UNIVERSITI PUTRA MALAYSIA

ISOLATION AND MOLECULAR CHARACTERIZATION OF MYCOTOXIGENIC FUNGI FROM PALM KERNEL CAKE AND EFFECTS OF TEMPERATURE ON AFLATOXIN PRODUCTION

SITI MARDHIYAH RAZALI

IPTSM 2016 7

ISOLATION AND MOLECULAR CHARACTERIZATION OF MYCOTOXIGENIC FUNGI FROM PALM KERNEL CAKE AND EFFECTS OF TEMPERATURE ON AFLATOXIN PRODUCTION

By

SITI MARDHIYAH RAZALI

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

June 2016

This thesis is dedicated with deepest love

to my beloved parents, Razali Mat Zin and Ramlah Daud.

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

ISOLATION AND MOLECULAR CHARACTERIZATION OF MYCOTOXIGENIC FUNGI FROM PALM KERNEL CAKE AND EFFECTS OF TEMPERATURE ON AFLATOXIN PRODUCTION

By

SITI MARDHIYAH RAZALI

June 2016

Chair : Assoc. Prof. Nor Ainy Mahyudin, PhD
Faculty: Institute of Tropical Agriculture and Food Security

The widespread contamination of animal feed with mycotoxin is not a new issue worldwide. This toxin is produced by mycotoxigenic fungi as secondary metabolites and it is harmful to human and animal. Apart from economic lost; the mycotoxin can have adverse health effects to humans due to the carcinogenicity, teratogenicity, and mutagenicity potentials of the toxins. Palm Kernel Cake (PKC) is the largest animal feed production in Malaysia. PKC is a by-product of palm kernel oil processing and it has been exported as animal feed. The improper storage facilities for PKC may lead to conditions which enhance the aflatoxigenic fungi growth and thus the production of aflatoxins in PKC.

The purpose of this study is to isolate and characterize toxigenic fungi from PKC, to determine the effect of media and temperatures on the fungal growth, and to study the effect of PKC storage temperatures on aflatoxins production.

For the first objective, characterization of fungi was conducted by using 3 different media culture; Dichloran Rose Bengal Chloramphenicol (DRBC) Agar, Dichloran 18% Glycerol (DG18) Agar and Malt Extract Agar (MEA) from PKC that is stored under 3 different temperatures of 4˚C, 25˚C and 60˚C. Identification of fungi was carried out based on macroscopy and microscopy as well as molecular identification. Incidences of four mycotoxigenic fungi were found from PKC (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicilium citrinum*).

In order to characterize polymorphism of the isolates, RAPD assay was performed by using OPA 3 as the primer. A dendogram was generated by GelCompare 4.2 software. The dendogram tree was clustering based on the amplified segments

grouped the isolates according to their treatments of temperature and media culture. The identification of the isolates are obtained based on the banding pattern from the generated dendogram. The software resulted in constructed dendogram to reveal the percentage of similarities between the typable isolates (*A. fumigatus, A. niger* and *P. citrinum*) within ranged from 20% to 80%.

The effect of storage temperature on the strains enumeration is reported through this work. The distributing strains are influenced by the storage temperature of PKC matrices. The findings clearly viewed that *Aspergillus* species profused at 25^0C PKC storage while it is restricted at low and high temperature.

The validated HPLC method for detection of aflatoxins from spiked PKC with *A. flavus* was greatly evaluated. Acceptability of linearity of this methods was achieved with correlation coefficients of linear range of 0.998 (AFB_1), 1 (AFB_2), 0.9987 (AFG_1) and 0.999 (AFG_2). A good recovery also found within ranged 82.5% to 102.8%. The findings however showed no aflatoxin detected at any of incubated temperature of PKC from day 0 until the day of 28. Therefore, another experiment is conducted to detect the availability of the toxins in the selected isolate of *A. flavus.* As expected, there are no aflatoxins recognized from the strain. This finding suggested that the isolated *A. flavus* species was not associated with aflatoxin producing eventhough it was under the favorable condition for fungi growth. In conclusion, the collected PKC is free with aflatoxigenic strains thus the risk to be contaminated by aflatoxins is low.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGASINGAN DAN PENCIRIAN MOLEKUL KULAT MYCOTOXIGENIC DARI HAMPAS ISIRONG SAWIT DAN KESAN SUHU TERHADAP PENGHASILAN AFLATOKSIN

Oleh

SITI MARDHIYAH RAZALI

Jun 2016

Pengerusi : Prof. Madya Nor Ainy Mahyudin, PhD
Fakulti : Institut Pertanian Tropika dan Sekuriti Makanan

Pencemaran meluas terhadap makanan haiwan dengan mikotoksin bukan lagi isu yang baru di seluruh dunia. Toksin ini dihasilkan oleh kulat yang boleh menghasilkan mycotoxin sebagai metabolit sekunder dan ia adalah berbahaya kepada manusia dan haiwan. Selain ekonomi merosot; mikotoksin boleh menyumbang kepada kesan kesihatan yang buruk kepada manusia kerana potensi kekarsinogenan, teratogen dan mutagen daripada toksin. Pemprosesan Hampas Isirong Sawit (PKC) adalah pengeluaran makanan haiwan yang terbesar di Malaysia. PKC ialah hasil sampingan pemprosesan minyak isirong sawit dan telah dieksport sebagai makanan haiwan. Kemudahan penyimpanan yang tidak wajar bagi PKC boleh membawa kepada keadaan yang meningkatkan pertumbuhan kulat aflatoxigenic dan dengan itu meningkatkan pengeluaran aflatoksin dalam PKC .

Tujuan kajian ini adalah untuk mengasingkan dan mencirikan kulat toxigenic dari PKC , untuk menentukan kesan media dan suhu terhadap pertumbuhan kulat , dan untuk mengkaji kesan PKC suhu penyimpanan pada pengeluaran aflatoksin.

Bagi objektif pertama , pencirian kulat telah dijalankan dengan menggunakan 3 budaya media yang berbeza iaitu Dichloran Rose Bengal Chloramphenicol (DRBC) Agar, Dichloran 18% Glycerol (DG18) Agar and Malt Extract Agar (MEA) dari PKC yang telah disimpan di bawah 3 suhu berbeza iaitu pada 4˚C, 25˚C and 60˚C. Pengenalan kulat telah dijalankan berdasarkan teknik makroscopi dan mikroskopi serta pengenalpastian secara molekular. Insiden empat kulat mycotoxigenic telah didapati daripada PKC iaitu (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus dan Penicilium citrinum*).

Dalam usaha untuk mencirikan polymorphism daripada pencilan-pencilan kulat, RAPD assay telah dijalankan dengan menggunakan OPA 3 sebagai primer. Dendogram telah dijana oleh perisian GelCompare 4.2. Pokok dendogram telah dikelompok berdasarkan segmen pencilan yang dikuatkan dan dikumpulkan mengikut rawatan mereka terhadap suhu dan agar media. Pengenalpastian pencilan diperolehi berdasarkan corak band yang diperolehi dari dendogram yang dihasilkan. Perisian itu menghasilkan dendogram untuk mendedahkan peratusan persamaan antara perincian yang boleh dibaca iaitu (*A. fumigatus*, *A. niger* dan *P. citrinum*) adalah di antara 20% hingga 80%.

Kesan suhu penyimpanan pada penghitungan strain yang juga dilaporkan melalui kerja-kerja ini. Taburan strain adalah dipengaruhi oleh suhu penyimpanan matriks PKC. Hasil kajian jelas dilihat bahawa spesies *Aspergillus* didapati sesuai pada PKC yang disimpan pada suhu 25^0C dalam masa yang sama ia terhad pada suhu rendah dan tinggi.

Kaedah HPLC yang telah disahkan untuk mengesan aflatoksin dari PKC yang telah di hidupkan dengan *A. flavus* telah dinilai dengan berjaya. Linearity yang boleh diterima melalui kaedah ini dicapai dengan pekali korelasi linear adalah 0.998 (AFB_1), 1 (AFB_2), 0.9987 (AFG_1) and 0.999 (AFG_2). Pemulihan yang baik juga ditemui dalam adalah di antara 82.5% hingga 102.8%. Penemuan bagaimanapun tidak menunjukkan sebarang aflatoksin yang dapat dikesan di mana-mana suhu dieram daripada PKC dari hari 0 sehingga hari 28. Oleh itu, eksperimen lain dijalankan untuk mengesan adanya toksin dalam pencilan yang dipilih iaitu *A. flavus*. Seperti yang dijangka, tiada aflatoksin diiktiraf dari strain *A. flavus*. Penemuan ini mencadangkan bahawa pencilan dari spesies *A. flavus* yang tidak berkaitan dengan penghasilan aflatoksin walaupun ia adalah di bawah keadaan yang menggalakkan bagi pertumbuhan kulat. Kesimpulannya, PKC yang telah dikumpulkan didapati bebas dari kulat yang menghasilkan aflatoxins oleh itu ia risiko untuk dicemarkan oleh aflatoxins adalah rendah.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious, The Most Merciful. All praise be to Allah S.W.T., Lord of The Worlds for giving me His love, as well as the strength, courage and perseverance to complete this thesis. May the peace and blessing of Allah be upon Prophet Muhammad S.A.W. This thesis requires a lot of hard work and effort, and it would not be possible to complete this thesis without the contributions of many significant individuals.

I owe huge gratitude to my supervisor, Assoc. Prof. Dr. Nor Ainy Mahyudin, for her valuable knowledge and continuing guidance, expensive motivation and continuing support which helps me complete this study. I could not ask for a better supervisor. I thank to all members in my supervisory committee, Prof. Dr. Jinap Selamat and Prof. Dr. Fatimah Abu Bakar for their encouragement and experience sharing through out my research. I have owed so much Dr. Lee Hai Yen and Dr. Muhammad Zukhrufuz who have shared their struggles and valuable experience in their journey.

I also wish to express my gratitude to the technical staff at Faculty of Food Science and Technology and the Institute of Tropical Agriculture, Universiti Putra Malaysia, for their assistance in carrying out the laboratory work.

To my beloved parents, Mr. Razali Mat Zin and Ms. Ramlah Daud, I sincerely thank you for your unconditional love as well as endless prayers and support me in every way, which serve as a big source of inspiration. I thank my brothers and in laws, Muhammad Ridhuan, Muhammad Nazmi and Ahmad Khairuddin for motivating me to higher achievement. To my lovely sisters, Siti Raihanah and Karimah Nadiah thank you very much for admiring my words and believing me in completing my study. To my little angels, Khayra, Raiqal and Aisyah, all of you are the sushine in my soul.

To my dearest sister , Zayyani, a very special thank you for putting your heart and soul and be my witness seeing me at my worst and best along this arduous journey. I could not find the words to truly convey my deep love and appreciation to you except for a very special thank you. Dear Siti Asiah, thank you for the privilege and honor of being able to call you my best buddy and thank you for being you and for letting me be me. I love you deeply and I always will. I appreciate my supportive friends, Farah Asilah, Muhammad Aden, Noor Jaini, Hafiz, A'isyah, Selvi and for those not mentioned here, they are forever part of my work.

Again, I wish to thank everyone for being there for me throughout my studies – this thesis will be impossible without each and every one of you. Thank you.

I certify that a Thesis Examination Committee has met on 17 June 2016 to conduct the final examination of Siti Mardhiyah Binti Razali on her) thesis entitled "Isolation and Molecular Characterization of Mycotoxigenic Fungi from Palm Kernel Cake and Effects of Temperature on Aflatoxin Production" 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

Abdulkarim Sabo Mohammed, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Anjas Asmara @ Ab. Hadi Samsudin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Norlidah Abdullah, PhD
Professor
University of Malaya
Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 23 August 2016

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Nor Ainy Mahyuddin, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chaiman)

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

_____
BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- the thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by; Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be owned from supervisor and deputy vice –chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature:_____Date: _____

Name and Matric No.: Siti Mardhiyah Binti Razali, GS33688

Declaration by Members of Supervisory Committee

This is confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____	Signature: _____
Name of Chairman of Supervisory Committee: _____	Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACN	Acetonitrile
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AOAC	Association of Analytical Communities
CFU	Colony Forming Unit
CFU/g	Colony Forming Unit per gram
DG18	Dichloran 18% Glycerol
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide
DRBC	Rose Bengal Chloramphenicol
HCL	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
LCMS/MS	Liquid Chromatography Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
M	Molar
MEA	Malt Extract Agar
MeOH	Methanol
mg/kg	Milligram per kilogram
$MgCl_2$	Magnesium Chloride
ml	millliliter
mm	millimeter
ng/g	nanogram per gram
ng/ml	Nanogram per milliliter
OTA	Ochratoxin A
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PHRED	Post column photochemical derivation
PKC	Palm Kernel Cake
ppb	parts per billion
RAPD	Randomly Amplified Polymorphism DNA

rpm	Revolutions per minute
spp.	Species
TE	Tris-EDTA
ug/kg	Microgram/ kilogram
UV	Ultra violet
v/v	Volume per volume
w/v	Weight per volume
µl	microliter
µm	micrometer

CHAPTER 1

INTRODUCTION

Mycotoxins are secondary metabolites produced by mycotoxigenic fungi in conditions that are favourable for fungal growth. These fungi can produce mycotoxins during pre-harvest and post-harvest of agricultural crops. Mycotoxin contamination can also occur in animal feeds, especially when the feeds are not packed properly, resulting in the growth of mycotoxigenic fungi. Mycotoxin contamination of agricultural crops and animal feeds is a serious issue since it has undesirable consequences towards human and animal health. For this reason, a large number of studies have been carried out on the detection of mycotoxins in agricultural crops and animal feeds. Beg et al. (2006) conducted a survey on mycotoxin contamination in samples of yellow maize, soybean meal and wheat bran which are used in the preparation of poultry feeds at a poultry feed production unit in Kuwait. Lee et al. (2010) conducted a study to determine the presence of beauvericin produced by *Fusarium* spp. in animal feeds and the level of the mycotoxin contamination in Korea. Even more alarming, some fungi are able to produce various types of mycotoxins and some mycotoxins are produced by various fungal species. *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. are among the common types of mycotoxigenic fungi. Some of the common mycotoxins found in animal feeds are aflatoxins and fumonisins.

Aflatoxins are mainly produced by *A. flavus* and it is one of the mycotoxins typically found in animal feeds. A wide variety of agricultural products can be contaminated by aflatoxins such as corn, rice, wheat, spices and nuts (Busman et al., 2015). To date, 18 types of aflatoxins have been identified – however, only four of these are of primary concern, namely AFB_1, AFB_2, AFG_1 and AFG_2 (Decastelli et al., 2007). AFB_1 is the most toxic aflatoxin among the above-mentioned aflatoxins and it has been classified under 'Group 1: Carcinogenic to humans' by the International Agency for Research on Cancer (IARC) (IARC, 1993). *A. flavus* is a fungal species commonly associated with aflatoxin production. However, it shall be noted that there are other fungal species which are aflatoxins producers and therefore, there are various sources of aflatoxins production.

Aflatoxins contamination of agricultural crops and animal feeds is a serious issue since aflatoxins contribute to diseases in both humans and animals. A number of researchers have reviewed the effects of mycotoxins on human and animal health over the years. Animal aflatoxicosis is poisoning in animals resulting from the ingestion of aflatoxins in contaminated animal feeds and it is characterized by a reduction in weight gain, anorexia, poor feed utilization, lower growth rate, reduction in egg weight and production, listlessness, susceptibility to microbial and environmental stresses, as well as higher mortality rates (Leeson et al., 1995). The route of exposure to aflatoxins involves the metabolism of aflatoxins contaminated feed ingredients in the cow liver, transmission of the metabolites into milk in the mammary glands of the cow and subsequent excretion (Busman et al., 2015). The

factors which influence the magnitude of toxicity in humans and animals resulting from the ingestion of aflatoxins contaminated foods or feeds include the species, mechanism or modes of action, metabolism, as well as defence mechanisms (Hussein & Brasel, 2001). Aflatoxins can acutely cause liver necrosis, bile duct proliferation, edema, as well as lethargy (Williams et al., 2004; Paterson 2007). The potential carcinogenicity of aflatoxins has led to the establishment of very low tolerances of aflatoxins in foods (including peanuts and related products) by government regulatory agencies. This move is made to prevent the trade of commodities which are contaminated by aflatoxins beyond the tolerance limits (van Egmond and Jonker, 2004).

Malaysia is one of the major producers of palm oil and palm oil products in the world. The increasing demand for palm oil over the years has led to the increase in the production of palm kernel cake (PKC). Lee Oil Mills located in Klang, Malaysia, receives 250 tonnes which is equivalent to eight lorries of palm kernel per day. These palm kernels are used to produce 46 and 54% of palm kernel oil and PKC, respectively. In 2009, Malaysia produced 17.56 million tonnes of palm oil, from which 2.31 million tonnes of PKC were produced on 4.69 million hectares of planted land and it is projected that these values will increase over the years (MPOB, 2009). PKC is produced from ground palm kernels. The palm kernels are pressed several times to produce palm kernel oil and its by-product, which is PKC. PKC can be obtained by means of mechanical extraction (expeller) or solvent extraction. In the latter method, n-hexane is used as the solvent during the extraction process. However, mechanical extraction using a screw press is widely used by palm oil manufacturers in Malaysia because of its low cost. For this reason, PKC is also known as palm kernel expeller.

In general, oil palm fruits contain a number of bioactive compounds and they are high in nutritional value. Hence, it is unsurprising that PKC, which is a by-product of the palm oil milling process, is a rich source of nutrients, particularly fibre and protein. According to Abdeshahian et al. (2010), PKC contains crude fibre and crude protein within a range of 12–18% and 15–18%, respectively. For this reason, PKC is widely used as a nutritious ingredient in animal feeds for farm animals (Awaludin, 2001).

The occurrence of mycotoxins in animal feeds has been studied extensively by researchers in different countries including South Korea, Sweden and Portugal. Mycotoxins contamination of animal feeds has undesirable consequences in the long term since mycotoxins are not only detrimental to human and animal health, but they also lead to significant economic losses due to the low productivity of animal husbandry. The negative impact of mycotoxins contamination includes the condemnation of highly contaminated crops, the implementation of costly preventive measures and mycotoxins screening programmes, reduction in production efficiency, detriment in animal health due to exposure to mycotoxins contaminated feeds, as well as detriment in human health resulting from the ingestion of mycotoxins contaminated foods (Hooft et al. 2011) of animal feeds from manufacturers to vendors and end users may consume a significant amount of time, and this increases

the risk of the animal feeds being contaminated with mycotoxigenic fungi. In addition, there are no dedicated transportation and storage facilities which will prevent mycotoxins contamination in animal feeds and this aggravates the situation even further.

Feeding poultry with aflatoxins contaminated feeds results in high feed conversion ratios, poor weight gain, depressed appetite, lower quantity of meat from the carcass, lower resistance to diseases as well as high mortality rates (Leeson et al., 1995). Raising poultry with aflatoxins contaminated feeds will not only result in lower productivity and inferior product quality for farmers, but it also has adverse effects on consumer health due to the toxic residues present in the meat. Bintvihok et al. (2002) detected the presence of aflatoxins in the liver, muscles and eggs of poultry. Begum et al. (2001) detected higher levels of AFB_1 in the liver compared to kidneys and meat of poultry, which indicates that the liver is the primary site for metabolism of aflatoxins.

Aflatoxins contamination of foods and feeds can occur during pre-harvest and post-harvest stages, and the level of contamination is highly dependent on biotic and abiotic factors since these factors will influence the growth of aflatoxigenic fungi which in turn, results in the production of aflatoxins. Aflatoxins can be produced by aflatoxigenic fungi during the post-harvest stage whenever agricultural crops are harvested during floods and unseasonal rains in the absence of reliable facilities for crop storage. In addition, mechanical, insect or bird damage of grains, drought stresses and excessive rainfall may lead to fungal growth during the pre-harvest stage and thus, the production of aflatoxins in agricultural crops.

At present, PKC is widely used as one of the main ingredients in animal feeds in Malaysia due to its high nutritional value. More importantly, PKC helps in reducing the costs of animal feeds. However, the lack of proper temperature storage transportation and storage facilities for PKC may result in conditions which favour the growth of aflatoxigenic fungi and hence, the production of aflatoxins in PKC. At present, there is lack of studies pertaining to the determination of aflatoxins by aflatoxigenic fungi in Malaysia, which is forms the motivation of this study.

Hence, the aim of this study is to identify the types of fungi present in PKC samples collected from different palm oil mills in Selangor. Since the growth of fungi is influenced by factors such as the growth medium and storage temperature, this study is also focused on investigating the effect of these factors on the growth of fungi in PKC. Three types of culture media (*i.e.* Dichloran Rose Bengal Chloramphenicol (DRBC) Agar, Dichloran 18% Glycerol (DG18) Agar and Malt Extract Agar (MEA)) are used for this purpose and the PKC samples are stored at three different temperatures (4, 25 and 60°C). Finally, this study is focused on determining the presence of aflatoxins in PKC samples spiked with *A. flavus* (which is an aflatoxigenic fungus known to produce the most potent aflatoxins in agricultural crops and animal feeds) stored at different temperatures (4, 25 and 45°C) over a specific number of days up to 28 days.

The objectives of this study are set as follows:

1) to identify fungi isolated from PKC
2) to determine the effect of media and temperatures on the fungal growth
3) to study the effect of PKC storage temperatures on aflatoxins production

REFERENCES

Abarca, M.L., Bragulat, M.R., Castella, G. and Caba˜nes, F.J. (1994). Mycoflora and aflatoxin-producing strains in animal mixed feeds. *Journal of Food Protection*. 57: 256–258

Abdeshahian, P., Samat, N., Hamid, A.A. and Yusoff, W.M.W. (2010). Utilization of palm kernel cake for production of beta-mannanase by Aspergillus niger FTCC 5003 in solid substrate fermentation using an aerated column bioreactor. *Journal of Industrial Microbiology & Biotechnology*, 37: 103–109.

Abdeshahian, P., Samat, N., Hamid, A.A., Mohtar, W. and Yusoff, W. (2011). Solid Substrate Fermentation for Cellulase Production Using Palm Kernel Cake as a Renewable Lignocellulosic Source in Packed-bed Bioreactor, 244: 238–244.

Abdullah, N., Nawawi, A. and Othman, I. (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. *Mycopathologia*. 143: 53–58

Abdul-Raouf, U.M., Hwang, C.A. and Beuchat, L.R. (1994). Comparison of combinations of diluents and media for enumerating *Zygosaccharomyces rouxii* in intermediate water activity foods. *Letters of Applied Microbiol*ogy. 19: 28-31.

Abu Seadah, A.A. and El Shikh, M.E. (2008). RAPD typing of *Aspergillus chevalieri, Aspergillus nidulans, Aspergillus tetrazonus* (quadrilineatus) and their teleomorphs using 5'-d[AACGCGCAAC]-3' and 5'-d[CCCGTCAGCA]-3' primers. *Molecular Biology Reports*. 35: 89–95.

Abu, H.O. and Yeong, S.W. (1999). By-products as animal feedstuffs. In: Singh, G., Lim, K.H., Teo, L., Lee, D.K. (Eds.), Oil Palm and the Environment: A Malaysian Perspective. Malaysian Oil Palm Growers' Council, Malaysia, pp. 225–239

Abu, H.O., Hamdan, M. and Jamaludin, A.B. (1997). The economics of production of animal feed from oil palm fibres. In: Harun, J. (Ed.), Utilisation of Oil Palm Residues: Progress Towards Commercialisation, Oil Palm Tree Utilisation Committee, Malaysia, pp. 160–172.

Akiyama, H., Goda, Y., Tanaka, T. and Toyoda, M. (2001). Determination of aflatoxins B1, B2, G1 and G2 in spices using a multifunctional column clean-up. *Journal of Chromatography*. 932: 153–157.

Akpan, H.D., Udofia, A.A., Akpan, E.J. and Joshua, A.A. (2005). The effect of phytase and zink supplementation on palm kernel cake toxicity in sheep. *Pakistan Journal of Nutrition*. 4: 148–153

Al-Gabr., Hamid Moh Zheng., Tianling., Yu, Xin. (2013). Occurrence and quantification of fungi and detection of mycotoxigenic fungi in drinking water

in Xiamen City, China.

American Society for Microbiology (ASM), (2004). Clinical microbiology workforce issues, Washington

Atasie, V.N. and Akinhanmi, T.F. (2009). Extraction, compositional studies and physico - chemical characteristics of palm kernel oil. *Pakistan Journal of Nutrition*. 6: 1680–5194.

Awaludin,R .(2001). Feeding trials in Malaysia: palm kernel cake as a feedstuff for cattle. *Palm Oil Developments*. 34: 7–10

Beg, M.U., Beg, K.R., and Ali, L.N. and Saeed, T. (2006). Mycotoxins in Poultry Feed in Kuwait. *Archives of Environmental Contamination and Toxicology*. 50: 594-602

Begum, F., Rehman, A., Mahila, G. and Nuzhat, J. (2001). Distribution of aflatoxin B1 from poultry feed to different body tissues of broilers. *Pakistan Veterinary Journal*. 21: 121-123

Bellí, N., Marín, S., Sanchis, V. and Ramos, A.J. (2004). Influence of water activity and temperature on growth of isolates of *Aspergillus* section *Nigri* obtained from grapes. *International Journal of Food Microbiology*. 96: 19–27.

Beuchat, L.R. (2003). Dichloran Glycerol (DG18) agar, 369–385.

Beuchat, L.R. and Cousin, M.A. (2001). Yeasts and molds. In: E Pouch-Downes and K. Ito (Eds.), Compendium of Methods for the Microbiological Examination of Foods, Am. Public Health Assoc. Washington, DC, pp. 209-215

Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F. and Sharma, K.K. (2015). Biotechnological advances for combating *Aspergillus flavus* and aflatoxin contamination in crops. Plant Science. *An International Journal of Experimental Plant Biology*. 234: 119–132.

Bidochka, M.J., Mcdonald, M.A., St. Leger, R.J. and Roberts, D.W. (1994). Differentiation of species and strains of entomopathogenic fungi by random amplified polymorphic DNA (RAPD). *Current Genetics*. 25: 107-113,

Binder, E.M. (2007). Managing the risk of mycotoxins in modern feed production. *Animal Feed Science and Technology*. 133: 149–166.

Bintvihok, A., Bunlunara, W. and Kaewamatawong, T. (2002). Aflatoxin detoxification by esterified glucomannans in ducklings. *Journal of Health Research.* 16: 135–148.

Boff, C., Brun, C.P., Miron, D., Zoppas, B.C.D. a. and Pasqualotto, A.C. (2012). Technical note: the effect of different incubation temperatures on the recovery of *Aspergillus* species from hospital air. *American Journal of Infection Control*, 40: 1016–7.

Buncic, S., Avery, S.M., Rocourt, J. and Dimitrijevic, M. (2001). Can food-related environmental factors induce different behaviour in two key serovars, 4b and 1/2a, of *Listeria monocytogenes*? *International Journal of Food Microbiology*. 65: 201-212.

Busman, M., Bobell, J.R.and Maragos, C.M. (2015). Determination of the aflatoxin M1 (AFM_1) from milk by direct analysis in real time – mass spectrometry (DART-MS). *Food Control*. 47:592–598.

Chang P.K, Horn B.W. and Dorner JW. (2005). Sequence breakpoints in the aflatoxin biosyntheis gene cluster and flanking regions in nonaflatoxigenic *Aspergillus flavus* isolates. *Fungal Genetics and Biology*. 42: 914–923

Chelkowski, J. (1991). Mycological quality of mixed feeds and ingredients. In: J. Chelkowski (ed.), Cereal Grain, Mycotoxins, Fungi and Quality in Drying and Storage *(Elsevier, Amsterdam*), 217–227

Chilvers, K.F., Reed, R.H. and Perry, J.D., (1999). Phototoxicity of rose bengal in mycological media implications for laboratory practice. *Letters in Appied Microbiology*. 28: 103–107.

Chin, F.Y. (2002). Utilization of palm kernel cake (PKC) as feed in Malaysia. Asian Livestock Magazine XXVI 4: 19–23

Cotty, P.J. and Mellon, J.E. (2006). Ecology of aflatoxin producing fungi and biocontrol of aflatoxin contamination Adaptations of aflatoxin-producing fungi Aflatoxins in the Sonoran desert, 22: 110–117.

Dalcero, A, Magnoli, C., Chiacchiera, S.,Palacios, G. and Reynoso, M. (1997). Mycoflora and incidence of aflatoxin B1, zearalenone and deoxinyvalenol in poultry feeds in Argentina, *Mycopathologia*, 137: 179–184

Dashti, B., Al-hamli, S., Alomirah, H., Al-zenki, S., Abbas, A.B. and Sawaya, W. (2009). Levels of aflatoxin M1 in milk , cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. *Food Control*, 20: 686–690.

Deak, T., Chen, J., Golden, D.a, Tapia, Tornai-Lehoczki, J., Viljoen, B.C.and Beuchat, L.R., (2001). Comparison of dichloran 18% glycerol (DG18) agar with general purpose mycological media for enumerating food spoilage yeasts. *International Journal of Food Microbiology*., 67: 49–53.

Decastelli, L., Lai, J., Gramaglia, M., Monaco, A., Nachtmann, C., Oldano, F., et al. (2007). Aflatoxins occurrence in milk and feed in Northern Italy during 2004e 2005. *Food Contro*l. 18: 1263-1266

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S.and Klich, M.A. (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annual Review of Phytopathology*. 25: 249–270.

Ding, X., Li, P., Bai, Y. and Zhou, H. (2012). Aflatoxin B1 in post-harvest peanuts and dietary risk in China. *Food Control*. 23: 143–148.

Doester, A.M., Cotty, J.P., and Michailides, T.J. (2009). Descriptive of a distinctive aflatoxin producing strain of *Aspergillus nomius* that produces submerged sclerotia. *Mycophatologia* 168: 193-201

Dutta, T. K., and Das, P. (2001). Isolation of aflatoxigenic strains of As*pergill*us and detection of aflatoxin B 1 from feeds in India *Mycopathologia*. 151: 29–33.

Ehrlich, and Kenneth, C. (2014) .Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: advantages and limitations. *Frontiers in Microbiology*. 5: 50

Ehrlich, K.C., Yu, J., and Cotty, P.J. (2005). Aflatoxin biosynthesis gene clusters and flanking regions. *Journal of Applied Microbiology*. 99: 518-527

El-Kady, I.a., El-Maraghy, S.S.M. and Mostafa, M.E. (1995). Natural occurrence of mycotoxins in different spices in Egypt. *Folia Microbiologica*, 40: 297–300.

Ellis, W.O., Smith, J.P., Simpson, B.K. and Oldham. J.H. (1991). Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection and methods of control. Crit. *Reviews in Food Science and Nutrition*. 30: 403-439

Ellis, W.O., Smith, P.J., Simpson, B.K., Khanizadeh, S. and Oldham, J.H. (1993). Control of growth and aflatoxin production of *Aspergillus flavus* under modified atmosphere packaging conditions. *International Journal of Food Microbiology*. 10: 9–21

Enumeration, G. P. (1992). Recommended Methods For Food, 343–348.

Erlich, K. and Cotty, P. (2002). Variability in nitrogen regulation of aflatoxin production by *Aspergillus flavus* strains. *Applied Microbiology Biotechnology*. 60: 174-178

European Communities No. 657/2002, of 12 August. 2002. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Of J Eur Union. L221/8:129.

Freire, C.O., Kozakiewicz, Z. and Paterson, R.R.M. (2001). Mycoflora and mycotoxins in Brazilian black pepper , white pepper and Brazil nuts, *Mycopathologia*. 149: 13–19.

Gao, J., Liu, Z. and Yu, J. (2007). Identification of *Aspergillus* section *Flavi* in maize in northeastern China. *Mycopathologia*. 164: 91–95.

Garcia, D., Ramos, A.J., Sanchis, V. and Marin, S. (2009). Predicting mycotoxins in foods: A review. *International Journal of Food Microbiology*. 26: 757-769

Giorni, P., Magan, N., Pietri, a, Bertuzzi, T. and Battilani, P. (2007). Studies on *Aspergillus* section *Flavi* isolated from maize in northern Italy. *International Journal of Food Microbiology*, 113: 330–8.

Gonzalez-Mendoza, D., Argumendo-Delira, R., MoralesT-Trejo, A., Pulido-Herrera, A., Cervantes-Diaz, L., Grimaldo-Juarez, O. and Alarcon, A. (2010). A rapid method for isolation of total DNA from pathogenic filamentous plant fungi. *Genetics and Molecular Research*. 9: 162-166.

Gorski, L., Flaherty, D. and Mandrell, R.E., (2006). Competitive fitness of *Listeria monocytogenes* serotype 1/2a and 4b strains in mixed cultures with and without food in the U. S. Food and Drug Administration enrichment protocol. *Applied and Environmental Microbology*. 72: 776–783

Hernandez, P. and Beuchat, L.R. (1995). Evaluation of diluents and media for enumerating *Zygosaccharomyces rouxii* in blueberry syrup. *International ournal of Food Microbiology*. 25: 11-18

Hishamuddin, M.A. (2000). Malaysian palm kernel cake as animal feed. *Palm Oil Developments*. 34: 4–6.

Hooft,, J.M., El, A.H., Elmor, I., Encarnação, P. and Bureau, D.P. (2011). Rainbow trout (Oncorhynchus mykiss) is extremely sensitive to the feed- borne *Fusarium* mycotoxin deoxynivalenol (DON). *Aquaculture.* 311: 224–232

Hsieh, D.P. and Wong, J.J. (1994). Pharmacokinetics and excretion of aflatoxins. In The Toxicology of Aflatoxins. Human Health, Veterinary, and Agricultural Significance, Eaton DL, Groopman JD (eds). Academic Press, Toronto, Ont., pp. 373–88.

Hussein, H.S. and Brasel, J.M. (2001). Toxicity , metabolism , and impact of mycotoxins on humans and animals. *Toxicology*. 167: 101–134.

Hutagulung, R.I. and Mahyuddin, M.D. Nutritive values and feeding systems on palm kernel cake and palm press fibre for ruminants. Proceedings 3rd Asian-Australasian Association of Animal Production Societies, 1985.

International Agency for Research on Cancer (IARC). (1993). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on Evaluation of Carcinogenic Risks to Humans, 56,397e444.

Jelan, Z.A., Ishak, Y. and Yaakub, T. (1991). Feed lotting of cattle on palm kernel cake in small holder farming system. Proceedings 14th Annual Conference Malaysian Society Animal Production, pp. 99–102.

Joshua, H. (1993). Determination of aflatoxins by reversed-phase high- performance liquid chromatography with post-column in-line photochemical derivitization and fluorescence detection. *Journal of Chromatography. A*. 654: 247-254.

Joubrane, K., El Khoury, A., Lteif, R., Rizk, T., Kallassy, M., Hilan, C. and Maroun, R. (2011). Occurrence of aflatoxin B1 and ochratoxin A in Lebanese cultivated wheat. *Mycotoxin Research*, 27: 249–57.

Kaaya, A.N., Kyamuhangire, W. and Kyamanywa, S. (2006). Factors affecting aflatoxin con- tamination of harvested maize in the three agro-ecological zones of Uganda, *Journal Applied Science*. 6: 2401–2407

Keller, N.P., Nesbitt, C., Sar, B., Philips, T.D., and Burrow, G.B. (1997). pH regulation of sterigmatocystin and aflatoxin biosynthesis in *Aspergillus* spp. *Phytopathology*. 87: 643-648.

King, A.D., Hocking, A.D. and Pitt, J.I. (1979). Dichloran rose bengal medium for enumeration isolation of molds from foods. *Applied and Environmental Microbiology*. 37: 959-964.

Koehler, P.E., Beuchat, L.R. and Chhinnan, M.S. (1985). Influence of temperature and water activity on aflatoxin production by *Aspergillus flavus* in cowpea (Vigna unguiculata) seeds and meal. *Journal of Food Protection*. 48: 1040– 1043.

Kolade, O.O., Coker, A.O., Sridhar, M.K.C. and Adeoye, G.O. (2006). Palm kernel waste management through composting and crop production. *Journal of Environmental Health Research* 5: 81–85.

Kumagai, S. (1989). Intestinal absorption and excretion of aflatoxin in rats. *Toxicology and Applied Pharmacology*. 97: 88–97.

Lee, K., Kim, B.H. and Lee, C. (2010). Occurrence of *Fusarium* mycotoxin beauvericin in animal feeds in Korea. *Animal Feed Science and Technology*. 157: 190-194.

Leeson, S., Diaz, G. and Summers. J.D. (1995). Aflatoxins. In: Leeson, S., Diaz, G., Summers, J.D. (Eds.), Poultry Metabolic Disorders and Mycotoxins. University Books, Canada, Ont., pp. 248–279.

Luchese, R.H. and Harrigan, W.F. (1993). Biosynthesis of aflatoxin the role of nutritional factors. *Journal of Applied Bacteriology* 74: 5–14

Malaysian Palm Oil Board (MPOB), Economics & Industry Development Division. http://econ.mpob.gov.my/economy/ Overview_2009.pdf

Marin, S., Ramos, A.J. and Sanchis, V. (2012). Modelling *Aspergillus flavus* growth and aflatoxins production in pistachio nuts. *International Journal of Food Microbiology*. 32: 378-388

McClenny, N. (2005). Laboratory detection and identification of *Aspergillus* species by microscopic observation and culture: the traditional approach. *Journal of Medical and Veterinanry Mycology* 43: 125-128.

Meyer, W., Koch, A., Niemann, C., Beyermann, B., Epplen, J.T. and Borner, T.

(1991) Differentiation of species and strains among filamentous fungi by DNA fingerprinting. *Current Genetics*. 19:239-242.

Muhammad, S., Shehu, K, and Amusa, N.A., (2004). Survey of the market diseases and aflatoxin contamination of tomato (Lycopersicon escolentum Mill.) fruits in Sokoto, northwestern Nigeria. *Nutrition and Food Science*. 34: 72-76.

Ng, K.L., Ayob, M.K., Said, M., Osman, M.A., and Ismail, A. (2013). Optimization of enzymatic hydrolysis of palm kernel cake protein (PKCP) for producing hydrolysates with antiradical capacity. *Industrial Crops and Products*. 43: 725–731.

Ng, W.K., Lim, H.A., Lim, S.L. and Ibrahim, C.O. (2002). Nutritive value of palm kernel meal pretreated with enzyme or fermented with Trichoderma koningii (Oudemans) as a dietary ingredient for red hydrid tilapia (Oreochromis sp.). *Aquaculture Research* 33: 1199–1207.

Orunmuyi, M., Bawa, G.S., Adeyinka, F.D., Daudu, O.M. and Adeyinka, I.A., (2006). Effects of graded levels of palm kernel cake on performance of grower rabbits. *Pakistan Journal of Nutrition* 5: 71–74.

Osman, N.A., Abdelgadir, A.M., Moss, M.O., and Bener, A., (1999). Aflatoxin contamination of rice in the United Arab Emirates. *Mycotoxin Research*. 15: 39-44.

Panigrahi, S. and Waite, B.S. (1998). Use of rations with up to forty per cent palm kernel meal for egg production. *British Poultry Science* 39: 37–38.

Park, K.Y., and Bullerman, L.B. (1983). Effect of cycling temperature on aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus* in rice and cheese. *Journal of Food Science*. 48: 889–896

Parra, R. and Magan, N. (2004). Modelling the effect of temperature and water activity on growth of *Aspergillus niger* strains and applications for food spoilage moulds. *Journal of Applied Microbiology*. 97: 429–38.

Paterson, R.R.M. (2007). Aflatoxins contamination in chili samples from Pakistan. *Food Control*. 18: 817–820

Pini, G., Faggi, E., Donato, R., Sacco, C. and Fanci, R. (2007). Invasive pulmonary aspergillosis in neutropenic patients and the influence of hospital renovation. Mycoses 51: 117-22

Pitt, J.I. and Hocking, A.D (Eds). (1997). Fungi and Food Spoilage. Second ed. Blackie Academic Press, London.

Pitt, J.I., Hocking, A.D., and Glenn, D.R. (1983). An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. *Journal of Applied Bacteriology* 54: 109-114.

Pittet, A. (1998). Natural occurrence of mycotoxins in foods and feeds – an updated review, *Revue De Medecine Veterinaire*. 149: 479–492

Pomeran, Y., and Meloan, C. (1994). Food Analysis: Theory and practice, New York, Chapman and Hall

Rahmani, A., Selamat, J., and Soleimany, F. (2011). Food Additives & Contaminants : Part A Optimization and validation of a HPLC method for simultaneous determination of aflatoxin B 1 , B 2 , G 1 , G 2 , ochratoxin A and zearalenone using an experimental design. *Food Additives and Contaminant.* DOI: 10.1080/19440049.2011.576436

Ramachandran, S., Singh, S.K., Larroche, C., Soccol, C.R. and Pandey, A. (2007). Oil cakes and their biotechnological applications–A review. *Bioresource Technology*. 98: 2000–2009.

Razuan, R., Chen, Q., Zhang, X., Sharifi, V. and Swithenbank, J. (2010). Pyrolysis and combustion of oil palm stone and palm kernel cake in fixed-bed reactors. *Bioresource Technology*, 101: 4622–4629.

Reddy, K.R.N. et al., (2010). An overview of mycotoxin contamination in foods and its implications for human health, *Toxin Reviews*. 29: 3–26.

Reobuck B.D. and Maxuitenko Y.Y. (1994). Biochemical mechanism and biological implications of the toxicity of aflatoxins as related to aflatoxin carcinogens. In The Toxicology of Aflatoxins. Human Health, Veterinary, and Agricultural Significance, Eaton DL, Groopman JD (eds). Academic Press, Toronto, Ont., pp. 27-73.

Rhule, S.W.A. (1996). Growth rate and carcass characteristics of pigs fed on diets containing palm kernel cake. *Animal Feed Science and Technology*. 61: 167–172.

Rosa C.A.R., J.M.M. Ribeiro, M.J. Fraga, M. Gatti, L.R. Cavaglieri, C.E. Magnoli, A.M. Dalcero, and C.W.G. Lopes. (2006). Mycoflora of poultry feeds and ochratoxin-producing ability of isolated *Aspergillus* and *Penicillium* species. *Veterinary Microbiology*. 113: 89-96.

Rustom, I.Y.S. (1997). Aflatoxin in food and feed: Occurrence, legisla- tion and inactivation by physical methods. *Food Chemistry*. 59: 57–67

Sahib, A. (2009). Fungal diversity and aflatoxins in poultry feed : ecology and implications for control of aflatoxins ingestion and distribution in broilers ' tissues, PhD Thesis, NWFP Agricultural University Peshawar, Pakistan.

Samson, R.A., E.S. Hoekstra, F. Lund, O. Filtenborg, and J.C. Frisvad. (2002). Mycological Media for food- and indoor fungi. In: R. A. Samson, E. S. Hoekstra, J. C. Frisvad and O. Filtenborg, Editors, Introduction to Food- and Airborne Fungi, Centraalbureau voor Schimmecultures, Utrecht.

Saw, H. Y., Janaun, J. and Subbarao, D. (2008). Hydration properties of palm kernel cake. *Journal of Food Engineering*, 89: 227–231.

Scott, W.J. (1957). Water relations of food spoilage microorgan- isms. *Advances in Food Research*. 7: 83–127.

Sebunya, T.K. and D.M. Yourtee. (1990). *Aflatoxigenic aspergilli* in foods and feeds in Uganda. *Journal of Food Quality.* 13: 97-107.

Sharma, M. (2001). Determination of aflatoxins in domestic pet foods (dog and cat) using immunoaf ® nity column and HPLC. 93:109–114.

Shehu, K. and Bello, M.T., (2011). Effect of Environmental Factors on the Growth of *Aspergillus* Species Associated with Stored Millet Grains in Sokoto. *Nigerian Journal of Basic and Applied Science*. 19: 218–223.

Sobolev, V.S. and Dorner. J.W. (2002). Cleanup procedure for determination of aflatoxins in major agricultural commodities by liquid chromatography. *Journal of AOAC International*. 85: 642-645.

Stroka, J.A.E., U. Jorissen, and J. Glbert. (2001). Immunoaffinity column clean up with liquid chromatography using Post-column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder: collaborative study. *Journal of AOAC International.* 83: 320-340

Takahashi, T. (1997). Airborne fungal colony forming units in outdoor and indoor environments in Yokohama, Japan. *Mycopathologia*. 139: 23–33

Tan, Y.A., Sambanthamurthi, R., Sundram, K. and Wahid, M.B. (2007). Valorisation of palm by-products as functional components. *European Journal of Lipid Science and Technology*. 109: 380–393.

Tan, Y.N., Ayob, M.K., Ahmad, W. and Yaacob, W. (2013). Purification and characterisation of antibacterial peptide-containing compound derived from palm kernel cake. *Food Chemistry*. 136: 279–284.

Tang, T.S., (2000). Quality and characteristics of Malaysian palm kernel cake/ expellers. *Palm Oil Developments*. 34: 1–3.

Taniwaki, M.H., Iamanaka, B.T. and Banhe, A.A. (1999). Comparison of culture media to recover fungi from flour and tropical fruit pulps. *Journal of Food Mycology*. 2: 291-302.

van Egmond, H.P. and Jonker, M.A. (2004). Worldwide regulations on aflatoxins – the situation in 2002. *Journal of Toxicology* 23: 273–293.

Waltking, A.E., and Wilson, D. (2006). Liquid Chromatographic analysis of aflatoxin using post-column photochemical derivitization: collaborative study. *Journal of AOAC International*. 89: 678-692.

Warris, A., Voss, A. and Verweij, P.E. (2001). Hospital sources of *Aspergillus* species: New routes of transmission. *Journal of Medical Mycology*. 18: 156-62

Weidenborner, M., Wieczorek, C., Appel, S. and Kunz, B. (2000). Whole wheat and white wheat flour-the mycobiota and potential mycotoxins. *International Journal of Food Microbiology*. 17: 103-107

West, K. (2009). Methods for Isolation , Enumeration and Identification (pp. 19–52).

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. 18: 6531–6535.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and inter- ventions. *The American Journal of Clinical Nutrition.* 80: 1106–1122

Yan Yan, F., Krishniah, D., Rajin, M. and Bono, A. (2009). Cellulose extraction from palm kernel cake using liquid phase oxidation. *Journal of Engineer Scence and Technology*. 4: 57–68.

Ying, L.S. and Zhang, L.H. (2004). Anti-fatigue effect of PKC peptides on mice. *Polypeptides and Anti-cancer International Forum*, 10: 73–75

Zahari, M.W. and Alimon, A.R. (2003). Use of palm kernel cake and oil palm by-products in compound feed. *Palm Oil Developments*. 40: 5–9