UNIVERSITI PUTRA MALAYSIA

EFFECT OF PACKAGING AND STORAGE ON Aspergillus spp. AND AFLATOXIN PRODUCTION IN PEANUTS

JOSHUA MARK A/L JOHN

FSTM 2021 11

EFFECT OF PACKAGING AND STORAGE ON *Aspergillus* spp. AND AFLATOXIN PRODUCTION IN PEANUTS

By

JOSHUA MARK A/L JOHN

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

November 2020

DEDICATION

This work is dedicated to the Lord, for His strength and guidance along this journey. I would like to thank my loving family who kindly and assuredly supported me throughout my MSc. journey, always showering me with kind encouragements, patience, insight and love throughout my research period. A huge thank you to my closest friends who were a pillar of strength and were always by my side during all times throughout my journey.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

EFFECT OF PACKAGING AND STORAGE ON *Aspergillus* spp. AND AFLATOXIN PRODUCTION IN PEANUTS

By

JOSHUA MARK A/L JOHN

November 2020

Chairman : Professor Jinap binti Selamat, PhD
Faculty : Food Science and Technology

Peanuts are most susceptible to mycotoxins contamination especially aflatoxins, which are class one carcinogenic mycotoxins vastly produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They can induce both mutagenic and hepatogenic effect causing conditions such as immunosuppression and hepatic carcinoma. Due to the high temperature and humidity conditions in tropical and subtropical regions, high presence of aflatoxins can be expected. The purpose of the present study was to investigate, a) The effects of different packaging materials, temperatures and water activities on the aflatoxin B1 production by *Aspergillus flavus* and *A. parasiticus* in stored peanuts, b) To optimize the best storage conditions for the reduction of aflatoxin B_1, total aflatoxins and growth of *Aspergillus flavus* and *A. parasiticus* in stored peanuts. On the first objective, peanut samples were randomly collected from supermarkets around Serdang, Selangor. The samples were separated into three different packaging materials (low density polyethylene [LDPE], polypropylene [PP] and polyethylene laminated aluminium [PELA]), inoculated and stored under temperatures of 25°C and 30°C, water activities (a_w) of 0.80 and 0.95. For *A. flavus* stored in PELA, no AFB_1 was detected (100% reduction) at 25°C for both a_w tested. For *A. parasiticus* stored in PELA, no AFB_1 was detected at 25°C (0.85 a_w) and 30°C (0.74 a_w). Lowest concentration of AFB_1 was detected in PELA for both *A. flavus* and *A. parasiticus*, followed by PP and LDPE. For the second objective, peanut samples were separated into two packaging materials, PELA and PP, inoculated directly with *A.flavus* and *A.parasiticus* spores in concentration ranging from 10^1 to 10^6 spores per ml and stored under temperature of 30°C and relative humidity of 65 and 95% for 42 days (6 weeks). Results showed that significant ($P < 0.05$) response surface models with high coefficient of determinations (R^2) with a range from 0.849 to 0.997 were able to be fitted to assess the AFB_1 and AFT formation levels as functions of storage condition variables with more than 84% of variability of AFB_1 and AFT formation could be explained by the significant ($p < 0.05$) nonlinear functions of time and initial spore inoculum levels. The main effects of time and initial spore inoculum level has significant ($p < 0.05$) effect on the formation of both AFB_1 and AFT formation in the artificially inoculated air packed stored peanuts. For PP-

FL 65, PP-AP-65, PELA-FL-65, PELA-AP-65, PP-FL-95, PP-AP-95, PELA-FL-95 and PELA-AP-95 the quadratic effects of both time and initial spore inoculum level showed significant ($p < 0.05$) effect on fungal growth. For the interaction effects, no significant ($p > 0.05$) effect was shown for all experimental runs and that both time and initial spore inoculum level had significant ($p < 0.05$) effect on values of all target AF's and *Aspergillus* spp. growth. Optimum storage conditions for the best reduction of *Aspergillus* spp. growth and AF's formation were able to be identified.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PEMBUNGKUSAN DAN PENYIMPANAN KE ATAS *Aspergillus* spp. DAN PENGHASILAN AFLATOXIN DALAM KACANG TANAH

Oleh

JOSHUA MARK A/L JOHN

November 2020

Pengerusi : Profesor Jinap binti Selamat, PhD
Fakulti : Sains dan Teknologi Makanan

Kacang tanah mudah terdedah kepada pencemaran mikotoksin terutamanya aflatoxin, yang merupakan mikotoksin karsinogenik kelas satu yang dihasilkan terutamanya oleh *Aspergillus flavus* dan *Aspergillus parasiticus*. Ia boleh memberi kesan mutagenik dan karsinogenik, lalu menyebabkan pengimunotindasan dan karsinoma hepatogenik. Keadaan suhu dan kelembapan yang tinggi di kawasan tropika dan subtropika menyebabkan kehadiran tahap aflatoxin yang tinggi. Tujuan kajian semasa ini adalah untuk menyiasat a) Kesan pengunaan bahan pembungkusan yang berlainan, suhu dan aktiviti air bagi mengekang penghasilan Aflatoksin B_1 oleh *Aspergillus flavus* dan *A.*parasiticus dalam kacang tanah yang disimpan, b) mengkaji pengoptimuman keadaan penyimpanan yang terbaik bagi pengurangan Aflatoksin B_1, aflatoksin keseluruhan dan pertumbuhan kulat dalam kacang tanah yang disimpan. Dalam objektif pertama, sampel telah dikutip secara rawak dari pasaraya sekitar Kawasan Serdang, Selangor. Selepas itu, sampel tersebut diasingkan ke dalam tiga bahan penyimpanan (polietilena berketumpatan rendah [LDPE], polipropilena [PP] dan aluminum berlapis polietilena[PELA]), diinokulasi dan disimpan pada suhu 25°C dan 30°C, aktiviti air (a_w) 0.80 dan 0.95. Bagi sampel *A.flavus* yang disimpan dalam PELA, tiada AFB_1 dikesan (pengurangan 100%) pada 25°C bagi kedua-dua a_w yang diuji. Bagi *A. parasiticus* yang disimpan dalam PELA, tiada AFB_1 dikesan pada 25°C (0.85 a_w) dan 30°C (0.74 a_w). Konsentrasi AFB_1 terendah dikesan dalam PELA bagi kedua-dua spesies *A. flavus* dan *A. parasiticus*, diikuti oleh PP dan LDPE. Bagi objektif kedua, sampel kacang tanah diasingkan kepada dua bahan pembungkusan, iaitu PELA dan PP, diinokulasi terus dengan spora *A.flavus* and *A.parasiticus* dalam konsentrasi antara 10^1 hingga 10^6 se ml dan disimpan dalam suhu 30°C dan kelembapan relatif 65 dan 95% untuk 42 hari (6 minggu). Keputusan yang diperoleh menunjukkan tindak balas permukaan model yang ketara ($p < 0.05$) dengan pekali penentuan (R^2) yang tinggi iaitu julat antara 0.849 hingga 0.997 dapat dimuatkan bagi menilai penghasilan AFB_1 dan AFT sebagai pemboleh ubah keadaan penyimpanan dengan lebih 84% kebolehubahan penghasilan AFB_1 dan AFT boleh diterangkan oleh fungsi tidak linear masa dan tahap penginokulasian spora awal yang ketara ($p < 0.05$). Kesan utama masa dan tahap

penginokulasian spora awal mempunyai kesan yang ketara ($p < 0.05$) ke atas penghasilan kedua-dua AFB_1 dan AFT dalam kacang tanah yang diinokulasi secara tiruan dan dibungkus dalam udara biasa. Bagi PP-FL 65, PP-AP-65, PELA-FL-65, PELA-AP-65, PP-FL-95, PP-AP-95, PELA-FL-95 and PELA-AP-95, kesan kuadratik bagi kedua- dua masa dan tahap penginokulasian spora awal menunjukkan kesan yang ketara ($p>0.05$) ke atas pertumbuhan kulat. Bagi kesan interaksi, tiada kesan yang ketara ($p>0.05$) ditunjukkan bagi semua ujian eksperimen dan yang kedua-dua masa dan tahap penginokulasian spora awal mempunyai kesan yang ketara ($p>0.05$) ke atas nilai semua AF's sasaran dan pertumbuhan *Aspergillus.* spp.. Keadaan penyimpanan optimum bagi pengurangan tertinggi pertumbuhan *Aspergillus* spp. dan penghasilan AF's dapat dikenal pasti.

ACKNOWLEDGEMENTS

First and foremost, I would like to take this opportunity to express my greatest appreciation and deepest gratitude to my supervisor Professor Dr Jinap Selamat for her endless guidance, virtue of patience, seamless motivation and support throughout my journey in the research. I would also like to thank AP Dr Nor Khaizura Ab Rashid and AP Dr Nur Hanani Zainal Abedin for being excellent co-supervisors and providing a great amount of dedication, attention and support throughout the course of my work.

I am deeply indebted to to MyBrain 15 for honoring me with a scholarship during my study period. I am also grateful to Universiti Putra Malaysia for funding of research materials and providing the facilities to conduct the research via the IPB Putra Grant.

My deepest appreciation and love go to my family members especially my parents John and Latchmi, siblings Jonathan and Jennifer, and my closest friends Vignesh, Jeevan, Marcus and Barathi for being with me along the way. A big thank you to all my labmates for being there through good times and bad, with kind support and advice.

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

Jinap binti Selamat, PhD
Professor
Faculty Food Science and Technology
Unversiti Putra Malaysia
(Chairman)

Nor Khaizura binti Mahmud @ Ab Rashid, PhD
Associate Professor
Faculty Food Science and Technology
Universiti Putra Malaysia
(Member)

Nur Hanani Zainal Abedin, PhD
Associate Professor
Faculty Food Science and Technology
Universiti Putra Malaysia
(Member)

_____

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 June 2021

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this 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 obtained from supervisor and the office of 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: Joshua Mark a/l John, GS41239

Declaration by Members of Supervisory Committee

This is to 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) were adhered to.

Signature:	
Name of Chairman of Supervisory Committee:	Professor Dr. Jinap binti Selamat
Signature:	
Name of Member of Supervisory Committee:	Associate Professor Dr. Nor Khaizura binti Mahmud @ Ab Rashid
Signature:	
Name of Member of Supervisory Committee:	Associate Professor Dr. Nur Hanani Zainal Abedin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFPA	*Aspergillus flavus parasiticus agar*
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFs	Aflatoxins
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Aspergillus flavus (FL)	*A.flavus*
Aspergillus parasiticus (AP)	*A.parasiticus*
CCD	Central Composite Design
CP	Centre point
EC	European Commission
Em	Emission
Ex	Excitation
FAO	Food and Agricultural Organization
FLD	Fluorescence detection
IAC	Immunoaffinity Column
IARC	International agency for research on cancer
LOD	Limit of detection
LOQ	limit of quantification
ng/ µL	Nanogram/ microlitre
ND	not detected

PDA	potato dextrous agar
PE	polyethylene
PELA	polyethylene laminated aluminium
PP	polypropylene
PHRED	photochemical reactor for enhanced detection
Rh	Relative humidity
RSM	Response surface methodology
R^2	Correlation
R^2 (adj)	Adjusted R^2
Tween 80	Polysorbitan 80
UV	Ultra Violet
λ	Wavelength

CHAPTER 1

INTRODUCTION

1.1 Background of study

Aflatoxins have attracted a substantial amount of attention over the course of the last four decades. AFs are a class of chemicals which are very potent teratogenic, hepatotoxic, mutagenic and a very well-known carcinogenic to humans. Mycotoxin contamination in food is known to cause both acute and/or chronic health issues in humans and animals alike. The main systemic targets of mycotoxins are organs such as the kidney, liver, immune system and the nervous system (IARC,1993).

The ever presence of aflatoxins in various food is nearly inevitable, in spite of decades of research and proper good manufacturing practices throughout food production, and distribution especially during the storage phase, mycotoxins remain and contaminate wide range of food and feed (Bryden,2007). On a demographic view, the continents that have reported the most amount of human aflatoxicosis are Africa and Asia.

In the context of Malaysia, peanuts are a part of the dietary process. They can be taken in their raw state, although it is more commonly served in its boiled, roasted or salted form (Arya et al., 2016). Leong et al., 2010 reported that adults from the region of Penang take in an average of 0.77g of nuts daily. When comparing among ethnicity, the study showed that Indians consume the single highest amount of peanuts at 0.94g/day. The consumption can cause the consumers to be exposed to a range of levels of AFs, from nanograms to micrograms per day.

As above per mentioned, Malaysia is a region with a rather high peanut consumption rate. The increased mycotoxin contamination of peanuts in tropical region countries puts the consumers in a serious deal of health problems. The extreme hazard brought by AFs to humans has propagated the establishments of proper control and acceptable measures and tolerance levels, both implemented by international organizations and national authoritative bodies. For Malaysia, the maximum allowed levels for total AFs in nuts and nut product are 15 ng/g and at 5 ng/g for spices (MOH, 2004; Malaysian Food Regulation, 1985).

Malaysia possesses a climate of a tropical zone with elevated temperatures and high humidity, that puts peanuts under the stresses of *Aspergillus* spp. and AF's contamination, mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Peanuts are one of the mainly affected crops by *Aspergillus* spp. due to the fact that this fungi is ubiquitious and the edible section of the peanut plant grows underground making it extra susceptible. Elevated a_w levels in the food commodity as a result of a direct equilibrium

interaction with the high relative humidity of the surrounding atmosphere further increases the likelihood of *Aspergillus* spp. growth and AF's production (Naresh Magan and Aldred, 2007; Naresh Magan and Lacey, 1984). The ideal temperature range for *Aspergillus* spp. proliferation and aflatoxin production is from 26.7-43.3°C, and a relative humidity of 62-99% (Sumner and Lee, 2012).The combination of high temperatures and increased a_w results in high proliferation of *Aspergillus* spp. and subsequent AF's production (Medina et al., 2015; Bueno et al., 2004). Packaging plays a vital role in curbing *Aspergillus* spp. growth and AF's production. Peanuts are usually stored in bulk or packed in either jute or polypropylene woven sacks during their storage in farms while at the market places, the shelled peanuts are most common ones to be packed in the above mentioned materials. These packaging materials were found to have an effect on the AF's levels in the stored peanuts (Mutegi et al., 2013). This is due to the above-mentioned packaging materials being non-airtight, with strong evidence that the mentioned storage methods further causing *Aspergillus* spp. proliferation and AF's production (Hell et al., 2000; Udoh et al., 2000). These adversities in conditions requires the need for proper packaging and storage methods of peanuts against the elements, to curb fungal proliferation and aflatoxins in stored peanuts in Malaysia.

1.2 Importance of study

Worldwide tainting of peanuts with mycotoxins is a real problem, both in currently developing and well developed countries, being a major issue for human health in whole, added to a very substantial economic detriment. Problems such as loss of human and animal life: healthcare and veterinary care costs, loss of livestock, regulatory and research costs are very much associated with mycotoxin contamination of peanuts. Probst et al. (2007) reported that in 2004, an episode of acute aflatoxicosis killed 125 people in Kenya and in October 1988, a number of children who consumed Chinese noodles; locally known as "loh see fun", succumbed to acute hepatic encephalopathy in the state of Perak (Lye et al., 1995). There have been several reported cases of aflatoxin contamination in Malaysia. Leong, et al. (2011) mentioned that Aflatoxin B_1 (AFB_1) was detected in 57% of nut and nut related products in Penang with contamination levels ranging from 0.40 to 222µg/kg while Arzandeh et al. (2010) reported that out of 84 raw peanut kernels samples randomly collected from Malaysian supermarkets, 78.57% samples were contaminated with aflatoxin in concentrations ranging from not detected to 79.28ng/g. With the majority of peanut producers, exporters and consumers being situated in tropical climatic regions and *Aspergillus* spp. being a readily available storage fungi, the aspect of packaging and proper storage techniques plays a very important role against *Aspergillus* spp. proliferation and subsequent AF's production. A storage condition of lower relative humidity using the packaging material with the lowest water vapour transmission rate, low permeation to O_2 and high moisture barrier should yield the lowest contamination with *Aspergillus* spp. and subsequent aflatoxin contamination.

1.3 Objectives

The objectives of this study were defined as the following:

i) To investigate the effects of different packaging materials, storage temperatures, and water activities (a_w) on aflatoxin B_1 production by *Aspergillus flavus* and *A.parasiticus* in stored peanuts

ii) To determine the optimum storage conditions for the highest reduction of aflatoxin B_1, total aflatoxins and fungal growth in stored peanuts.

1.4 Research Problems

i) Malaysia possesses a climate of a tropical zone with elevated temperatures and high humidity, that puts the peanuts under the stresses of Aspergillus spp. and AF's contamination, mainly by Aspergillus flavus and Aspergillus parasiticus.

ii) These adversities in conditions require the need for proper packaging and storage methods of peanuts against the elements, to curb fungal proliferation and aflatoxins in stored peanuts in Malaysia.

REFERENCES

Abbas, H.K.,Weaver, M.A., Zablotowicz, R.M., Horn, B.W., Shier, W.T. (2005). Relationships between aflatoxin production and sclerotia formation among isolates of *Aspergillus* Section Flavi from the Mississippi Delta. Eur J Plant Pathol 112, 283–287.

Abdel-Hadi A, Schmidt-Heydt M, Parra R, Geisen R, Magan N (2012) A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by *Aspergillus flavus*. J R Soc Interface 9:757-767.

Abdulkadar A, Al-Ali AA, Al-Kildi AM, Al-Jedah JH. (2004). Mycotoxins in food products available in Qatar. Food Control 15:543–8.

Abdullah, N., Nawawi, A., & Othman, I. (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. *Mycopathologia*, *143*(1), 53-58.

Abriba, C., Lennox, J. A., Asikong, B. E., Asitok, A., Ikpoh, I. S., Henshaw, E. E., and Eja, M. E. (2013). Isolation of aflatoxin producing species of *Aspergillus* from foodstuffs sold in calabar markets, Cross River state, Nigeria. J Microbiol Biotechnol Res, *3*(1), 8–13. Retrieved from http://scholarsresearchlibrary.com/JMB-vol3-iss1/JMB-2013-3-1-8-13.pdf

Adebiyi, A. P., Adeyemi, I. A., and Olorunda, A. O. (2002). Effects of processing conditions and packaging material on the quality attributes of dry-roasted peanuts. J Sci Food Agric., *82*(13), 1465–1471. https://doi.org/10.1002/jsfa.1192

Afsah-Hejri L, Jinap S, Arzandeh S, Mirhosseini H. 2011. Optimization of HPLC conditions for quantitative analysis of aflatoxins in contaminated peanut. Food Control 22:381–8.

Agag B.I (2004) Mycotoxins in food and feeds 1: Aflatoxins. Assiut University BES 7:173-206.

Ali, N. (2000). Aflatoxins in Malaysian food. Mycotoxins, 50, 31–35.

Arrus, K., Blank, G., Abramson, D., Clear, R., and Holley, R. A. (2005). Aflatoxin production by *Aspergillus flavus* in Brazil nuts. J Stored Prod Res, 41(5), 513–527. https://doi.org/10.1016/j.jspr.2004.07.005

Arya, S. S., Salve, A. R., and Chauhan, S. (2016). Peanuts as functional food: a review. J Food Sci Technol, 53(1), 31–41. https://doi.org/10.1007/s13197-015-2007-9

Arzandeh S, Selamat J, Lioe H. (2010). Aflatoxin in raw peanut kernels marketed in Malaysia. J Food Drug Anal 18:44–50.

Atkinson, A. C., & Donev, A. N. (1992). Optimum experimental designs (p. 344).

Barros G, Chiotta M.L, Torres A, Chulze S (2006) Genetic diversity in *Aspergillus parasiticus* population from the peanut agroecosystem in Argentina. Lett Appl Microbiol 42:560–566

Beasley, V. R., & Lambert, R. J. (1990). The apparently minimal hazard posed to human consumers of products from animals fed trichothecene-contaminated grains. Vet Hum Toxicol, *32*(Suppl.), 27-39.

Bhat, R.V., Vasanthi, S., Rao, R. N., Rao, V. S., Nagaraja, K. (1996). Aflatoxin B1 contamination in groundnut samples collected from different geographical regions of India: A multicentre study. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 13(3), 325-331.

Birzele, B., Prange, A. and Kramer, J. (2000) Deoxynivalenol and ochratoxin A in German wheat and changes of level in relation to storage parameters. Food Addit Contam, 17: 1027– 1035.

Bock, C.H., Mackey, B. and Cotty, P.J., (2004). Population dynamics of *Aspergillus flavus* in the air of an intensive cultivated region of south-west Arizona. Plant Pathol, 53: 422-433

Bryden WL. (2007). Mycotoxins in the food chain: human health implications. Asia Pac J Clin Nutr. 2007;16 Suppl 1:95-101. Review.

Bueno, D. J., Silva, J. O., and Oliver, G. (2004). Fungal isolation and enumeration in foods. Methods Mol Biol (Clifton, N.J.), 268(5), 127–131. https://doi.org/10.1385/1-59259-766-1:127

Bulaong, S. S. P., and Dharmaputra, O. S. (2002). Fungal population, aflatoxin and free fatty acid contents of peanuts packed in different bag types. Biotropia, 19,1–25.

Bullerman, L. B., and Bianchini, A. (2007). Stability of mycotoxins during food processing. Int J Food Microbiol, *119*(1-2),140–146.

Burlingame, B., & Pineiro, M. (2007). The essential balance: Risks and benefits in food safety and quality. Burlingame, B., & Pineiro, M. (2007). The essential balance: Risks and benefits in food safety and quality. J Food Compost Anal, *20*(3-4), 139-146., *20*(3-4), 139-146.

Butts, C. L., Chastain, J. T., Dorner, J. W., Sanders, T. H., Lamb, M. C., & Rowland, D. L. (2005). Storing farmers stock peanuts in monolithic domes. In 2005 ASAE Annual Meeting (p. 1). Trans ASABE.

C. Popa, (2016). Breathing Disorders Using Photoacoustics Gas Analyzer. J Med Imaging Health Inform, Vol. 6, pp. 1893-1895.

Carvajal-Moreno, M., Vargas-Ortiz, M., Hernández-Camarillo, E., Ruiz-Velasco, S., & Rojo-Callejas, F. (2019). Presence of unreported carcinogens, aflatoxins and their hydroxylated metabolites, in industrialized Oaxaca cheese from Mexico City. Food Chem. Toxicol., *124*, 128-138.

Cepeda, A., Franco, C. M., Fente, C. A., Vázquez, B. I., Rodriguez, J. L., Prognon, P., & Mahuzier, G. (1996). Postcolumn excitation of aflatoxins using cyclodextrins in liquid chromatography for food analysis. J. Chromatogr. A, *721*(1), 69-74.

Chiou, C.-H., Miller, M., Wilson, D. L., Trail, F., and Linz, J. E. (2002). Chromosomal location plays a role in regulation of aflatoxin gene expression in Aspergillus parasiticus. J Appl Environ Microbiol, *68*(1), 306–315. https://doi.org/10.1128/AEM.68.1.306

Cho, S. H., Lee, C. H., Jang, M. R., Son, Y. W., Lee, S. M., Choi, I. S., and Kim, D. B. (2008). Aflatoxins contamination in spices and processed spice products commercialized in Korea. Food Chem., *107*(3), 1283-1288.

Chun, H. S., Kim, H. J., Ok, H. E., Hwang, J. B. and Chung, D. H. (2007). Determination of aflatoxin levels in nuts and their products consumed in South Korea. Food Chem. 102: 385-391.

Cotty, P. J., and Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int. J. Food Microbiol., 119(1-2), 109-115.

Craufurd, P. Q., Prasad, P. V. V., Waliyar, F., & Taheri, A. (2006). Drought, pod yield, pre-harvest *Aspergillus* infection and aflatoxin contamination on peanut in Niger. Field Crops Res., *98*(1), 20-29.

Darko, C. B. (2017). Effects of Storage Conditions of *Aspergillus* Growth and Aflatoxin Production in Peanuts. A Study in Ghana (Doctoral dissertation, Virginia Tech).

Davis, N. D., Diener, U. L. and Agnihotri, V. P. (1967). Production of aflatoxins B1 and GI in a chemically defined medium. Mycopathol Mycol Appl 31, 251-256.

Del Pilar Monge, M., Magnoli, C. E., and Chiacchiera, S. M. (2012). Survey of *Aspergillus* and *Fusarium* species and their mycotoxins in raw materials and poultry feeds from Córdoba, Argentina. Mycotoxin Res, 28(2), 111–122. https://doi.org/10.1007/s12550-011-0121-8

Detroy RW, Hesseltine CW (1970) Structure of a new transformation product of aflatoxin B1. Can J Biochem Physio 48:830–832

Diener, U. L. and Davis, N. D., (1970) Limiting temperature and relative humidity for aflatoxin production by Aspergillus flavus in stored peanuts, J. Am. Oil Chem. Soc., 47, 347.

Drew, S. W., & Demain, A. L. (1977). Effect of primary metabolites on secondary metabolism. Ann. Rev. Microbiol., 31(1), 343-356.

Dorner, J. W., and Cole, R. J. (1997). A method for determining kernel moisture content and aflatoxin concentrations in peanuts. J Am Oil Chem Soc, 74(3), 285–288. https://doi.org/10.1007/s11746-997-0137-6

Dorner, J. W., Cole, R. J., Connick, W. J., Daigle, D. J., McGuire, M. R., and Shasha, B. S. (2003). Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. Biol Control, 26(3), 318–324. https://doi.org/10.1016/S1049-9644(02)00139-1

Eaton, D. L., Bammler, T. K., & Kelly, E. J. (2001). Interindividual differences in response to chemoprotection against aflatoxin-induced hepatocarcinogenesis: implications for human biotransformation enzyme polymorphisms. Biological Reactive Intermediates Vi, 559-576.

Eaton, D. L., & Groopman, J. D. (Eds.). (2013). The toxicology of aflatoxins: human health, veterinary, and agricultural significance. Elsevier.

Egner, P. A., Wang, J. B., Zhu, Y. R., Zhang, B. C., Wu, Y., Zhang, Q. N., ... & Kensler, T. W. (2001). Chlorophyllin intervention reduces aflatoxin–DNA adducts in individuals at high risk for liver cancer. Proceedings of the National Academy of Sciences, 98(25), 14601-14606.

Ehrlich, K. C., Yu, J., & Cotty, P. J. (2005). Aflatoxin biosynthesis gene clusters and flanking regions. J Appl Microbiol, 99(3), 518-527.

Ellis, W. O., Smith, J. P., Simpson, B. K., Ramaswamy, H., & Doyon, G. (1994). Effect of gas barrier characteristics of films on aflatoxin production by *Aspergillus flavus* in peanuts packaged under modified atmosphere packaging (MAP) conditions. Food Res Int, 27(6), 505-512.

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 Rev Food Sci Nutr, 30(4), 403–439.

Ellis, W. O., Smith, J. P., Simpson, B. K., Khanizadeh, S., & Oldham, J. H. (1993). Control of growth and aflatoxin production of *Aspergillus flavus* under modified atmosphere packaging (MAP) conditions. Food Microbiol, 10(1), 9-21.

Ellis, W. O., Smith, J. P., Simpson, B. K., Ramaswamy, H., & Doyon, G. (1994). Growth of and aflatoxin production by *Aspergillus flavus* in peanuts stored under modified atmosphere packaging (MAP) conditions. Int J Food Microbiol, 22(2-3), 173-187.

Ellis, W.O., Smith, J.P., Simpson, B.K. and Doyon, G. (1994) Effect of films of different gas transmission rates on aflatoxin production by *Aspergillus flavus* in peanuts packaged under modified atmosphere packaging (MAP) conditions. Food Res Int, 27, 505-12.

Ellis, E. M., & Hayes, J. D. (1995). Substrate specificity of an aflatoxin-metabolizing aldehyde reductase. Biochem J, 312(2), 535-541.

European Commission. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJEU, 364(1), 5-24.

Fani O (2013) Comparison of aflatoxin B_1 production by *Aspergillus flavus* and *Aspergillus parasiticus* under various conditions of temperature, light and pH. Armaghane-danesh J 18:210-218.

Fernández-Cruz, M. L., Mansilla, M. L., & Tadeo, J. L. (2010). Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. J.Adv.Res., 1(2), 113-122.

Fink- Grernmels, J. (1999). Mycotoxins: their implications for human and animal health. Vet Q 21(4), 115-120.

Franzolin, M. R., Gambale, W., Cuero, R. G., and Correa, B. (1999). Interaction between toxigenic *Aspergillus flavus* Link and mites (Tyrophagus putrescentiae Schrank) on maize grains: Effects on fungal growth and aflatoxin production. J Stored Prod Res, 35(3), 215–224. https://doi.org/10.1016/S0022-474X(99)00006-5

Freitas, V. P., and Brigido, B. M. (1998). Occurrence of aflatoxins B1, B2, G1, and G2 in peanuts and their products marketed in the region of Campinas, Brazil in 1995 and 1996. Food Addit Contam, 15(7), 807–811. https://doi.org/10.1080/02652039809374714

Gareis, M., Bauer, J., Montgelas, A.V., Gedek, B., Stimulation of Aflatoxin B1 and T-2 Toxin Production by Sorbic Acid (1984). Appl Environ Microbiol, Feb. 1984,p.416-418

Gautier, M., Normand, A. C., and Ranque, S. (2016). Previously unknown species of Aspergillus. Clin Microbiol Infect, 22(8), 662–669. https://doi.org/10.1016/j.cmi.2016.05.013

Giorni, P., Battilani, P., Pietri, A., Magan, N., 2008. Effect of a_w and CO2 level on *Aspergillus flavus* growth and aflatoxin production in high moisture maize post-harvest. Int J Food Microbiol 122, 109–113

Gordon, A. (2017). Introduction: effective implementation of food safety and quality systems: prerequisites and other considerations. In Food Safety and Quality Systems in Developing Countries (pp. 1-19). Academic Press.

Gottlieb, D., & Tripathi, R. K. (1968). The physiology of swelling phase of spore germination in Penicillium atrovenetum. Mycologia, 60(3), 571-590.

Groopman, J.D., Hall, A.J., Whittle, H., Hudson, G.J., Wogan, G.N., Montesano, R. and Wild, C.P. (1992) Molecular dosimetry of aflatoxin-N7-guanine in human urine obtained in the Gambia, West Africa. Cancer Epidemiol. Biomarkers Prev., 1, 221-227

Gunterus A, Roze LV, Beaudry R, Linz JE (2007) Ethylene inhibits aflatoxin biosynthesis in *Aspergillus parasiticus* grown on peanuts. Food Microbiol 24:658-663.

Guynot, M.E., Marin, S., Sanchis, V., Ramos, A.J., 2003. Modified atmospheres packaging form prevention of mould spoilage of bakery product with different pH and water activity levels. J Food Prot 10, 1864–1972.

Gqaleni, N., Smith, J.E., Lacey, J., Gettinby, G., (1997). Effects of temperature, water activity and incubation time on production of aflatoxins and cyclopiazonic acied by an isolate of *Aspergillus flavus* in surface agar culture. Appl. Environ. Microbiol. 63, 1048-1053.

Hell, K., Cardwell, K.F., Setamou, M., and Poehling, H.M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. J Stored Prod Res 36, 365–382.

Hema, R., Kumaravel, S., & Elanchezhiyan, N. (2009). Antimicrobial activity of some of the South-Indian spices and herbals against food pathogens. Glob J Pharmacol, 3(1), 38-40.

Herzallah, S., Alshawabkeh, K., & Fataftah, A. A. (2008). Aflatoxin decontamination of artificially contaminated feeds by sunlight, γ-radiation, and microwave heating. J Appl Poult Res, 17(4), 515-521.

Hesseltine CW, Sorenson WG, Smith M (1970). Taxonomic studies of aflatoxin–producing strains in *Aspergillus flavus* group. Mycologia 62: 123–132.

Horn, B. W., & Wicklow, D. T. (1983). Factors influencing the inhibition of aflatoxin production in corn by *Aspergillus niger*. Can. J. of Microbiol. 29(9), 1087-1091.

Horn, C. P., Dalal, R. C., Birch, C. J., & Doughton, J. A. (1996). Sowing time and tillage practice affect chickpea yield and nitrogen fixation. 2. Nitrogen accumulation, nitrogen fixation and soil nitrogen balance. Australian Journal of Experimental Agriculture, 36(6), 701-706.

Hotchkiss, J. H. (1995). Safety considerations in active packaging. In Active food packaging (pp. 238-255). Springer, Boston, MA.

Hsieh, D. P., & Wong, J. J. (1994). Pharmacokinetics and excretion of aflatoxins. The Toxicology of Aflatoxins: Human health, veterinary, and agricultural significance, 73-88.

Hu, F., Ran, Y., Zhou, Z., & Gao, M. (2006). Preparation of bioconjugates of CdTe nanocrystals for cancer marker detection. Nanotechnology, 17(12), 2972.

IARC. (1993). Aflatoxin In Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC monograph on the evaluation of carcinogenic risk to humans, Vol. 56 (pp. 245-395). Lyon: International Agency for Research on Cancer

IARC (1995) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 62, Wood Dust and Formaldehyde, Lyon, IARC Press, pp. 217–362

ILSI Europe Report on Packaging Materials: 3. Polypropylene as a Packaging Material for Foods and Beverages. https://ilsi.eu/publication/packaging-materials-3-polypropylene-as-a-packaging-for-food-and-beverages/

Ito, Y., Peterson, S.W., Wicklow, D., Goto, T., 2001. *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section flavi. Mycol. Res. 105, 233–239.

Jaime-Garcia, R., and Cotty, P. J. 2006. Spatial relationships of soil texture and crop rotation to *Aspergillus flavus* community structure in South Texas. Phytopathology 96;599-607.

Jinap, S., De Rijk, T. C., Arzandeh, S., Kleijnen, H. C. H., Zomer, P., Van der Weg, G., & Mol, J. G. J. (2012). Aflatoxin determination using in-line immunoaffinity chromatography in foods. Food Control, 26(1), 42-48.

Jinks, J. L. 1969. Selection for adaptability to new environment in *Aspergillus glaucus*. J. Gen. Microbiol. 20: 223-236.

Joglekar, A. M., May, A. T., Graf, E., & Saguy, I. (1987). Product excellence through experimental design. Food product and development: From concept to the marketplace, 211.

John, P. J. (2010). A handbook on food packaging. Daya Publishing House. .

Johnson, W.W. and Guengerich, F.P. (1997) Reaction of aflatoxin B1 exo-8,9-epoxide with DNA: Kinetic analysis of covalent binding and DNA-induced hydrolysis. Proc. natl Acad. Sci. USA, 94, 6121–6125

Joshua, H. (1993) Determination of aflatoxins by reversed-phase high- performance liquid chromatography with post-column in-line photochemical derivatization and fluorescence detection. J Chromatogr A, 654 (1993) 247-254

Juan, C., Zinedine, A., Moltó, J. C., Idrissi, L., and Mañes, J. (2008). Aflatoxins levels in dried fruits and nuts from Rabat-Salé area, Morocco. Food Control, 19(9), 849–853. https://doi.org/10.1016/j.foodcont.2007.08.010

Karian, H. (Ed.). (2003). Handbook of polypropylene and polypropylene composites, revised and expanded. CRC press.

Kennedy, L., & Devereau, A. D. (1994). Observations on large-scale outdoor maize storage in jute and woven polypropylene sacks in Zimbabwe. In Proc. 6th International Working Conference on Stored-Product Protection (Canberra, Australia). CAB International, Wallingford, UK (pp. 290-295).

Khuri, A. I., & Cornell, J. A. (2018). Response surfaces: designs and analyses. Routledge.

Koehler, P. E., Hanlin, R. T., and Beraha, L. (1975). Production of aflatoxins B1 and G1 by *Aspergillus flavus* and *Aspergillus parasiticus* isolated from market pecans. Appl Microbiol , 30(4), 581–583.

Kong, Q., Shan, S., Liu, Q., Wang, X., & Yu, F. (2010). Biocontrol of *Aspergillus flavus* on peanut kernels by use of a strain of marine *Bacillus megaterium*. Int J Food Microbiol, 139(1-2), 31-35.

Kumagai, S., Nakajima, M., Tabata, S. E., Ishikuro, E., Tanaka, T., Norizuki, H., et al. (2008). Aflatoxin and ochratoxin A contamination of retail food and intake of these mycotoxins in Japan. Food Addit Contam , 25(9), 1101-1106.

Kumar, V., Basu, M. S., and Rajendran, T.P. (2008). Mycotoxin research and mycoflora in some commercially important agriculture commodities. Crop Prot, 27(6), 891-905.

Lahouar, A., Marin, S., Crespo-Sempere, A., Saïd, S., and Sanchis, V. (2016). Effects of temperature, water activity and incubation time on fungal growth and aflatoxin B1 production by toxinogenic *Aspergillus flavus* isolates on sorghum seeds. Rev Argent Microbiol, 48(1), 78–85. https://doi.org/10.1016/j.ram.2015.10.001

Lamberti, M., & Escher, F. (2007). Aluminium foil as a food packaging material in comparison with other materials. Food Rev Int, 23(4), 407-433.

Lawellin, D. W., Grant, D. W., and Joyee, B. K., (1977). Enzyme – Linked Immunosorbent analysis of aflatoxin B1. Appl. Environ. Microbiol. 34: 94.

Leblanc, JC, Tard, A, Volatier, JL and Verger, P. (2005). Estimated dietary exposure to principal food mycotoxins from the first French total diet study. Food Addit Contam, 22: 652–672.

Leong, Y. H., Ismail, N., Latiff, A. A.. Rosma, A. (2010) Aflatoxin occurrence in nuts and commercial nutty products in Malaysia. Food Control 21(3):334-338. DOI: 10.1016/j.foodcont.2009.06.002

Leong, Y. H., Ismail, N., Latiff, A. A., Nurul Izzah, A., Narazah, M. Y., and Nurul Ain, A. B. (2011). Nuts consumption pattern among Malaysian adults: A socio-demographic and dietary behaviour perspective. International Food Research Journal, 18(1), 319–328.

Liu, X., Guan, X., Xing. F., Lv, C., Dai, X.,Liu, Y. (2016) Effect of water activity and temperature on the growth of *Aspergillus flavus*, the expression of aflatoxin biosynthetic genes and aflatoxin production in shelled peanuts. Food Control 82 (2017) 325-332 https://doi.org/10.1016/j.foodcont.2017.07.012

Lu, H., Feng, Z. and Feng, X. (2008). Polymorphisms of CYP3A7 Gene of 210 Persons in Guangxi with Heavy Aflatoxin B1 Contamination, Carcinogenesis, Teratogenesis and Mutagenesis 20, 294-298.

Lye, M.S.; Ghazali, A.A.; Mohan, J.; Alwin, N. and Nair, R.C. (1995): "An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia." Am. J. Trop. Med. Hyg., 53 (1): 68-72

Malaysian Food Regulations (1985). Regulation 39 (Microorganisms and Their Toxins); Fifteenth Schedule; Table II (Mycological Contaminant), pp. 242.

Malaysian Meteorological Department (2017) Daily report on relative humidity for meteorological stations in Malaysia. Malaysian Ministry of Science, Technology and Innovations. www.met.gov.my/relativehumidity. Accessed Jan 2017

Magan, N., and Lacey, J. (1984). Effect of water activity, temperature and substrate on interactions between field and storage fungi. Transactions of the British Mycological Society, 82(1), 83–93. https://doi.org/10.1016/S0007-1536(84)80214-4

Magan, N., and Aldred, D. (2007). Post-harvest control strategies: Minimizing mycotoxins in the food chain. Int J Food Microbiol , 119(1–2), 131–139. https://doi.org/10.1016/j.ijfoodmicro.2007.07.034

Mason, R. L., Gunst, R. F., & Hess, J. L. (2003). Statistical design and analysis of experiments: with applications to engineering and science (Vol. 474). John Wiley & Sons.

Mateles, R. I., & Adye, J. C. (1965). Production of aflatoxins in submerged culture. Appl Microbiol, 13(2), 208-211.

Mayer, Z., Bagnara, A., Färber, P., and Geisen, R. (2003). Quantification of the copy number of nor-1, a gene of the aflatoxin biosynthetic pathway by real-time PCR, and its correlation to the cfu of *Aspergillus flavus* in foods. Int J Food Microbiol, 82(2), 143–151. https://doi.org/10.1016/S0168-1605(02)00250-7

Medina, Á., Rodríguez, A., Sultan, Y., and Magan, N. (2015). Climate change factors and Aspergillus flavus : effects on gene expression, growth and aflatoxin production. World Mycotoxin J, 8(2), 171–179. https://doi.org/10.3920/WMJ2014.1726

Meka, P., & Stehling, F. C. (1994). Heat sealing of semicrystalline polymer films. I. Calculation and measurement of interfacial temperatures: Effect of process variables on seal properties. J. Appl. Polym. Sci., 51(1), 89-103.

Milani, J.M. (2013) Ecological conditions affecting mycotoxin production in cereals: a review. VET MED-CZECH, 58, 2013 (8): 405–411.

Mirhosseini, H., Tan, C., Hamid, N., Yusof, S., and Chern, B. (2009). Characterization of the influence of main emulsion components on the physicochemical properties of orange beverage emulsion using response surface methodology. Food Hydrocoll., 23(2), 271-280.

Misra, J.B. (2004) A mathematical approach to comprehensive evaluation of quality in groundnut. J Food Compost Anal 17 (2004) 69–79

MOH (Ministry of Health Malaysia). (2006). Food consumption statistics in Malaysia. p.6.

Montgomery, D. (2001). Design and analysis of experiments (5th ed.). New York, USA: John Wiley and Sons Inc.

Moss, M. O., & Frank, M. (1987). Prevention: effects of biocides and other agents on mycotoxin production. Natural toxicants in food: progress and prospects/edited by DH Watson.

Mutegi, C., Wagacha, M., Kimani, J., Otieno, G., Wanyama, R., Hell, K., & Christie, M. E. (2013). Incidence of aflatoxin in peanuts (*Arachis hypogaea* Linnaeus) from markets in Western, Nyanza and Nairobi Provinces of Kenya and related market traits. J Stored Prod Res, 52, 118-127.

Myers, R. H., Montgomery, D. C., Vining, G. G., Borror, C. M., & Kowalski, S. M. (2004). Response surface methodology: a retrospective and literature survey. J. Qual. Technol., 36(1), 53-77.

Mykkänen, H., Zhu, H., Salminen, E., Juvonen, R. O., Ling, W., Ma, J., ... & El-Nezami, H. (2005). Fecal and urinary excretion of aflatoxin B1 metabolites (AFQ1, AFM1 and AFB- N7- guanine) in young Chinese males. Int J Cancer, 115(6), 879-884.

Magan, N. Cayley, G.R., Lacey, J. (1984) Effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. Appl. Environ. Microbiol., 47(5), 1113-1117.

Magan, N., Aldred, D. (2007) Post-harvest control strategies: Minimizing mycotoxins in the food chain. Int. J. Food Microbiol. 119 (2007) 131–139

Nakai, V. K., Rocha de, L. O., Gonçalez, E., Fonseca, H., Ortega, E. M. M., and Corrêa, B. (2008). Distribution of fungi and aflatoxins in a stored peanut variety. Food Chem. , 106, 285-290.

Nayak, S., Sashidhar, R.B. Metabolic intervention of aflatoxin B1 toxicity by curcumin. J. Ethnopharmacol. 127 (2010) 641–644.

Nkama, I., Nobbs, J. H., and Muller, H. G. (1987) Destruction of aflatoxin B, in rice exposed to light, J. Cereal Sci., 5, 167.

Norlia, M., Nor-Khaizura, M. A. R., Selamat, J., Abu Bakar, F., Radu, S., & Chin, C. K. (2018). Evaluation of aflatoxin and *Aspergillus sp*. contamination in raw peanuts and peanut-based products along this supply chain in Malaysia. Food Addit Contam Part A, 35(9), 1787-1802.

Northolt, M. D., Van Egmond, H.P., and Paulsch, W.E., (1977). Differences between *Aspergillus flavus* strains in growth and aflatoxin B1 production in relation to water activity and temperature, J. Food Prot. 40: 778.

Okano K, Tomita T, Chonan M (2003). Aflatoxins inspection in groundnuts imported into Japan in 1994–2000. Mycotoxins, 53: 25–29.

Okello, D. K., Monyo, E., Deom, C. M., Ininda, J., and Oloka, H. K. (2013). Groundnuts production guide for Uganda: Recommended practices for farmers. National Agricultural Research Organisation, Entebbe. ISBN, 978-9970.72

Omer, R.E., Bakker, M.I., van't Veer, P., Hoogenboom, R.L.A.P., Polman, T.H.G., Alink, G.M., Idris, M.O., Kadaru, A.M.Y. and Kok, F.J. (1998) Aflatoxin and liver cancer in Sudan. Nutr. Cancer, 32, 174–180

Park, K.Y., Bullerman, L.B., (1983). Effects of Substrate and Temperature on Aflatoxin Production by *Aspergillus parasiticus* and *Aspergillus flavus*. J. Food. Prot. 1983 Mar;46(3):178-184. doi: 10.4315/0362-028X-46.3.178

Park, D. L., & Liang, B. (1993). Perspectives on aflatoxin control for human food and animal feed. Trends Food Sci. Technol., 4(10), 334-342.

Parvathi, K., Uma, V., Begum, M. S., and Wesely, E. G. (2014). Detection of fungal pathogens from maize seeds of central Tamil Nadu, India. J. Sci., 4(11), 682–685.

Passone, M.A., Resnik, S., Etcheverry, G.M. (2007) Antiaflatoxigenic property of food grade antioxidants under different conditions of water activity in peanut grains. Int. J. Food Microbiol. 118 (2007) 8–14.

Paterson, R. R., & Lima, N. (2010). Toxicology of mycotoxins. Molecular, clinical and environmental toxicology, 31-63.

Payne, G.A.and Brown, M.P. (1998). Genetics And Physiology of Aflatoxin Biosynthesis. Annu. Rev. Phytopathol. 1998. 36:329–362

Peterson S.W. (2000). Phylogenetic relationships in Aspergillus based on rDNA sequence analysis. In: Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification, Samson RA, Pitt JI, eds. Amsterdam: Harwood Academic Publishers: 323–356.

Peterson S.W. (2008). Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia, 100(2), 2008, pp. 205–226.

Pietrasik, Z., and Li-Chan, E. (2002). Response surface methodology study on the effects of salt, microbial transglutaminase and heating temperature on pork batter gel properties. Food Res. Int. , 35(4), 387-396.

Pildain, M.B., Vaamonde, G., Cabral, D., 2004. Analysis of population structure of *Aspergillus flavus* from peanut based on vegetative compatibility, geographic origin, mycotoxin and sclerotia production. Int. J. Food Microbiol. 93, 31–40

Pitt JI, Hocking AD, Bhudhasamai K, Miscamble BF, Wheeler KA, Tanboon-Ek P (1993) The normal mycoflora of commodities from Thailand. 1.Nuts and oilseeds. Int J Food Microbiol 20:211–226.

Pitt, J.I. and A.D. Hocking. 1996. Current knowledge of fungi and mycotoxin assessment of food commodities in South East Asia. In: Highley, E. and G.I. Johnson (eds.). pp. 5-10. ACIAR Technical Reports 37. Mycotoxin Contamination in Grains. Paper presented at the 17"' ASEAN Technical Seminar on Grain Postharvest Technology. Lumut, Malaysia, 25-27 July 1995.

Pitt, J. I., & Hocking, A. D. (2009). Fungi and food spoilage (Vol. 519, p. 388). New York: Springer.

Polychronaki, N. (2007). Biomarkers of aflatoxin exposure and a dietary intervention: studies in infants and children from Egypt and Guinea and young adults from China (Lasten altistuminen aflatoksiinille äidinmaidon välityksellä kehitysmaissa ja aflatoksiinialtistuksen estäminen probiootti-intervention avulla) (Doctoral dissertation, Kuopion yliopisto).

Popa, C., Dumitras D.C., Patachia M. and Banita S. (2014) Testing fruit quality by photoacoustic spectroscopy assay Laser Phys. 24 (105702).

Popa C. (Achim), Petrus M. (2017). Heavy metals impact at plants using photoacoustic spectroscopy technology with tunable CO2 laser in the quantification of gaseous molecules. Microchem. J. Vol. 134,p. 390-399.

Pratiwi, C., Rahayu, W. P., Lioe, H. N.,Herawati, D., Broto, W. and Ambarwati, S. (2015). The effect of temperature and relative humidity for *Aspergillus flavus* BIO 2237 growth and aflatoxin production on soybeans. Int Food Res J 22(1): 82-87.

Probst, C., Njapau, H., and Cotty, P.J. (2007) Outbreak of an Acute Aflatoxicosis in Kenya in 2004: Identification of the Causal Agent. Appl. Environ. Microbiol., Apr. 2007, p. 2762–2764

Proctor, A. D., Ahmedna*, M., Kumar, J. V., & Goktepe, I. (2004). Degradation of aflatoxins in peanut kernels/flour by gaseous ozonation and mild heat treatment. Food Additives and Contaminants, 21(8), 786-793.

Reddy, K.R.N., Reddy, C.S., Muralidharan, K., (2009). Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food Control 20, 173–178.

Revoredo, C. L., and Fletcher, S. M. (2002). World Peanut Market : An Overview of the Past 30 Years. Research Bulletin, The University of Georgia, (437).

Rodrigues, P., Soares, C., Kozakiewicz, Z., Paterson, R. R. M., and Lima, N. (2007). Identification and characterization of *Aspergillus flavus* and aflatoxins. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 527–534.

Rustom, I. Y. S. (1997). Aflatoxin in food and feed: Occurrence legislation and inactivation by physical methods. Food Chem., 59, 57–67.

Saleemullah, Iqbal, A., Khalil, I. A., and Shah, H. (2006). Aflatoxin contents of stored and artificially inoculated cereals and nuts. Food Chem., 98(4), 699–703. https://doi.org/10.1016/j.foodchem.2005.06.034

Samapundo, S., Devlieghere, F., De Meulenaer, B., and Debevere, J. (2007). Growth kinetics of cultures from single spores of *Aspergillus flavus* and *Fusarium verticillioides* on yellow dent corn meal. Food Microb., 24(4), 336–345. https://doi.org/10.1016/j.fm.2006.07.020.

Sanchis V, Magan N (2004) Environmental profiles for growth and mycotoxin production. In: Magan N, Olsen M (ed) Mycotoxins in Food: Detection and Control. Cambridge, UK: Woodhead Publishing Ltd., pp 174-89.

Sangare-Tigori, B., Moukha, S., Kouadio, H.J., Betbeder, A.M., Dano, D.S., Creppy, E.E. (2006). Co-occurrence of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone in cereals and peanuts from Côte d'Ivoire. Food Addit. Contam. 23, 1000-1007.

Scheidegger K.A., Payne G.A. (2003). Unlocking the secrets behind secondary metabolism: a review of *Aspergillus flavus* from pathogenicity to functional genomics. J Toxicol Toxin Rev 22:423– 259

Schroeder HW, Hein H (1967) Aflatoxins: production of the toxins in vitro in relation to temperature. Appl Microbiol 15:441–445

Scudamore, K. A., Patel, S., and Breeze, V. (1999), Surveillance of stored grain from the 1997 harvest in the United Kingdom for ochratoxin A. Food Additives and Contaminants,16, 281- 290.

Seifert, K.A.and Levesque, C.A. (2004). Phylogeny and molecular diagnosis of mycotoxigenic fungi. European Journal of Plant Pathology 110: 449–471, 2004.

Shakerardekani, A., and Karim, R. (2013b). Effect of different types of plastic packaging films on the moisture and aflatoxin contents of pistachio nuts during storage. J. Food Sci. Technol., 50(2), 409–411. https://doi.org/10.1007/s13197-012-0624-0

Sharma, A., Behere, A. G., Padwal-Desai, S. R., and Nadkarni, G. B. (1980). Influence of inoculum size of *Aspergillus parasiticus* spores on aflatoxin production. Appl. Environ. Microbiol., 40(6), 989–993.

Sharma, R. A., and Farmer, P. B. (2004). Biological relevance of adduct detection to the chemoprevention of cancer. Clin. Cancer Res., 10(15), 4901-4912.

Siriacha, P., Kawashima, K., Saito, M., Tonboon-Ek, P., and Buangsuwon, D. (1990). Prevention of Thai maize from the infection by *Aspergillus flavus* and aflatoxin contamination in various packages. In the Proceedings of the Japanese Association of Mycotoxicology, 32, 41–46.

Smith, J. E., & Moss, M. O. (1985). Mycotoxins. Formation, analysis and significance. John Wiley & Sons Ltd..

Somashekar, D., Rati E.R, Anand S., Chandrashekar A. (2004) Isolation, enumeration and PCR characterization of aflatoxigenic fungi from food and feed samples in India. Food Microbiol 21:809– 813

Soon, N. K., Chin, L. H., and Ahmad, A. R. (2017). Quality assurance practices of the food manufacturers in Malaysia. Advanced Science Letters, 23(1), 317–321.

Srivatanakul, P., Ohshima, H., Khlat, M., Parkin, M., Sukaryodhin, S,, Brouet, I. and Bartsch, H. (1991) Opisthorchis viverrini infestation and endogenous nitrosamines as risk factors for cholangiocarcinorna in Thailand. lut. J. Cancer, 48, 821-825

Stehling FC, Meka P (1994) Heat sealing of semicrystalline polyer films. II. Effect of melting distribution on heat sealing behavior of polyolefins. J Appl Polym Sci 51:105–119.

Sulaiman, M. R., Chye, F. Y., Hamid, A., and Yatim, A. M. (2007). The occurrence of aflatoxins in raw shelled peanut samples from three districts of Perak, Malaysia. Elec. J. Env. Agricult. Food Chem., 6(5), 2045–2052.

Sumner P. E., and Lee D. (2012). Reducing Aflatoxin in Corn during Harvest and Storage. Atlanta, GA: The University of Georgia, Georgia College of Agriculture and Environmental Sciences.

Sun, D., She, J., Gower, J., Stokes, C., Windham, G., Baird, R., and Mlsna, T. (2016). Effects of Growth Parameters on the Analysis of *Aspergillus flavus* Volatile Metabolites. Separations, 3(2), 13.

Sweeney, M.J., Dobson, A.D.W., (1998). Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. Int. J. Food Microbiol. 43, 141–158.

Szymanska, K., Chen, J. G., Cui, Y., Gong, Y. Y., Turner, P. C., Villar, S., and Hainaut, P. (2009). TP53 R249S mutations, exposure to aflatoxin, and occurrence of hepatocellular carcinoma in a cohort of chronic hepatitis B virus carriers from Qidong, China. Cancer Epidemiology and Prevention Biomarkers, 18(5), 1638-1643.

TFO (2010) Session 4: plastic packaging films and laminates; properties, specifications and purchasing. Presented during the TFO Canada workshop of procurement of packaging for exports.Guyana, pp 1–39.

Truckness MW (2000) AOAC Official Method 991.31, Aflatoxin in corn, raw peanuts, and peanut butter. J AOAC Int 49:22–24.

Trung, T. S., Tabuc, C., Bailly, S., Querin, A., Guerre, P., and Bailly, J. D. (2008). Fungal mycoflora and contamination of maize from Vietnam with aflatoxin B1 and fumonisin B1. World Mycotoxin J., 1(1), 87–94. https://doi.org/10.3920/WMJ2008.x010.

Turner, N. W., Subrahmanyam, S., and Piletsky, S. A. (2009). Analytical methods for determination of mycotoxins: a review. Anal. Chim. Acta, 632,168-180.

Udoh, J.M., Cardwell, K.F., and Ikotun, T. (2000) Storage structures and aflatoxin content of maize in five agro-ecological zones of Nigeria. J. Stored Prod. Res. 36, 187-201.

USDA, United States Department of Agriculture, (2007). Rule 7 CFR Part 981. Almonds grown in California, outgoing quality control requirements. Docket n. FV06-981-1 FR.

Varga, J., Juhász, Á., Kevei, F., and Kozakiewicz, Z. (2004). Molecular diversity of agriculturally important *Aspergillus* species. In Molecular Diversity and PCR-detection of Toxigenic *Fusarium* Species and Ochratoxigenic Fungi (pp. 627-640). Springer, Dordrecht.

Villa P, Markaki P. 2009. Aflatoxin B1 and ochratoxin A in breakfast cereals from athens market: occurrence and risk assessment. Food Control 20:455–61.

Wang, J. S., & Groopman, J. D. (1999). DNA damage by mycotoxins. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 424(1-2), 167-181.

Wei, D.-L., and Jong, S.-C. (1986). Production of aflatoxins by strains of the *Aspergillus flavus* group maintained in ATCC. Mycopathologia, 93(1), 19–24.

Wicklow, D. T., and Cole, R. J. (1982). Tremorgenic indole metabolites and aflatoxins in sclerotia of *Aspergillus flavus*: an evolutionary perspective. Can J Bot, 60(5), 525-528.

Wicklow, D. T., Bobell, J. R., and Palmquist, D. E. (2003). Effect of intraspecific competition by *Aspergillus flavus* on aflatoxin formation in suspended disc culture. Mycol. Res. , 107(5), 617-623.

Wild, C. P., and Turner, P. C. (2002). The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis, 17, 471–481.

Wild, C.P.and Gong Y.Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis 31:71-82.

Woloshuk, C. P. and Prieto, R. (1998) Genetic organisation and function of the aflatoxin B1 biosynthetic genes. FEMS Microbiol. Lett. 160: 169–176.

Wu, Q., Jezkova, A., Yuan, Z., Pavlikova, L., Dohnal, V., and Kuca, K. (2009). Biological degradation of aflatoxins. Drug Metab. Rev. , 41(1), 1-7.

Yang, J., Li, J., Jiang, Y., Duan, X., Qu, H., Yang, B., Sivakumar, D. (2014). Natural occurrence, analysis, and prevention of mycotoxins in fruits and their processed products. Crit. Rev. Food Sci. Nutr., 54(1), 64–83. https://doi.org/10.1080/10408398.2011.569860.

Yentűr, G., Er, B., Gűr Ozkan, M., and Bayhan Oktem, A. (2006). Determination of aflatoxins in peanut butter and sesame samples using high-performance liquid chromatography method. Eur. Food Res. Technol., 224(2), 167-170

Yousef, A. E., and Marth, E. H. (1981). Growth and synthesis of aflatoxin by *Aspergillus parasiticus* in the presence of sorbic acid. J. Food Prot., 44(10), 736-741.

Yousef, A. E., and Marth, E. H. (1983). Incorporation of [14 C] acetate into aflatoxin by resting cultures of *Aspergillus parasitic*us in the presence of antifungal agents. Eur. J. Appl. Microbiol. , 18(2), 103-108.

Zarba A, Wild CP, Hall AJ, Montesano R, Hudson GJ, Groopman JD. (1992). Aflatoxin M1 in human breast milk from The Gambia, West Africa, quantified by combined monoclonal antibody immunoaffinity chromatography and HPLC. Carcinogenesis 13:891–894.

Zhang, T., Shi, Z.Q., Hu, L.B., Cheng, L.G., Wang, F. (2007). Antifungal compounds from Bacillus subtilis B-FS06 inhibiting the growth of *Aspergillus flavus*. World J. Microbiol. Biotechnol. 24, 783–788.

Zhang L, Sun D.W., Zhang Z (2017). Methods for measuring water activity (a_w) of foods and its applications to moisture sorption isotherm studies. Crit Rev Food Sci Nutr 57:1052-1058.

BIODATA OF STUDENT

Joshua Mark a/l John was born on 7th March 1990 in Kuala Lumpur, Malaysia. He completed his primary education level of education at Sekolah Rendah Kebangsaan Taman Klang Jaya, Klang and secondary level at Sekolah Menengah Kebangsaan Raja Mahadi, Klang. He furthered his education at Sekolah Menengah Kebangsaan Shahbandaraya, Klang for Form 6, Sijil Tinggi Pelajaran Malaysia (STPM). Upon completion of STPM, he was offered a Bachelor in Biomedical Science (Hons.) at Management and Science University, Malaysia in the year 2009. He graduated with a second class upper in 2013.

In 2014, he was given a scholarship to pursue his Master Degree in Food Science at Universiti Putra Malaysia.

LIST OF PUBLICATIONS

John, J.M., Jinap, S., Nor Khaizura, M.A.R., Samsudin N.I.P., Hanani Z.A.N. (2019) The effects of different packaging materials, temperatures and water activities to control aflatoxin B_1 production by *Aspergillus flavus* and *A. parasiticus* in stored peanuts. *J Food Sci Technol* **56,** 3145–3150 (2019) doi:10.1007/s13197-019-03652-6 – published

John, J.M., Jinap, S., Nor Khaizura, M.A.R., Samsudin N.I.P., Hanani Z.A.N The effects of the gas barrier characteristics of different food grade packaging materials, storage time, relative humidity, and inoculum level on the growth of *Aspergillus flavus* and *A.parasiticus* and aflatoxins production in air packed stored peanuts- in preparation for submission

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION: Second Semester 2020/2021

TITLE OF THESIS / PROJECT REPORT:

EFFECT OF PACKAGING AND STORAGE ON *Aspergillus* spp. AND AFLATOXIN PRODUCTION IN PEANUTS

NAME OF STUDENT: JOSHUA MARK A/L JOHN

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as:

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
√	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for:

☐	**PATENT**	Embargo from _____ until _____ (date) (date)

Approved by:

_____	_____
(Signature of Student)	(Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.:	Name:
Date:	Date:

[Note: If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]