillus flavus* DAN PENGHASILAN AFLATOKSIN

Oleh

WAEL MOUSA

Mei 2012

Pengerusi: Farinazleen Mohammed Ghazali, PhD

Fakulti: Fakulti Sains Dan Teknologi Makanan

Tujuan utama kajian semasa adalah untuk membina strategi lepas tuai bersepadu untuk mengawal pertumbuhan dan penghasilan aflatoksin oleh *Aspergillus flavus* pada padi dan beras. Permulaannya, kandungan kelembapan keseimbangan padi dikaji pada 20, 30, 40 dan 50 °C pada kelembapan relatif (RH) di antara 0.113 dan 0.976 menggunakan teknik gravimetrik. Isoterma menunjukkan lengkungan sigmoidal Jenis II am dan mempamerkan histerisis di mana ia adalah lebih ketara pada suhu lebih rendah. Pada RH tetap, ciri penyerapan adalah suhu-kebergantungan di mana kapasiti penyerapan padi meningkat apabila suhu menurun. Antara model-model yang dinilai untuk keupayaan mereka yang sesuai dengan data penyerapan, persamaan Oswin adalah yang terbaik diikuti dengan model-model polinomial tertib ketiga, GAB, Smith, Chung-Pfost dan Henderson. Oleh itu, model Oswin telah dipilih untuk menganggar kandungan air diperlukan untuk menghidrat semula padi kering kepada aktiviti air (a_w) diingini dalam kajian selanjutnya. Berikutnya, pertumbuhan dua aflatoksigenik *A. flavus* pada padi dan penghasilan aflatoksin dikaji mengikut reka bentuk faktorial penuh dengan tujuh tahap a_w di antara 0.82 dan

0.99 dan tujuh suhu di antara 10 dan 43 °C. Pertumbuhan kulat, dinyatakan sebagai diameter koloni (mm), diukur setiap hari dan penghasilan aflatoksin telah dianalisis menggunakan HPLC isokratik dengan pengesan pendafluoran. Kadar pertumbuhan koloni yang maksimum daripada kedua-dua isolat telah dianggarkan dengan menyesuaikan model utama Baranyi kepada data pertumbuhan. Tiga model sekunder berpotensi yang sesuai; Rosso, polinomial dan Davey, telah dinilai keupayaan mereka untuk menerangkan kadar pertumbuhan radial sebagai fungsi suhu dan a_w. Kedua-dua strain gagal untuk tumbuh pada suhu marginal (10 dan 43 °C) tanpa mengira a_w yang dikaji, dan pada tahap a_w 0.82, tanpa mengira suhu. Meskipun ramalan kesemua model yang dikaji menunjukkan persetujuan yang baik dengan kadar pertumbuhan yang diperhatikan, model Davey terbukti sebagai peramal terbaik untuk data eksperimentasi. Aflatoksin telah dikesan pada a_w di antara 0.86-0.99 dengan a_w optimal pada 0.98 dan suhu optimal dalam lingkungan 25-30 °C. Kemudian, kesan a_w (0.82-0.92) dan suhu (15-42 °C) pertumbuhan dan penghasilan aflatoksin oleh *A. flavus* pada beras putih dan beras perang juga dikaji. Empat model sekunder telah digunakan secara mutlak untuk kesan gabungan a_w dan suhu terhadap kadar pertumbuhan. Kesemua model telah disahihkan menggunakan data eksperimentasi bebas. Berpandukan kepada indeks penilaian, perlaksanaan mmodel Davey dalam menghuraikan data eksperimentasi adalah yang tertinggi, diikuti dalam susunan menurun oleh model-model polinomial, Gaussian dan Rosso. Anggaran suhu pertumbuhan optima adalah di antara 30-34 °C. Tiada pertumbuhan mahupun aflatoksin telah dikesan pada a_w 0.82 pada beras putih manakala pertumbuhan dan aflatoksin telah dikesan pada a_w di antara 25-35 °C pada beras perang. Jumlah aflatoksin yang tertinggi telah dibentuk pada nilai a_w (0.90-0.92) yang lebih tinggi dan suhu 20 °C selepas 21 hari inkubasi untuk kedua-dua jenis beras di mana penghasilan aflatoksin secara konsisten di dalam julat nilai a_w yang lebih luas berlaku di antara 25-30 °C. Keputusan juga menunjukkan bahawa beras perang cenderung untuk menyokong pertumbuhan *A. flavus* dan penghasilan aflatoksin yang lebih tinggi berbanding beras putih. Model logistik yang menghuraikan sempadan pertumbuhan dan penghasilan aflatoksin *A. flavus* juga telah dibangunkan. Eksperimen telah dijalankan pada a_w di antara 0.80 dan 0.99 dan suhu di antara 10 °C dan 45 °C pada agar tepung beras (RA) untuk jangka masa empat minggu. Darjah persetujuan di antara data ramalan dan pemerhatian dalam terma kesejajaran adalah > 97% dan > 98% untuk untuk pertumbuhan dan penghasilan aflatoksin, masing-masing. Kebarangkalian pertumbuhan dan penghasilan aflatoksin pada 21 hari adalah hampir sama dengan kebarangkalian pada 28 hari. Model-model logistik polinomial yang telah dibangunkan telah disahihkan dengan data yang diperolehi daripada eksperimen ulangan terhadap padi. Model-model tersebut dengan berjayanya mampu meramal kebarangkalian dengan kadar kesejajaran 85.2% dan 88.9% untuk pertumbuhan dan penghasilan aflatoksin, masing-masing. Manakala kesemua kes salah pengesanan telah didapati sebagai positif palsu. Kemudian, potensi pembungkusan atmosfera terubah suai dengan 20-80% CO_2 (diseimbangkan dengan nitrogen) dalam mengawal pertumbuhan dan penghasilan aflatoksin terhadap padi pada a_w (0.92-0.98) yang berbeza relatif kepada kawalan (0% CO_2) telah diselidik menggunakan dua isolat kulat yang dinyatakan di atas. Kecuali pada a_w 0.92, CO_2 sebanyak 80% gagal untuk merencat pertumbuhan kulat sepenuhnya. Bagaimanapun, pada kesemua tahap a_w yang dikaji, parameter-parameter pertumbuhan seperti yang dianggar dengan fungsi Baranyi dan penghasilan aflatoksin telah dipengaruhi dengan peningkatan dalam CO_2 di mana kadar pertumbuhan dan penghasilan aflatoksin adalah berhubung kait secara negatif dengan CO_2 manakala fasa lag berhubung kait secara positif dengan CO_2. Pada a_w 0.98, atmosfera yang diperkaya dengan 20% dan 80% CO_2 membawa kepada sekurang-kurangnya 59% dan 88% pengurangan dalam pertumbuhan dan 47% dan 97% dalam penghasilan aflatoksin, masing-masing. Pada a_w 0.95, fasa-fasa lag kedua-dua isolat secara purata meningkat dengan faktor 1.7-12.0 apabila tahap CO_2 di dalam ruang kepala adalah di antara 20-80% berbanding kawalan. Akhirnya, keberkesanan tiga minyak esen (kayu manis, bunga cengkih dan thyme) dan tiga antioksidan [butylated hidroksianisol (BHA), propyl paraben (PP) dan butylated hidroksitoluena (BHT)] dalam mengawal pertumbuhan dan pembentukan aflatoksin oleh *A. flavus* yang dibiakkan pada agar tepung beras dan bijirin padi pada a_w (0.92, 0.95 dan 0.98) yang berbeza telah dinilai. Dua daripada antioksidan (BHA dan PP) dan ketiga-tiga minyak esen mempamerkan kesan perencatan yang signifikan terhadap pertumbuhan dan pembentukan aflatoksin pada in vitro dan pada bijirin padi. Tanpa mengira a_w pada padi, penggunaan minyak-minyak esen pada 500 $\mu g\ g^{-1}$ mengurangkan pertumbuhan dan pembentukan aflatoksin dengan > 55% dan > 80%, berbanding dengan > 75% dan > 82% dengan pengunaan antioksidan (BHA, PP), masing-masing. Anggaran 50% dos-dos berkesan (ED_{50}) yang diperlukan untuk mengurangkan pertumbuhan dan pembentukan aflatoksin pada agar tepung beras adalah lebih rendah berbanding yang digunakan pada padi dan yang diperlukan untuk merencatkan pertumbuhan aflatoksin adalah lebih rendah berbanding yang diperlukan untuk pertumbuhan.

ACKNOWLEDGEMENTS

"In the Name of Allah, the Most Merciful and the Most Beneficent"

All praise do to allah, Lord of the universe. Only by his grace and mercy this thesis was completed. First and for most I would like to express my profound gratitude to my honourable supervisor Dr. Farinazleen Mohamad Ghazali for her invaluable advice, guidance, encouragement. I would like to wish her unending success in her family and professional lives. I am extremely grateful to my supervisory committee members, Prof. Dr. Jinap Selamat, Pof. Dr. Hasanah Mohd. Ghazali and Prof. Dr. Son Radu for their valuable contribution and suggestions.

I am grateful to my colleagues in the laboratory of food safety and quality; Kabir Umar, Elham Farahany, Sahar Arzandeh, Gisia Daniali, Wendy Lim, Diyana Syamim, Afsaneh Farhadian, Dr. Parvaneh Hajeb for being supportive friends and wish them all the very best for the future. I am indebted to the staff of the Faculty of Food Science and Technology, University Putra Malaysia who assisted me in one way or the other. Special thanks to Norliza Othman for her assistance through my study.

I would like to express my sincere gratitude to my parents, brothers and sisters for their prayers, unending encouragement and continuous moral support. Last but not least, my beloved wife, Myssa Mousa and my dear kids (Abdulrahman and Abdullah) deserve my deepest and heartfelt gratitude for their bearing with me for the many months I spent away from home.

I certify that a Thesis Examination Committee has met on 15 May 2012 to conduct the final examination of Wael A. S. Mousa on his thesis entitled "An Integrated Modelling Approach to Postharvest Control of *Aspergillus flavus* Growth and Aflatoxin Production in Paddy Grains and Rice" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohd Yazid bin Abd Manap, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Fatimah binti Abu Bakar, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Cheah Yoke Kqueen, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

R. Russell M. Paterson, PhD
Professor
Centre of Biological Engineering
University of Minho
Portugal
(External Examiner)

____

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 28 June 2012

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Farinazleen Mohamad Ghazali, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Jinap Selamat, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Hasanah Mohd. Ghazali, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Son Radu, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 28 June 2012

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

WAEL MOUSA
Date: 15 May 2012

TABLE OF CONTENTS