EVALUATION, CHARACTERISATION AND MODELLING OF Aspergillus flavus IN AFLATOXIN PRODUCTION IN PEANUTS ALONG THE SUPPLY CHAIN

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EVALUATION, CHARACTERISATION AND MODELLING OF *Aspergillus flavus* IN AFLATOXIN PRODUCTION IN PEANUTS ALONG THE SUPPLY CHAIN

By

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

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

Aflatoxin contamination is a major food safety issue in raw peanuts and peanut-based products worldwide. Thus, an extensive study on aflatoxins and *Aspergillus* spp. in peanuts along the supply chain in Malaysia is needed in order to protect the consumers against the harmful effects of aflatoxins. Generally, this study was aimed to evaluate the aflatoxin contamination, identify, characterise and model the growth of aflatoxigenic *A. flavus* isolated from peanuts collected from the importers, manufacturers, and retailers. In the present study, aflatoxins were found to be significantly higher ($p < 0.05$) in raw peanuts and peanut-based products from the retailers (< LOD – 1021.4 µg/kg) followed by the manufacturers (< LOD – 181.9 µg/kg) while samples from the importers were free from aflatoxins. Total fungal count was relatively higher in raw peanuts (log 0.3 – 3.6 CFU/g) as compared to peanut-based products (log 0.6 – 2.3 CFU/g) in which samples from the importers recorded the highest contamination level for aflatoxigenic *Aspergillus* spp. (log 2.2 ± 1.1 CFU/g). On the basis of morphological, chemical, and molecular identification, all isolates were identified as *Aspergillus* section *Flavi*. Specifically, 127 isolates were confirmed as *A. flavus*, and one isolate as *A. tamarii*. Six chemotype profiles were proposed indicating the diversity of toxigenic potential. About 58.6%, 68.5%, and 100% of the isolates were positive for aflatoxin, cyclopiazonic acid and aspergillic acid production, respectively. The maximum likelihood (ML) phylogenetic tree using ITS and β-tubulin gene resolved the species into two different clades in which all *A. flavus* (both aflatoxigenic and non-aflatoxigenic) were grouped in the same clade and *A. tamarii* in a different clade. Aflatoxin biosynthesis genes namely *aflR*, *aflP* (*omtA*), *aflD* (*nor-1*), *aflM* (*ver-1*), and *aflC* (*pksA*) were detected in all aflatoxigenic *A. flavus* while the non-aflatoxigenic *A. flavus* failed to amplify at least one of the genes that was tested. The analysis of variance showed a significant effect of strain, temperature and water activity (a_w) on the fungal growth and aflatoxin production ($p < 0.05$). The maximum growth rate, μ_{max} (mm/day) of two aflatoxigenic *A. flavus*, (A8R and A82R)

on PMEA was estimated by using the primary model of Baranyi and the μ_{max} was then fitted to the secondary model; second order polynomial and linear Arrhenius-Davey to describe the growth rate as a function of temperature and a_w. In general, the growth rate of *A. flavus* increased with increasing temperature and a_w until reaching the optimum temperature and further increase in a_w beyond this point resulted in decrease growth rate. The growth of *A. flavus* was observed at the minimum a_w of 0.85 under the optimum temperature (32 – 33°C) and the minimum temperature of 20°C with 0.94 a_w. A similar pattern was observed in aflatoxin production but in a narrower range of temperature (25 – 35°C) and a_w (0.92 – 0.98 aw). In conclusion, *A. flavus* was the predominant species that contaminate peanuts and subsequently produce aflatoxins during the storage period. Therefore, proper storage structures and conditions for peanuts during storage are very important in order to control the growth of aflatoxigenic *A. flavus* and aflatoxin contamination.

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

PENILAIAN, PENCIRIAN DAN PERMODELAN *Aspergillus flavus* TERHADAP PENGHASILAN AFLATOKSIN DALAM KACANG TANAH DI SEPANJANG RANTAIAN BEKALAN

Oleh

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Aflatoksin merupakan isu keselamatan makanan yang utama dalam kacang tanah mentah dan produk berasaskan kacang tanah di seluruh dunia. Oleh itu, kajian menyeluruh mengenai kontaminasi aflatoksin dan *Aspergillus* spp. dalam kacang tanah di sepanjang rantaian bekalan amat diperlukan untuk melindungi pengguna daripada kesan bahaya aflatoksin. Secara amnya, kajian ini bertujuan untuk menilai tahap kontaminasi aflatoksin, mengenalpasti, mencirikan dan memodelkan pertumbuhan *A. flavus* yang bersifat aflatoksigenik daripada kacang tanah yang dikumpul dari pengimport, pengilang, dan peruncit. Dalam kajian ini, kontaminasi aflatoksin didapati lebih tinggi ($p < 0.05$) dalam kacang tanah mentah dan produk berasaskan kacang tanah daripada peruncit (< LOD – 1021.4 μg/kg) diikuti oleh pengeluar (< LOD – 181.9 μg/kg) manakala sampel dari pengimport bebas daripada aflatoksin. Jumlah kulat juga adalah lebih tinggi pada kacang tanah mentah (log 0.3 – 3.6 CFU/g) berbanding dengan produk berasaskan kacang tanah (log 0.6 – 2.3 CFU/g) di mana sampel dari pengimport mencatatkan tahap kontaminasi tertinggi bagi *Aspergillus* spp. yang aflatoksigenik (log 2.2 ± 1.1 CFU/g). Berdasarkan ciri-ciri morfologi, kimia dan molekul, semua isolat telah dikenalpasti sebagai *Aspergillus* section *Flavi*. Secara khusus, 127 isolat disahkan sebagai *A. flavus*, dan satu isolat sebagai *A. tamarii*. Enam profil *Chemotype* telah dicadangkan bagi menunjukkan kepelbagaian potensi toksigenik di mana kira-kira 58.6%, 68.5%, dan 100% daripada isolat adalah positif untuk aflatoksin, *cyclopiazonic acid* dan *aspergillic acid*. Pohon filogenetik *Maximum Likelihood* (ML) yang menggunakan jujukan ITS dan β-tubulin memisahkan spesies menjadi dua klad yang berbeza di mana semua *A. flavus* (termasuk yang aflatoksigenik dan tidak aflatoksigenik) dikelompokkan dalam klad yang sama dan *A. tamarii* dalam klad yang berasingan. Gen biosintesis aflatoksin, *aflR*, *aflP* (*omtA*), *aflD* (*nor-1*), *aflM* (*ver-1*), dan *pksA* dikesan dalam semua *A. flavus* yang aflatoksigenik manakala *A. flavus* yang tidak aflatoksigenik gagal menunjukkan kehadiran sekurang-kurangnya satu gen yang diuji. Analisis varians menunjukkan kesan signifikan strain, suhu dan a_w pada pertumbuhan kulat dan pengeluaran

aflatoksin ($p < 0.05$). Kadar pertumbuhan maksimum, μ_{max} (mm/hari) bagi dua *A. flavus* yang aflatoksigenik (A8R dan A82R) pada PMEA dianggarkan dengan menggunakan model utama Baranyi dan μ_{max} kemudiannya dimasukkan ke dalam model kedua; *second-order polynomial* dan *linear Arrhenius-Davey* untuk menggambarkan kadar pertumbuhan sebagai fungsi suhu dan a_w. Secara umumnya, kadar pertumbuhan *A. flavus* meningkat dengan peningkatan suhu dan a_w sehingga mencapai suhu optimum dan selepas itu kadar pertumbuhan akan menurun. *A. flavus* berupaya tumbuh pada tahap minimum 0.85 a_w di bawah suhu optimum (32 – 33°C) dan suhu minimum 20°C dengan 0.94 a_w. Corak yang sama diperhatikan dalam pengeluaran aflatoksin tetapi pada julat yang lebih sempit (25 – 35°C) dan a_w (0.92 – 0.98 a_w). Kesimpulannya, *A. flavus* adalah spesies utama yang mencemari kacang tanah dan seterusnya menghasilkan aflatoksin semasa tempoh penyimpanan. Oleh itu, struktur dan keadaan penyimpanan yang sesuai untuk kacang tanah adalah sangat penting untuk mengawal pertumbuhan *A. flavus* yang aflatoksigenik dan kontaminasi aflatoksin.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AFPA	*Aspergillus flavus* and *Aspergillus parasiticus* agar
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
a_w	Water activity
BLAST	Basic Local Alignment Search Tool
CAC	Codex Alimentarius Commission
CFU	Colony Forming Unit
CPA	Cyclopiazonic acid
CYA	Czapek Yeast Agar
CZ	Czapek Dox Agar
DG18	Dichloran 18% glycerol agar
EU	European Union
FAO	Food and Agriculture Organization of the United Union
FAOSTAT	FAO Statistical Databases (United Nations)
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
HCC	Hepatocellular carcinoma
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ITS	Internal Transcribed Spacer
kg	Kilogram
LOD	Limit of detection
LOQ	Limit of quantification
MEA	Malt Extract Agar
MeOH	Methanol
MOH	Ministry of Health
ML	Maximum Likelihood
mL	Millilitre

NCBI	National Center for Biotechnology Information
ng	Nanogram
PDA	Potato Dextrose Agar
PHRED	Photochemical reactor for enhanced detection
PMEA	Peanut Meal Extract Agar
ppb	Part per billion
USDA	The U.S. Department of Agriculture
WHO	World Health Organization
µL	Microlitre
°C	Degree celcius
°K	Degree Kelvin

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Peanuts are not a major agricultural product in Malaysia. Almost 95% of the peanuts in the Malaysian market are imported from other countries such as India, China and Vietnam (Afsah-Hejri et al., 2013). Aflatoxins are carcinogenic compounds that are produced mainly by *Aspergillus flavus* and *A. parasiticus* and have been found to be a major problem in peanuts. To date, several analogues of aflatoxins have been identified and characterised with aflatoxin B_1 (AFB_1) being classified as Group 1 carcinogen mostly associated with the development of liver cancer as reported by the International Agency for Research on Cancer (IARC, 1993). In addition, it is also known to cause chronic and serious health diseases known as aflatoxicosis, which is prevalent among children and the elderly. Several studies have found that liver is the main target of aflatoxin and liver cancer is dominant in developing countries that produce and consume peanuts (Liu and Wu, 2010).

The consumption of high concentration of aflatoxins in foods can be fatal. The largest and most severe case of acute aflatoxicosis outbreak was reported in the Eastern and Central Kenya due to aflatoxin's contamination in commercial maize products (Lewis et al., 2005). The outbreak was occurred in the rural area which resulting in 317 cases and 125 deaths. Aflatoxin outbreak in Malaysia was reported in 1988 which caused the deaths of 13 children upon consuming contaminated Chinese noodles known as *loh shi fun*. It was revealed through post-mortem reports that the death was associated with acute encephalopathy. A study by Lye et al. (1995) reported that an estimated 3 mg of aflatoxins were detected in a single serving of that noodle.

Upon realising the potential threats of aflatoxins to health, many countries have therefore established regulations on the amount of aflatoxins in foods and food products (Egmond and Jonker, 2004). Among these, peanuts receive the greatest attention as they are naturally susceptible to *Aspergillus* spp. contamination and the subsequent production of aflatoxins (Waliyar et al., 2015; Gnonlonfin et al., 2013). In this regard, Malaysia has set a maximum limit of 10 µg/kg and 15 µg/kg for total aflatoxin in ready-to-eat peanuts and raw peanuts for further processing, respectively (Food Act, 1983 Food (Amendment) (No.3) Regulations 2014). These regulations were established to help protect the consumers against the harmful effects of aflatoxins by preventing the compounds from entering the peanut supply chain in the country.

Several researchers have estimated the dietary exposure of aflatoxins among the Malaysian population (Chin et al., 2012; Leong et al., 2011; Arzandeh et al., 2010; Leong et al., 2010). For AFB_1, Chin et al. (2012) reported the dietary exposure of 24.3 to 34.0 ng/kg b.w./day. Among 236 food composites tested, peanuts were found to be the main contributor to aflatoxin contamination. Based on this finding, the liver cancer

risk among Malaysian was estimated to be 0.61 – 0.85% cancers/100,000 population/year which contributed to 12.4 –17.3% of the liver cancer cases.

Even though the current maximum regulatory limit was reported to be adequate in protecting Malaysians' health against aflatoxin, the chronic exposure is still a concern (Chin et al., 2012). Based on Malaysian Food Consumption Statistics 2014, the mean daily intake of peanuts for the total population and among the eaters were 1.86 g/day and 4.95 g/day, respectively as shown in Table 1.1. Generally, the Malays recorded the highest intake for both peanuts and peanut butter. High intake of aflatoxin-contaminated foods for a long period of time will lead to a chronic exposure and increase the risk of hepatocellular carcinoma (HCC), or commonly known as liver cancer.

Table 1.1: Peanuts and peanut butter consumption statistics in Malaysia, 2014.

	Estimated mean intake for total population (g/day)		**Estimated mean intake among eaters (g/day)**	
	Peanuts	**Peanut butter**	**Peanuts**	**Peanut butter**
Total	1.86	1.09	4.95	6.74
Urban	2.15	1.13	5.73	6.43
Rural	1.19	-	3.6	-
Male	2.49	1.22	6.28	8.79
Female	1.17	0.95	3.61	5.07
Ethnicity				
Malay	1.71	1.09	4.82	6.57
Chinese	-	0.97	-	5.30
India	-	-	-	-
Others	-	-	-	5.74

Source: Food Consumption Statistics of Malaysia, Ministry of Health, Malaysia 2014. (IPH, 2014).

A. flavus and *A. parasiticus* are the main aflatoxin' producers during pre- and post-harvest stages of peanuts. Various strategies have been applied to control the growth of these aflatoxigenic fungi and subsequently reduce the risk of aflatoxins in peanuts. Aflatoxin management strategies in the field have been reviewed extensively (Waliyar et al., 2015; Torres et al., 2014; Dorner, 2008). However, aflatoxins are still the main concern in the imported peanuts as the aflatoxins could not be eliminated from the products once they are contaminated (Torres et al., 2014; Zorzete et al., 2013; Nakai et al., 2008). Although the aflatoxin regulation in each country could help to protect the consumers from the risk of aflatoxins in the imported peanuts, the presence of aflatoxigenic fungi in the products might increase the chance of aflatoxin production and accumulation in peanuts during storage especially at the manufacturer' and retailer's stages.

As a peanut-importing country, Malaysia is more concern on aflatoxin production and contamination during storage as Malaysia's tropical weather favours fungal growth including that of aflatoxigenic fungi (Sulaiman et al., 2007). In addition, the precise identification and characterisation of the aflatoxigenic fungi that could survive and proliferate on the imported peanuts are less studied as compared to the peanuts in the field (Zhang et al., 2017; Pildain et al., 2008; Pildain et al., 2004). To date, there are very few available data in the literatures on the occurrence of aflatoxigenic fungi on the imported peanuts especially in Malaysia.

A study by Guezlane-tebibel et al. (2013) on imported peanuts from China marketed in Algiers reported that *Aspergillus* section *Flavi* occurred the highest with 79.3% of the isolates were highly toxigenic. Three strains of *Aspergillus* section *Flavi* (*A. flavus*, *A. minisclerotigenes* and *A. caelatus*) were identified through the polyphasic approach which included morphological, chemical and molecular techniques. These results indicated that these species were able to survive and contaminate the imported peanuts. In addition, the presence of aflatoxin biosynthesis genes in the aflatoxigenic strains of *Aspergillus* section *Flavi* could be used to confirm the ability of these fungi to produce aflatoxin (Lee et al., 2006).

The predictive modelling approach for fungi as proposed by Garcia et al. (2009) is used to describe the effect of temperature and water activity on the growth of aflatoxigenic fungi and aflatoxins production in peanuts during storage. Such model might be used in the food industry to predict whether the aflatoxigenic fungi are capable to grow when a product is stored under certain conditions (Dantigny et al., 2005). Besides, it could also be used to predict to what extends the fungal growth and aflatoxin might be affected if any changes in temperature or humidity occur during storage. This information is crucial in order to understand the behaviour of the aflatoxigenic fungi in peanuts under certain conditions which in turn could be used as the basis on the improvement of storage condition to ensure the safety and quality of raw peanuts. For *in vitro* study, a food-analogue agar medium using the respective food sample is usually used as the growth medium to study the kinetic growth of fungi such as the growth of *A. flavus* from corn on corn extract medium (Astoreca et al., 2012), *A. ochraceus* on green-coffee based medium (Pardo et al., 2005), and *A. carbonarius* on grape juice agar medium (Tassou et al., 2007).

1.2 Problem statement

In Malaysia, even though aflatoxin contamination in peanuts has been reported, no further studies have been conducted to address this issue (Arzandeh et al., 2010; Hong et al., 2010; Leong et al., 2010). Indeed, the exposure of aflatoxin in Malaysia has also been reported by several researchers (Mohd-Redzwan et al., 2013; Leong et al., 2011). The optimum temperature for *A. flavus* (28 – 35°C) and *A. parasiticus* (25 – 35°C) (Rustom, 1997) are very close to the average temperature in Malaysia (28 – 30°C). This indicates a high probability of the peanuts being contaminated under this condition. As an importing country, peanuts marketed in Malaysia might be contaminated during storage if the storage temperature, relative humidity and the moisture content of peanuts are not monitored.

The importers, manufacturers and retailers are the three main peanut stakeholders in the supply chain in Malaysia. To date, there is no report on the occurrence of aflatoxins in peanuts along the supply chain in Malaysia especially at the importer's and manufacturer's stages except for Farawahida et al. (2018) who reported on aflatoxin and *Aspergillus* spp. contamination in peanuts and peanuts sauces from different manufacturer. The available data on the occurrence of aflatoxins in foodstuffs are mainly on the samples collected from the retailers, and most of the findings revealed high levels of aflatoxin especially in peanuts and peanut-based products (Chin et al., 2012; Reddy et al., 2011; Arzandeh et al., 2010; Leong et al., 2010; Abidin et al., 2003; Ali, 2000). Therefore, more investigations are required in order to identify the critical points of aflatoxin contamination along the peanut supply chain in Malaysia. It is important to know at which stage the *Aspergillus* spp. and aflatoxin contamination begin, and what the possible causes are. Even though aflatoxins are not easily eliminated from the food supply chain, the information will be useful to be used as a database in the development of intervention strategies to control aflatoxin in foodstuffs.

Besides, the effect of storage condition such as temperature and water activity on the growth of aflatoxigenic fungi isolated from imported peanuts are also less documented as compared to the species isolated from peanuts at the post-harvest stage (Waliyar et al., 2015; Torres et al., 2014). In addition, previous researches were only focusing on the peanut-producing countries in the African region (Mutegi et al., 2013a; Wagacha et al., 2013a). According to Waliyar et al. (2015), the optimal bulk storage condition for peanut kernels at post-harvest stage was by maintaining the kernel moisture of <7.5%, temperature of 10°C, and relative humidity of 65%. For the unshelled peanuts, higher moisture content (9%), relative humidity (70%), and temperature (25 – 27°C) were allowed to prevent the aflatoxigenic fungal growth and allow a safe storage of peanuts up to one year for export purpose.

However, the optimal condition could not be maintained during shipping, transportation, and storage at the manufacturer's or retailer's premises due to the fluctuated temperature, inadequate ventilation and condensation which might occur along such processes (Wagacha and Muthomi, 2008). In this case, there is a possibility for re-emergence of the aflatoxigenic fungi in the peanuts once they reached the importing countries. Thus, it is important to identify and characterise the fungal species that could survive in the importing countries and evaluate their ability to re-produce the aflatoxin. Besides, the fungal predictive modelling is also the best approach to describe the effect of temperature and water activity on the growth of aflatoxigenic fungi isolated from the imported peanuts (Garcia et al., 2009).

Due to these reasons, extensive research focusing on the occurrence, identification, and characterisation of these contaminants in peanuts along the supply chain is highly needed in order to evaluate the safety of imported peanuts and to recommend relevant interventions related to the aflatoxin problem.

1.3 Significance of the study

The data and evidence obtained from this study will contribute to a better control of aflatoxin in Malaysia. Data and information from this study can be used by the decision maker or the authority such as the Ministry of Health to target for an intervention such as ensuring a proper enforcement of regulation and conducting a regular surveillance and monitoring in peanuts along the supply chain.

1.4 Objective

1.4.1 General objectives

Generally, this study was aimed to evaluate the aflatoxin contamination, identify, characterise, and model the growth of aflatoxigenic *Aspergillus* spp. found in raw peanuts and peanut-based products collected from the importers, manufacturers, and retailers along the supply chain in Malaysia.

1.4.2 Specific objectives

i. To determine the level of aflatoxins and *Aspergillus* spp. contamination in raw peanuts and peanut-based products and their critical point along the supply chain.
ii. To isolate, identify, and characterise the *Aspergillus* section *Flavi* based on the morphological and chemical approach.
iii. To determine the phylogenetic relationships among the *Aspergillus* section *Flavi* strains and detect the presence of aflatoxin biosynthesis genes.
iv. To model the growth of *A. flavus* as a function of temperature and water activity on peanut-based agar medium (*in vitro*) and determine the aflatoxin production.

1.5 Research approach

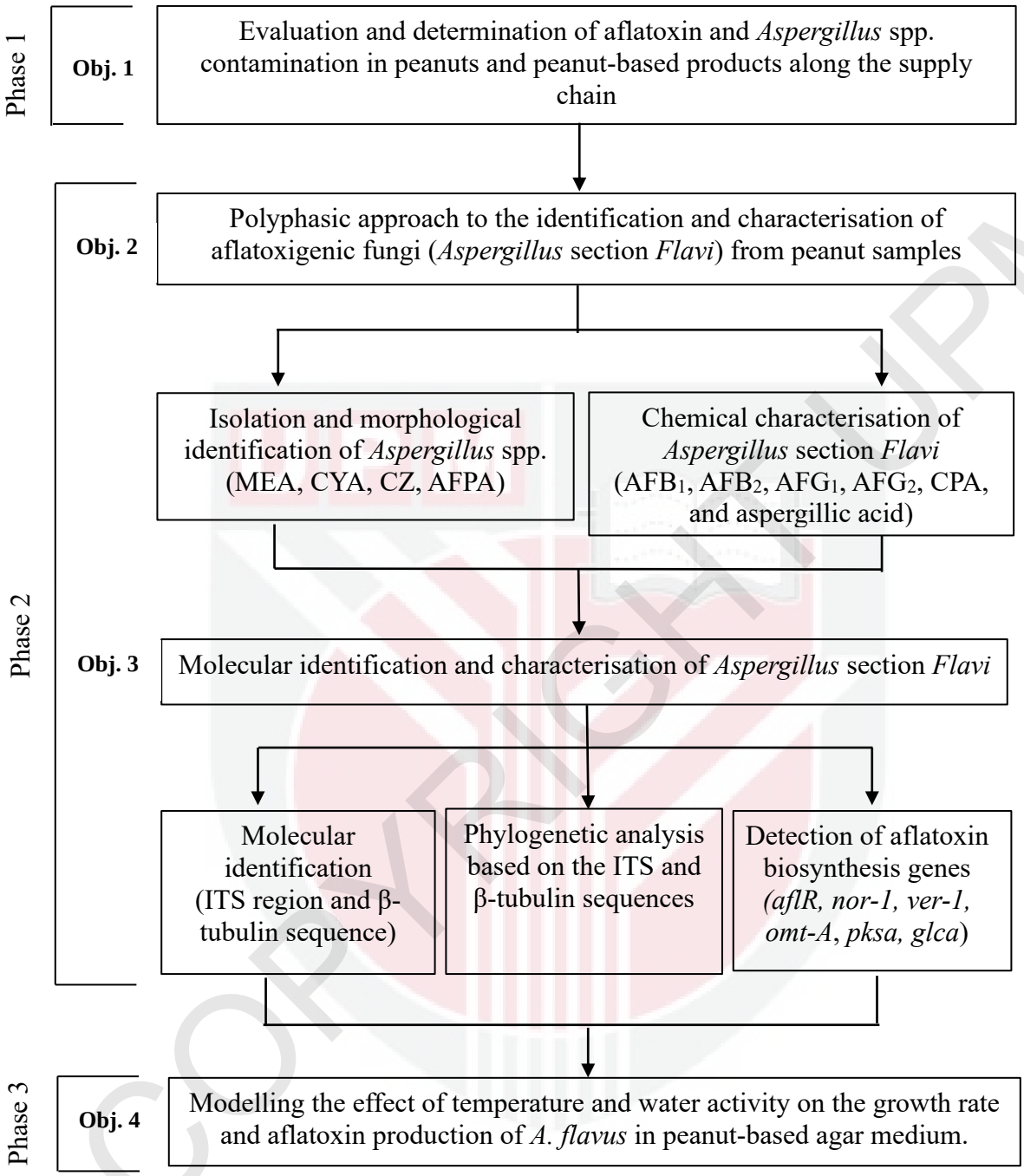

Figure 1.1: Flow chart of the study.

REFERENCES

Abbas, H. K., Accinelli, C., Zablotowicz, R. M., Abel, C. A., Bruns, H. A., Dong, Y., & Shier, W. T. (2008). Dynamics of mycotoxin and *Aspergillus flavus* levels in aging Bt and non-Bt corn residues under Mississippi no-till conditions. *Journal of Agricultural and Food Chemistry*, *56*, 7578–7585.

Abbas, H. K., Wilkinson, J. R., Zablotowicz, R. M., Accinelli, C., Abel, C. A., Bruns, H. A., & Weaver, M. A. (2009). Ecology of *Aspergillus flavus*, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. *Toxin Reviews*, *28*, 142–153.

Abdel-Hadi, A., Carter, D., & Magan, N. (2010). Temporal monitoring of the *nor-1 (aflD)* gene of *Aspergillus flavus* in relation to aflatoxin B_1 production during storage of peanuts under different water activity levels. *Journal of Applied Microbiology*, *109*, 1914–1922.

Abdel-Hadi, A., Carter, D., & Magan, N. (2011). Discrimination between aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* section *Flavi* group contaminationg Egyption peanuts using molecular and analystical techniques. *World Mycotoxin Journal*, *4*, 69–77.

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*. *Journal of The Royal Society Interface*, *9*, 757–767.

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

Abidin, H., Rosni, S. M., & Hazniza, A. (2003). Status of aflatoxin contamination in groundnut from five districts in Perak. *Journal of Tropical Agriculture and Food Science*, *31*, 199–205.

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*, 381–388.

Afsah-Hejri, L., Jinap, S., Hajeb, P., Radu, S., & Shakibazadeh, S. (2013a). A review on mycotoxins in food and feed: Malaysia case study. *Comprehensive Reviews in Food Science and Food Safety*, *12*, 629–651.

Afsah-Hejri, L., Jinap, S., & Radu, S. (2013b). Occurrence of aflatoxins and aflatoxigenic *Aspergillus* in peanuts. *Journal of Food, Agriculture and Environment*, *11*, 228–234.

Ainiza, W. M. W., Jinap, S., & Sanny, M. (2015). Simultaneous determination of aflatoxins and ochratoxin A in single and mixed spices. *Food Control*, *50*, 913–918.

Aisyah, S., Safika, & Jamin, F. (2015). Determination of aflatoxin B_1 in peanut food products by Enzyme-Linked Immunosorbent Assay (ELISA). *Jurnal Kedokteran Hewan*, *9*, 38–41.

Al-Muhtaseb, A. H., McMinn, W. A. M., & Magee, T. R. A. (2002). Moisture sorption isotherm characteristics of food Products: A review. *Food and Bioproducts Processing*, *80*, 118–128.

Alakali, J. S., & Satimehin, A. A. (2007). Moisture adsorption characteristics of Bambara groundnut (*Vigna subterranea*) powders. *Agricultural Engineering International*, *9*, 1–15.

Aldars-garcía, L., Berman, M., Ortiz, J., Ramos, A. J., & Marín, S. (2018a). Probability models for growth and aflatoxin B_1 production as affected by intraspecies variability in *Aspergillus flavus*. *Food Microbiology*, *72*, 166–175.

Aldars-garcía, L., Marín, S., Sanchis, V., Magan, N., & Medina, A. (2018b). Assessment of intraspecies variability in fungal growth initiation of *Aspergillus flavus* and aflatoxin B_1 production under static and changing temperature levels using different initial conidial inoculum levels. *International Journal of Food Microbiology*, *272*, 1–11.

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

Ambarwati, S., Dharmaputra, O. S., & Retnowati, I. (2011). Dietary exposure assessment for aflatoxin B_1 from processed peanut products in municipality of Bogor. *Biotropia*, *18*, 1–12.

Anukul, N., Vangnai, K., & Mahakarnchandkul, W. (2013). Significance of regulation limits in mycotoxin contamination in Asia and risk management programs at the national level. *Journal of Food and Drug Analysis*, *21*, 227–241.

Archer, P. (2016). Overview of the Peanut Industry Supply Chain. In H. T. Stalker & R. F. Wilson (Eds.), *Peanuts: Genetics, Processing, and Utilization* (pp. 253–266). VA, USA: Elsevier Inc.

Arzandeh, S., Selamat, J., & Lioe, H. (2010). Aflatoxin in raw peanut kernels marketed in Malaysia. *Journal of Food and Drug Analysis*, *18*, 44–50.

Astoreca, A., Vaamonde, G., Dalcero, A., Ramos, A. J., & Marín, S. (2012). Modelling the effect of temperature and water activity of *Aspergillus flavus* isolates from corn. *International Journal of Food Microbiology*, *156*, 60–67.

Azaman, N. N. M., Kamarulzaman, N. H., Shamsudin, M. N., & Selamat, J. (2016). Stakeholders' knowledge, attitude, and practices (KAP) towards aflatoxins contamination in peanut-based products. *Food Control*, *70*, 249–256.

Azri, F. A., Sukor, R., Hajian, R., Yusof, N. A., Bakar, F. A., & Selamat, J. (2017). Modification strategy of screen-printed carbon electrode with functionalized multi-walled carbon nanotube and chitosan matrix for biosensor development. *Asian Journal of Chemistry*, *29*, 31–36.

Azri, F. A., Sukor, R., Selamat, J., Bakar, F. A., Yusof, N. A., & Hajian, R. (2018). Electrochemical immunosensor for detection of aflatoxin B_1 based on indirect competitive ELISA. *Toxins*, *10*, 1–13.

Bakhiet, S. E. A., & Musa, A. A. A. (2011). Survey and determination of aflatoxin levels in stored peanut in Sudan. *Jourdan Journal of Biological Sciences*, *4*, 13–20.

Baquião, A. C., De Oliveira, M. M. M., Reis, T. A., Zorzete, P., Diniz Atayde, D., & Correa, B. (2013). Polyphasic approach to the identification of *Aspergillus* section *Flavi* isolated from Brazil nuts. *Food Chemistry*, *139*, 1127–1132.

Baranyi, J., Csernus, O., & Beczner, J. (2014). Error analysis in predictive modelling demonstrated on mould data. *International Journal of Food Microbiology*, *170*, 78–82.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology*, *23*, 277–294.

Barros, G. G., Chiotta, M. L., Reynoso, M. M., Torres, A. M., & Chulze, S. N. (2007). Molecular characterization of *Aspergillus* section *Flavi* isolates collected from peanut fields in Argentina using AFLPs. *Journal of Applied Microbiology*, *103*, 900–909.

Bediako, K. A., Ofori, K., Offei, S. K., Dzidzienyo, D., Asibuo, J. Y., & Amoah, R. A. (2019). Aflatoxin contamination of groundnut (*Arachis hypogaea* L.): Predisposing factors and management interventions. *Food Control*, *98*, 61–67.

Bekada, A. M. A., Benakriche, B., Hamadi, K., & Bensoltane, A. (2008). Modelling of effects of water activity , pH and temperature on the growth rate of *Mucor racemosus* isolated from soft camembert cheese. *World Journal of Agricultural Sciences*, *4*, 790–794.

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiology*, *10*, 189.

Bernáldez, V., Córdoba, J. J., Magan, N., Peromingo, B., & Rodríguez, A. (2017). The influences of ecophysiological factors on growth, *aflR* gene expression and aflatoxin B_1 production by a type strain of *Aspergillus flavus*. *LWT-Food Science and Technology*, *83*, 283–291.

Bhatnagar, D., Cary, J. W., Ehrlich, K., Yu, J., & Cleveland, T. E. (2006). Understanding the genetics of regulation of aflatoxin production and *Aspergillus flavus* development. *Mycopathologia*, *162*, 155–166.

Bragulat, M. R., Abarca, M. L., & Cabanes, F. J. (2001). An easy screening method for fungi producing ochratoxin A in pure culture. *International Journal of Food Microbiology*, *71*, 139–144.

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

Carvajal-campos, A., Manizan, A. L., Tadrist, S., Akaki, D. K., Koffi-nevry, R., Moore, G. G., … Puel, O. (2017). *Aspergillus korhogoensis*, a novel aflatoxin producing species from the Côte d' Ivoire. *Toxins*, *9*, 1–22.

Casselton, L., & Zolan, M. (2002). The art and design of genetic screens: Filamentous fungi. *Nature Reviews Genetics*, *3*, 683–697.

Chang, A. S., Sreedharan, A., & Schneider, K. R. (2013). Peanut and peanut products: A food safety perspective. *Food Control*, *32*, 296–303.

Chang, P., Ehrlich, K. C., & Fujii, I. (2009a). Cyclopiazonic acid biosynthesis of

Aspergillus flavus and *Aspergillus oryzae*. *Toxins*, *1*, 74–99.

Chang, P., Horn, B. W., & Dorner, J. W. (2009b). Clustered genes involved in cyclopiazonic acid production are next to the aflatoxin biosynthesis gene cluster in *Aspergillus flavus*. *Fungal Genetics and Biology*, *46*, 176–182.

Chang, P. K., & Ehrlich, K. C. (2010). What does genetic diversity of *Aspergillus flavus* tell us about *Aspergillus oryzae*? *International Journal of Food Microbiology*, *138*, 189–199.

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

Chauhan, Y. S., Wright, G. C., Rachaputi, R. C. N., Holzworth, D., Broome, A., Krosch, S., & Robertson, M. J. (2010). Application of a model to assess aflatoxin risk in peanuts. *Journal of Agricultural Science*, *148*, 341–351.

Chen, C. Y., Li, W. J., & Peng, K. Y. (2005). Determination of aflatoxin M_1 in milk and milk powder using high-flow solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, *53*, 8474–8480.

Chen, Y. C., Liao, C. D., Lin, H. Y., Chiueh, L. C., & Shih, D. Y. C. (2013). Survey of aflatoxin contamination in peanut products in Taiwan from 1997 to 2011. *Journal of Food and Drug Analysis*, *21*, 247–252.

Chin, C. K., Aminah, A., & Sugitha-Konishi, Y. (2012). Dietary intake of aflatoxins in the adult Malaysian population - an assessment of risk. *Food Additives & Contaminants: Part B*, *5*, 1–9.

Christensen, O. M., Kaufmann, H. H., & Feinstein, L. (1969). Characteristics of Field and Storage Fungi. In *Grain Storage: The role of fungi in quality loss* (pp. 17–35). Minnesota, USA: University of Minnesota Press.

Codex Stan CXS 193-1995. Codex Alimentarius International Food Standards, General Standard for Contaminants and Toxins in Food and Feed. Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO). Retrieved 9 March 2018 from www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCODEX%2BSTAN%2B193-1995%252FCXS_193e.pdf

Cole, R. J., Hill, R. A., Blankenship, P. D., & Sanders, T. H. (1986). Color mutants of *Aspergillus flavus* and *Aspergillus parasiticus* in a study of preharvest invasion of peanuts. *Applied and Environmental Microbiology*, *52*, 1128–1131.

Commission Regulation (EC) No. 165/2010 of 26 February 2010 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official Journal of the European Union. L50/8eL50/12, Luxembourg. Retrieved 8 August from http://www.fsai.ie/uploadedFiles/Reg165_2010.pdf.

Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of

mycotoxins in foodstuffs. Retrieved 28 August 2017 from http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:070:0012:0034:EN:PDF

Corthell, J. T. (2014). Agarose Gel Electrophoresis. In J. T. Corthell (Ed.), *Basic Molecular Protocols in Neuroscience: Tips, Tricks, and Pitfalls* (pp. 21–25). Academic Press.

Cotty, P. J. (1989). Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. *Phytopathology*.

Cotty, P. J., & Cardwell, K. F. (1999). Divergence of West African and North American communities of *Aspergillus* section *Flavi*. *Applied and Environmental Microbiology*, *65*, 2264–2266.

Criseo, G., Bagnara, A., & Bisignano, G. (2001). Differentiation of aflatoxin-producing and non-producing strains of *Aspergillus favus* group. *Letters in Applied Microbiology*, *33*, 291–295.

Cunha, S. C., Sá, S. V. M., & Fernandes, J. O. (2018). Multiple mycotoxin analysis in nut products: Occurrence and risk characterization. *Food and Chemical Toxicology*, *114*, 260–269.

Dallyn, H., & Fox, A. (1980). Spoilage of Materials of Reduced Water Activity by Xerophilic Fungi. In G. W. Gould & J. E. L. Corry (Eds.), *Society of Applied Bacteriology Technical Series* (pp. 129–139). UK: Academic Press.

Dantigny, P. (2016). Relevant issues in predictive mycology. *Current Opinion in Food Science*, *11*, 29–33.

Dantigny, P., Guilmart, A., & Bensoussan, M. (2005). Basis of predictive mycology. *International Journal of Food Microbiology*, *100*, 187–196.

Dantigny, P., Marín, S., Beyer, M., & Magan, N. (2007). Mould germination: Data treatment and modelling. *International Journal of Food Microbiology*, *114*, 17–24.

Davari, E., Mohsenzadeh, M., Mohammadi, G., & Rezaeian-Doloei, R. (2015). Characterization of aflatoxigenic *Aspergillus flavus* and *A. parasiticus* strain isolates from animal feedstuffs in northeastern Iran. *Iranian Journal of Veterinary Research*, *16*, 150–155.

Davey, K. R. (1989). A predictive model for combined temperature and water activity on microbial growth during the growth phase. *Journal of Applied Bacteriology*, *67*, 483–488.

Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N., & Panneerselvam, A. (2011). Aflatoxins and Aflatoxicosis in Human and Animals. In R. G. Guevara-González (Ed.), *Aflatoxins - Biochemistry and Molecular Biology* (pp. 221–254). Croatia: InTech.

Dijksterhuis, J., Houbraken, J., & Samson, R. A. (2013). Fungal Spoilage of Crops and Food. In F. Kempken (Ed.), *Agricultural Applications. The Mycota (A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research)* (pp. 36–56). Berlin, Heidelberg: Springer.

Dorner, J. W. (2008). Management and prevention of mycotoxins in peanuts. *Food Additives & Contaminants: Part A*, *25*, 203–208.

Edwards, S. G., O'Callaghan, J., & Dobson, A. D. W. (2002). PCR-based detection and quantification of mycotoxigenic fungi. *Mycological Research*, *106*, 1005–1025.

Egmond, H. P. van, & Jonker, M. A. (2004). Worldwide regulations on aflatoxins - The situation in 2002. *Journal of Toxicology - Toxin Reviews*, *23*, 273–293.

Egmond, H. P. van, Schothorst, R. C., & Jonker, M. A. (2007). Regulations relating to mycotoxins in food. *Analytical and Bioanalytical Chemistry*, *389*, 147–157.

Ehrlich, K. C. (2014). Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: advantages and limitations. *Frontiers in Microbiology*, *5*, 1–9.

Ehrlich, K. C., Chang, P., Yu, J., & Cotty, P. J. (2004). Aflatoxin biosynthesis cluster gene *cypA* is required for G aflatoxin formation. *Applied and Environmental Microbiology*, *70*, 6518–6524.

Ehrlich, K. C., Kobbeman, K., Montalbano, B. G., & Cotty, P. J. (2007). Aflatoxin-producing *Aspergillus* species from Thailand. *International Journal of Food Microbiology*, *114*, 153–159.

Einax, E., & Voigt, K. (2003). Oligonucleotide primers for the universal amplification of β-tubulin genes facilitate phylogenetic analyses in the regnum fungi. *Organisms Diversity and Evolution*, *3*, 185–194.

Emmott, A. (2012). *Technical Report : Value Chain Approach - Aflatoxin (Groundnuts) Final Report*. Southern Africa.

Erami, M., Hashemi, S., Pourbakhsh, S., Shahsavandi, S., Mohammadi, S., Shooshtari, A., & Jahanshiri, Z. (2007). Application of PCR on detection of aflatoxinogenic fungi. *Archives of Razi Institute*, *62*, 95–100.

Ezekiel, C. N., Sulyok, M., Babalola, D. A., Warth, B., Ezekiel, V. C., & Krska, R. (2013). Incidence and consumer awareness of toxigenic *Aspergillus* section *Flavi* and aflatoxin B_1 in peanut cake from Nigeria. *Food Control*, *30*, 596–601.

Ezekiel, C. N., Sulyok, M., Warth, B., Odebode, A. C., & Krska, R. (2012). Natural occurrence of mycotoxins in peanut cake from Nigeria. *Food Control*, *27*, 338–342.

Farawahida, A. H. (2018). *Prevalence and Control of Aspergillus spp. and Aflatoxins in Peanut Sauce during Food Procesing.* MSc Thesis, Universiti Putra Malaysia.

Farawahida, A. H., Jinap, S., Nor-Khaizura, M. A. R., & Samsudin, N. I. P. (2017). Reduction of *Aspergillus* spp. and aflatoxins in peanut sauce processing by oil-less frying of chilli powder and retort processing. *Food Additives & Contaminants: Part A*, *34*, 2242–2250.

FAOSTAT (2017). Food and Agriculture Data. Rome, Italy. Food and Agriculture Organization of the United Nations. Retrieved 2 May 2018 from http://www.fao.org/faostat/

Fonseca, H. (2002). Sampling plan for the analysis of aflatoxin in peanuts and corn: An update. *Brazilian Journal of Microbiology, 33*, 97–105.

Food Act 1983. Food (Amendment) (No. 3) Regulations 2014. Retrieved 25 August 2017 from http://fsq.moh.gov.my/v5/images/filepicker_users/5ec35272cb-78/Perundangan/Seranta/18062014/Draf-Pindaan-PPM-1985-Bil3-2014.pdf

FoSIM (2017). Food Safety Information System of Malaysia. Ministry of Health. Retrieved 25 August 2017 from http://fsis2.moh.gov.my/fosimv2/HOM/frmHOMPage.aspx

Frisvad, J. C., Hubka, V., Ezekiel, C. N., Hong, S. B., Nováková, A., Chen, A. J., … Houbraken, J. (2019). Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. *Studies in Mycology*, *93*, 1–63.

Frisvad, J. C., Thrane, U., & Pitt, R. S. I. (2006). Important mycotoxins and the fungi which produce them. *Advances in Experimental Medicine and Biology*, *571*, 3–31.

Gachomo, E. W., Mutitu, E. W., & Kotchoni, O. S. (2004). Diversity of fungal species associated with peanuts in storage and the levels of aflatoxins in infected samples. *International Journal of Agriculture & Biology*, *6*, 955–959.

Gallo, A., Stea, G., Battilani, P., Logrieco, A. F., & Perrone, G. (2012). Molecular characterization of an *Aspergillus flavus* population isolated from maize during the first outbreak of aflatoxin contamination in Italy. *Phytopathologia Mediterranea*, *51*, 198–206.

Gams, W., Christensen, M., Onions, A. H., Pitt, J. I., & Samson, R. A. (1986). Infrageneric Taxa of *Aspergillus*. In R. A. Samson & J. I. Pitt (Eds.), *Advances in Penicillium and Aspergillus Systematics* (pp. 55–62). Springer US.

Garcia, D., Ramos, A. J., Sanchis, V., & Marín, S. (2009). Predicting mycotoxins in foods: A review. *Food Microbiology*, *26*, 757–769.

Garcia, D., Ramos, A. J., Sanchis, V., & Marín, S. (2011). Modelling the effect of temperature and water activity in the growth boundaries of *Aspergillus ochraceus* and *Aspergillus parasiticus*. *Food Microbiology*, *28*, 406–417.

Geiser, D. M., Dorner, J. W., Horn, B. W., & Taylor, J. W. (2000). The phylogenetics of mycotoxin and sclerotium production in *Aspergillus flavus* and *Aspergillus oryzae*. *Fungal Genetics and Biology*, *31*, 169–179.

Geiser, D. M., Pitt, J. I., & Taylor, J. W. (1998). Cryptic Speciation and Recombination in the Aflatoxin-producing Fungus *Aspergillus flavus*. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 95, pp. 388–393).

Gibson, A. M., Baranyi, J., Pitt, J. I., Eyles, M. J., & Roberts, T. A. (1994). Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. *International Journal of Food Microbiology*, *23*, 419–431.

Gibson, A. M., & Hocking, A. D. (1997). Advances in the predictive modelling of fungal growth in food. *Trends in Food Science and Technology*, *8*, 353–258.

Ginting, E., Rahmianna, A. A., & Yusnawan, E. (2017). Aflatoxin and Nutrient Contents of Peanut Collected from Local Market and Their Processed Foods. In *IOP Conference Series: Earth and Environmental Science* (pp. 1–8). Indonesia: IOP.

Giorni, P., Magan, N., Pietri, A., Bertuzzi, T., & Battilani, P. (2007). Studies on *Aspergillus* section *Flavi* isolated from maize in northern Italy. *International Journal of Food Microbiology*, *113*, 330–338.

Glass, N. L., & Donalson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology*, *61*, 1323–1330.

Gnonlonfin, G. J. B., Hell, K., Adjovi, Y., Fandohan, P., Koudande, D. O., Mensah, G. A., … Brimer, L. (2013). A review on aflatoxin contamination and its implications in the developing world: A sub-Saharan African perspective. *Critical Reviews in Food Science and Nutrition*, *53*, 349–365.

Godet, M., & Munaut, F. (2010). Molecular strategy for identification in *Aspergillus* section *Flavi*. *FEMS Microbiology Letters*, *304*, 157–168.

Gonçalves, J. S., Ferracin, L. M., Vieira, M. L. C., Iamanaka, B. T., Taniwaki, M. H., & Fungaro, M. H. P. (2012). Molecular analysis of *Aspergillus* section *Flavi* isolated from Brazil nuts. *World Journal of Microbiology and Biotechnology*, *28*, 1817–1825.

Goto, T., Wicklow, D. T., & Ito, Y. (1996). Aflatoxin and cyclopiazonic acid production by a sclerotium-producing *Aspergillus tamarii* strain. *Applied and Environmental Microbiology*, *62*, 4036–4038.

Gourama, H., & Bullerman, L. B. (1995). *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds: A Review. *Journal of Food Protection*, *58*, 1395–1404.

Groopman, J. D., Kensler, T. W., & Wild, C. P. (2008). Protective interventions to prevent aflatoxin-induced carcinogenesis in developing countries. *Annual Review of Public Health*, *29*, 187–203.

Guezlane-tebibel, N., Bouras, N., Mokrane, S., Benayad, T., & Mathieu, F. (2013). Aflatoxigenic strains of *Aspergillus* section *Flavi* isolated from marketed peanuts (*Arachis hypogaea*) in Algiers (Algeria). *Annals of Microbiology*, *63*, 295–305.

Hoeltz, M., Einloft, T. C., Oldoni, V. P., Dottori, H. A., & Noll, I. B. (2012). The occurrence of aflatoxin B_1 contamination in peanuts and peanut products marketed in southern brazil. *Brazilian Archives of Biology and Technology*, *55*, 313–317.

Hong, L. S., Yusof, N. I. M., & Ling, H. M. (2010). Determination of aflatoxins B_1 and B_2 in peanuts and corn based products. *Sains Malaysiana*, *39*, 731–735.

Horn, B., Sorensen, R., Lamb, M., Sobolev, V., Olarte, R., Worthington, C., & Carbone, I. (2014). Sexual reproduction in *Aspergillus flavus* sclerotia naturally produced in corn. *Phytopathology*, *104*, 75–85.

Horn, B. W. (2007). Biodiversity of *Aspergillus* section *Flavi* in the United States: A review. *Food Additives and Contaminants*, *24*, 1088–1101.

Houbraken, J., Vries, R. P. De, & Samson, R. A. (2014). Modern taxonomy of biotechnologically important *Aspergillus* and *Penicillium* species. *Advances in Applied Microbiology*, *86*, 199–249.

IARC. (1993). Aflatoxins. In *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans* (Vol. 56, pp. 245–395). Lyon, France: International Agency for Research on Cancer.

Ibáñez-vea, M., Ana, L., Remiro, R., Murillo-arbizu, M. T., González-peñas, E., & Lizarraga, E. (2011). Validation of a UHPLC-FLD method for the simultaneous quantification of aflatoxins, ochratoxin A and zearalenone in barley. *Food Chemistry*, *127*, 351–358.

IPH. (2014). *National Health and Morbidity Survey 2014: Malaysian Adult Nutrition Survey (MANS): Vol. III: Food Consumption Statistics of Malaysia*. *Institute for Public Health, Ministry of Health, Malaysia*. Kuala Lumpur.

Ismail, N. A., Mohd, M. H., Mohd, N., Mohamed, I., & Zakaria, L. (2017). Fumonisin B_1-producing *Fusarium* species from agricultural crops in Malaysia. *Crop Protection*, *98*, 70–75.

Ito, Y., Peterson, S. W., Wicklow, D. T., & Goto, T. (2001). *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *Flavi*. *Mycological Research*, *105*, 233–239.

Jalili, M., & Jinap, S. (2012). Natural occurrence of aflatoxins and ochratoxin A in commercial dried chili. *Food Control*, *24*, 160–164.

Kachapulula, P. W., Akello, J., Bandyopadhyay, R., & Cotty, P. J. (2017). *Aspergillus* section *Flavi* community structure in Zambia influences aflatoxin contamination of maize and groundnut. *International Journal of Food Microbiology*, *261*, 49–56.

Kamarudin, N. A., & Zakaria, L. (2018). Characterization of two xerophilic *Aspergillus* spp. from peanuts (*Arachis hypogaea*). *Malaysian Journal of Microbiology*, *14*, 41–48.

Kamika, I., Ngbolua, K. te N., & Tekere, M. (2016). Occurrence of aflatoxin contamination in maize throughout the supply chain in the Democratic Republic of Congo. *Food Control*, *69*, 292–296.

Kamil, O. H., Lupuliasa, D., Draganescu, D., & Vlaia, L. (2011). Interrelations of drying heat and survival of different fungal spores within the tablets formulation. *Seria Stiintele Vietii*, *21*, 339–342.

Khayoon, W. S., Saad, B., Lee, T. P., & Salleh, B. (2012). High performance liquid chromatographic determination of aflatoxins in chilli, peanut and rice using silica based monolithic column. *Food Chemistry*, *133*, 489–496.

Kim, D. M., Chung, S. H., & Chun, H. S. (2011). Multiplex PCR assay for the detection of aflatoxigenic and non-aflatoxigenic fungi in meju, a Korean fermented soybean food starter. *Food Microbiology*, *28*, 1402–1408.

Klich, M. A. (2002). *Identification of Common Aspergillus species*. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures.

Klich, M. A. (2007a). *Aspergillus flavus*: The major producer of aflatoxin. *Molecular Plant Pathology*, *8*, 713–722.

Klich, M. A. (2007b). Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycoscience*, *48*, 71–80.

Klich, M. A., & Mullaney, E. J. (1987). DNA restriction enzyme fragment polymorphism as a tool for rapid differentiation of *Aspergillus flavus* from *Aspergillus oryzae*. *Experimental Mycology*, *11*, 170–175.

Klich, M. A., & Pitt, J. I. (1988). Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. *Transactions of the British Mycological Society*, *91*, 99–108.

Kooprasertying, P., Maneeboon, T., & Hongprayoon, R. (2016). Exposure assessment of aflatoxins in Thai peanut consumption. *Cogent Food & Agriculture*, *18*, 1–9.

Krithikadatta J. (2014). Normal distribution. *Journal of conservative dentistry: JCD*, *17*(1), 96–97. doi:10.4103/0972-0707.124171

Kumar, D., & Kalita, P. (2017). Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. *Foods*, *6*, 1–22.

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., & Sang G. Kang. (2017). Aflatoxins: A global concern for food safety, human health and their management. *Frontiers in Microbiology*, *7*, 1–10.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*, 1870–1874.

Lahouar, A., Marin, S., Crespo-Sempere, A., Saïd, S., & Sanchis, V. (2016). Effects of temperature, water activity and incubation time on fungal growth and aflatoxin B_1 production by toxinogenic *Aspergillus flavus* isolates on sorghum seeds. *Revista Argentina de Microbiologia*, *48*, 78–85.

Lansden, J. A., & Davidson, J. I. (1983). Occurrence of cyclopiazonic acid in peanuts. *Applied and Environmental Microbiology*, *45*, 766–769.

Lasram, S., Hamdi, Z., Chenenaoui, S., Mliki, A., & Ghorbel, A. (2016). Comparative study of toxigenic potential of *Aspergillus flavus* and *Aspergillus niger* isolated from Barley as affected by temperature, water activity and carbon source. *Journal of Stored Products Research*, *69*, 58–64.

Lee, C. Z., Liou, G. Y., & Yuan, G. F. (2006). Comparison of the *aflR* gene sequences of strains in *Aspergillus* section *Flavi*. *Microbiology*, *152*, 161–170.

Lee, S., Yoon, Y., Kim, D. M., Kim, D. S., Park, K. H., & Chun, H. S. (2014). Mathematical models to predict kinetic behavior and aflatoxin production of *Aspergillus flavus* under various temperature and water activity conditions. *Food Science and Biotechnology*, *23*, 975–982.

Leong, Y. H., Ismail, N., Latif, A. A., & Ahmad, R. (2010). Aflatoxin occurrence in nuts and commercial nutty products in Malaysia. *Food Control*, *21*, 334–338.

Leong, Y. H., Ismail, N., Latiff, A. A., Nurul Izzah, A., Narazah, M. Y., & Nurul Ain,

A. B. (2011). Nuts consumption pattern among Malaysian adults: A socio-demographic and dietary behaviour perspective. *International Food Research Journal*, *18*, 319–328.

Leong, Y. H., Rosma, A., Latiff, A. A., & Ahmad, N. I. (2011). Exposure assessment and risk characterization of aflatoxin B1 in Malaysia. *Mycotoxin Research*, *27*, 207–214.

Levinson, D. R. (2009). *Traceability in the Food Supply Chain*. *Department of Health and Human Services*. New York, USA.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., … Rubin, C. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicoses in Eastern and Central Kenya. *Environmental Health Perspectives*, *113*, 1763–1767.

Lipigorngoson, S., Limtrakul, P., Suttajit, M., & Yoshizawa, T. (2003). In-house direct cELISA for determining aflatoxin B_1 in Thai corn and peanuts. *Food Additives & Contaminants*, *20*, 838–845.

Lisker, N., Michaeli, R., & Frank, Z. R. (1993). Mycotoxigenic potential of *Aspergillus flavus* strains isolated from groundnuts growing in Israel. *Mycopathologia*, *122*, 177–183.

Liu, X., Guan, X., Xing, F., Lv, C., Dai, X., & Liu, Y. (2017). 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*, 325–332.

Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environmental Health Perspectives*, *118*, 818–824.

Lourenço, A., Durigon, E. L., Zanotto, P., Madeira, J. E. G. C., Almeida, A. P. De, & Correa, B. (2007). Genetic diversity of environmental *Aspergillus flavus* strains in the state of São Paulo, Brazil by random amplified polymorphic DNA. *Memórias Do Instituto Oswaldo Cruz*, *102*, 687–692.

Lye, M. S., Ghazali, A. A., Mohan, J., Alwin, N., & Nair, R. C. (1995). An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia. *American Journal of Tropical Medicine and Hygiene*, *53*, 68–72.

Ma, Z. Bin, Zhao, J. X., Wang, L. A., & Zheng, X. B. (2009). Cloning, prokaryotic expression, and bioactivity of the calmodulin gene of *Magnaporthe grisea*. *FEMS Microbiology Letters*, *300*, 107–114.

Machida, M., Yamada, O., & Gomi, K. (2008). Genomics of *Aspergillus oryzae*: learning from the history of koji mold and exploration of its future. *DNA Research*, *15*, 173–183.

Magan, N., & Lacey, J. (1985). Interactions between field, and storage fungi on wheat grain. *Transactions of the British Mycological Society*, *85*, 29–37.

Magrine, I. C. O., Ferrari, S. S. C., Souza, G. F., Minamihara, L., Kemmelmeier, C., Bando, E., & Machinski Jr., M. (2011). Intake of aflatoxins through the consumption of peanut products in Brazil. *Food Additives & Contaminants: Part*

B, *4*, 99–105.

Mahuku, G., Nzioki, H. S., Waliyar, F., Diarra, B., & Kodio, O. (2010). *Aflatoxin Prevalence Data Collection: Sampling Framework and Methodology*. *International Food Policy Research Institute*. Washington, D.C.

Manizan, A. L., Oplatowska-Stachowiak, M., Piro-Metayer, I., Campbell, K., Koffi-Nevry, R., Elliott, C., … Brabet, C. (2018). Multi-mycotoxin determination in rice, maize and peanut products most consumed in Côte d'Ivoire by UHPLC-MS/MS. *Food Control*, *87*, 22–30.

Manonmani, H. K., Anand, S., Chandrashekar, A., & Rati, E. R. (2005). Detection of aflatoxigenic fungi in selected food commodities by PCR. *Process Biochemistry*, *40*, 2859–2864.

Marín, S., Cuevas, D., Ramos, A. J., & Sanchis, V. (2008). Fitting of colony diameter and ergosterol as indicators of food borne mould growth to known growth models in solid medium. *International Journal of Food Microbiology*, *121*, 139–149.

Marin, S., Ramos, A. J., & Sanchis, V. (2005). Comparison of methods for the assessment of growth of food spoilage moulds in solid substrates. *International Journal of Food Microbiology*, *99*, 329–341.

Marín, S., Ramos, A. J., & Sanchis, V. (2012). Modelling *Aspergillus flavus* growth and aflatoxins production in pistachio nuts. *Food Microbiology*, *32*, 378–388.

Martins, L. M., Sant'Ana, A. S., Fungaro, M. H. P., Silva, J. J., Nascimento, M. da S. do, Frisvad, J. C., & Taniwaki, M. H. (2017). The biodiversity of *Aspergillus* section *Flavi* and aflatoxins in the Brazilian peanut production chain. *Food Research International*, *94*, 101–107.

Matumba, L., Poucke, C. Van, Monjerezi, M., Ediage, E. N., & De Saeger, S. (2015). Concentrating aflatoxins on the domestic market through groundnut export: A focus on Malawian groundnut value and supply chain. *Food Control*, *51*, 236–239.

Misra, J. B. (2004). A mathematical approach to comprehensive evaluation of quality in groundnut. *Journal of Food Composition and Analysis*, *17*, 69–79.

Mohd-Redzwan, S., Jamaluddin, R., Abd-Mutalib, M. S., & Ahmad, Z. (2013). A mini review on aflatoxin exposure in Malaysia: Past, present, and future. *Frontiers in Microbiology*, *4*, 1–8.

Mohd Redzwan, S., Rosita, J., Mohd Sokhini, A. M., Nurul 'Aqilah, A. R., Wang, J. S., Kang, M. S., & Zuraini, A. (2014). Detection of serum AFB1-lysine adduct in Malaysia and its association with liver and kidney functions. *International Journal of Hygiene and Environmental Health*, *217*, 443–451.

Montiel, D., Dickinson, M. J., Lee, H. A., Dyer, P. S., Jeenes, D. J., Roberts, I. N., … Archer, D. B. (2003). Genetic differentiation of the *Aspergillus* section *Flavi* complex using AFLP fingerprints. *Mycological Research*, *107*, 1427–1434.

Moore, G. G., Mack, B. M., & Beltz, S. B. (2015). Genomic sequence of the aflatoxigenic filamentous fungus *Aspergillus nomius*. *BMC Genomics*, *16*, 1–10.

Moore, G. G., Mack, B. M., Beltz, S. B., & Puel, O. (2018). Genome sequence of an

aflatoxigenic pathogen of Argentinian peanut, *Aspergillus arachidicola*. *BMC Genomics*, *19*, 1–12.

Mousa, W., Ghazali, F. M., Jinap, S., Ghazali, H. M., & Radu, S. (2011). Modelling the effect of water activity and temperature on growth rate and aflatoxin production by two isolates of *Aspergillus flavus* on paddy. *Journal of Applied Microbiology*, *111*, 1262–1274.

Mousa, W., Ghazali, F. M., Jinap, S., Ghazali, H. M., & Radu, S. (2013). Modeling growth rate and assessing aflatoxins production by *Aspergillus flavus* as a function of water activity and temperature on polished and brown rice. *Journal of Food Science*, *78*, 56–63.

Mutegi, C. K., Ngugi, H. K., Hendriks, S. L., & Jones, R. B. (2009). Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. *International Journal of Food Microbiology*, *130*, 27–34.

Mutegi, C. K., Ngugi, H. K., Hendriks, S. L., & Jones, R. B. (2012). Factors associated with the incidence of *Aspergillus* section *Flavi* and aflatoxin contamination of peanuts in the Busia and Homa bay districts of western Kenya. *Plant Pathology*, *61*, 1143–1153.

Mutegi, C. K., Wagacha, J. M., Christie, M. E., Kimani, J., & Karanja, L. (2013a). Effect of storage conditions on quality and aflatoxin contamination of peanuts (*Arachis hypogaea* L.). *International Journal of AgriScience*, *3*, 746–758.

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

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

Nascimento, M. S., Carminati, J. A., Silva, I. C. R. N., Silva, D. L., Bernardi, A. O., & Copetti, M. V. (2018). *Salmonella*, *Escherichia coli* and *Enterobacteriaceae* in the peanut supply chain: From farm to table. *Food Research International*, *105*, 930–935.

Ndungu, J. W., Makokha, A. O., Onyango, C. A., Mutegi, C. K., Wagacha, J. M., & Christie, M. E. (2013). Prevalence and potential for aflatoxin contamination in groundnuts and peanut butter from farmers and traders in Nairobi and Nyanza provinces of Kenya. *Journal of Applied Biosciences*, *65*, 4922–4934.

Nesci, A., Passone, M. A., Barra, P., Girardi, N., Garcia, D., & Etcheverry, M. (2016). Prevention of aflatoxin contamination in stored grains using chemical strategies. *Current Opinion in Food Science*, *11*, 56–60.

Nilsson, R. H., Ryberg, M., Abarenkov, K., Sjökvist, E., & Kristiansson, E. (2009). The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiology Letters*, *296*, 97–101.

Nishikawa, T., & Kambara, H. (1991). Analysis of limiting factors of DNA band separation by a DNA sequencer using fluorescence detection. *Electrophoresis*,

12, 623–631.

Nyirahakizimana, H., Mwamburi, L., Wakhisi, J., Mutegi, C. K., Christie, M. E., & Wagacha, J. M. (2013). Occurrence of *Aspergillus* species and aflatoxin contamination in raw and roasted peanuts from formal and informal markets in Eldoret and Kericho, Kenya. *Advances in Microbiology*, *03*, 333–342.

Ok, H. E. E., Kim, H. J., Shim, W. O. N. B. O., Lee, H., Bae, D., Chung, D., & Chun, H. S. (2007). Natural occurrence of aflatoxin B_1 in marketed foods and risk estimates of dietary exposure in Koreans. *Journal of Food Protection*, *70*, 2824–2828.

Okoth, S. A., Nyongesa, B., Joutsjoki, V., Korhonen, H., Ayugi, V., & Kang'ethe, E. K. (2016). Sclerotia formation and toxin production in large sclerotial *Aspergillus flavus* isolates from Kenya. *Advances in Microbiology*, *6*, 47–56.

Olarte, R. A., Horn, B. W., Dorner, J. W., Monacel, J. T., Singh, R., Stone, E. A., & Carbone, I. (2012). Effect of sexual recombination on population diversity in aflatoxin production by *Aspergillus flavus* and evidence for cryptic heterokaryosis. *Molecular Ecology*, *21*, 1453–1476.

Oliveira, C. A. F., Gonçalves, N. B., Rosim, R. E., & Fernandes, A. M. (2009). Determination of aflatoxins in peanut products in the Northeast Region of Sao Paulo, Brazil. *International Journal of Molecular Sciences*, *10*, 174–183.

Oplatowska-Stachowiak, M., Sajic, N., Xu, Y., Haughey, S. A., Mooney, M. H., Gong, Y. Y., … Elliott, C. T. (2016). Fast and sensitive aflatoxin B_1 and total aflatoxins ELISAs for analysis of peanuts, maize and feed ingredients. *Food Control*, *63*, 239–245.

Pardo, E., Ramos, A. J., Sanchis, V., & Marín, S. (2005). Modelling of effects of water activity and temperature on germination and growth of ochratoxigenic isolates of *Aspergillus ochraceus* on a green coffee-based medium. *International Journal of Food Microbiology*, *98*, 1–9.

Passone, M. A., Girardi, N. S., & Etcheverry, M. (2012). Evaluation of the control ability of five essential oils against *Aspergillus* section *Nigri* growth and ochratoxin A accumulation in peanut meal extract agar conditioned at different water activities levels. *International Journal of Food Microbiology*, *159*, 198–206.

Passone, M. A., Rosso, L. C., Ciancio, A., & Etcheverry, M. (2010). Detection and quantification of *Aspergillus* section *Flavi* spp. in stored peanuts by real-time PCR of *nor-1* gene, and effects of storage conditions on aflatoxin production. *International Journal of Food Microbiology*, *138*, 276–281.

Payne, G. A., Nierman, W. C., Wortman, J. R., Pritchard, B. L., Brown, D., Dean, R. A., … Yu, J. (2006). Whole genome comparison of *Aspergillus flavus* and *A. oryzae*. *Medical Mycology*, *44*, 9–11.

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

Pildain, B., Frisvad, J. C., Vaamonde, G., Cabral, D., Varga, J., & Samson, R. A. (2008). Two novel aflatoxin-producing *Aspergillus* species from Argentinean

peanuts. *International Journal of Systematic and Evolutionary Microbiology*, *58*, 725–735.

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. *International Journal of Food Microbiology*, *93*, 31–40.

Pitt, J. I., Dyer, S. K., & McCammon, S. (1991). Systemic invasion of developing peanut plants by *Aspergillus flavus*. *Letters in Applied Microbiology*, *13*, 16–20.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (Third Ed). New York, USA: Springer.

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

Prandini, A., Sigolo, S., Filippi, L., Battilani, P., & Piva, G. (2009). Review of predictive models for *Fusarium* head blight and related mycotoxin contamination in wheat. *Food and Chemical Toxicology*, *47*, 927–931.

Prencipe, S., Siciliano, I., Contessa, C., Botta, R., Garibaldi, A., Gullino, M. L., & Spadaro, D. (2018). Characterization of *Aspergillus* section *Flavi* isolated from fresh chestnuts and along the chestnut flour process. *Food Microbiology*, *69*, 159–169.

Probst, C., Callicott, K. A., & Cotty, P. J. (2012). Deadly strains of Kenyan *Aspergillus* are distinct from other aflatoxin producers. *European Journal of Plant Pathology*, *132*, 419–429.

Probst, C., Njapau, H., & Cotty, P. J. (2007). Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. *Applied and Environmental Microbiology*, *73*, 2762–2764.

Ramos, A. J., Labernia, N., Marin, S., Sanchis, V., & Magan, N. (1998). Effect of water activity and temperature on growth and ochratoxin production by three strains of *Aspergillus ochraceus* on a barley extract medium and on barley grains. *International Journal of Food Microbiology*, *44*, 133–140.

Rank, C., Klejnstrup, M. L., Petersen, L. M., Kildgaard, S., Frisvad, J. C., Held Gotfredsen, C., & Ostenfeld Larsen, T. (2012). Comparative chemistry of *Aspergillus oryzae* (RIB40) and *A. flavus* (NRRL 3357). *Metabolites*, *2*, 39–56.

Raper, K. B., & Fennell, D. I. (1965). *The genus Aspergillus*. Baltimore, USA: The Williams & Wilkins Company.

Razzaghi-abyaneh, M., Shams-ghahfarokhi, M., Allameh, A., Kazeroon-shiri, A., Ranjbar-bahadori, S., & Mirzahoseini, H. (2006). A survey on distribution of *Aspergillus* section *Flavi* in corn field soils in Iran: Population patterns based on aflatoxins , cyclopiazonic acid and sclerotia production. *Mycopathologia*, *161*, 183–192.

Razzazi-Fazeli, E., Noviandi, C. T., Porasuphatana, S., Agus, A., & Bohm, J. (2004). A survey of aflatoxin B_1 and total aflatoxin contamination in baby food, peanut and corn products sold at retail in Indonesia analysed by ELISA and HPLC.

Mycotoxin Research, *20*, 51–58.

Reddy, K. R. N., Farhana, N. I., & Salleh, B. (2011). Occurrence of *Aspergillus* spp. and aflatoxin B_1 in Malaysian foods used for human consumption. *Journal of Food Science*, *76*, 99–104.

Reis, T. A., Baquião, A. C., Atayde, D. D., Grabarz, F., & Corrêa, B. (2014). Characterization of *Aspergillus* section *Flavi* isolated from organic Brazil nuts using a polyphasic approach. *Food Microbiology*, *42*, 34–39.

Riba, A., Bouras, N., Mokrane, S., Mathieu, F., Lebrihi, A., & Sabaou, N. (2010). *Aspergillus* section *Flavi* and aflatoxins in Algerian wheat and derived products. *Food and Chemical Toxicology*, *48*, 2772–2777.

Rodrigues, P., Peterson, S. W., & Vena, A. (2012). Three new species of *Aspergillus* section *Flavi* isolated from almonds and maize in Portugal. *Mycologia*, *104*, 682–697.

Rodrigues, P., Santos, C., Venâncio, A., & Lima, N. (2011). Species identification of *Aspergillus* section *Flavi* isolates from Portuguese almonds using phenotypic, including MALDI-TOF ICMS, and molecular approaches. *Journal of Applied Microbiology*, *111*, 877–892.

Rodrigues, P., Venâncio, A., Kozakiewicz, Z., & Lima, N. (2009). A polyphasic approach to the identification of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* Section *Flavi* isolated from Portuguese almonds. *International Journal of Food Microbiology*, *129*, 187–193.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, *81*, 501–508.

Ruadrew, S., Craft, J., & Aidoo, K. (2013). Occurrence of toxigenic *Aspergillus* spp. and aflatoxins in selected food commodities of Asian origin sourced in the West of Scotland. *Food and Chemical Toxicology*, *55*, 653–658.

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

Sabran, M. R., Jamaludin, R., Sokhini, A. M. M., & Aqilah, A. R. N. (2012). Socio-demographic and socio-economic determinants of adults' knowledge on fungal and aflatoxin contamination in the diets. *Asian Pacific Journal of Tropical Biomedicine*, *2*, 1835–1841.

Saito, M., & Machida, S. (1999). A rapid identification method for aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus* by ammonia vapor. *Mycoscience*, *40*, 205–208.

Samapundo, S., Devlieghere, F., Geeraerd, A. H., De Meulenaer, B., Van Impe, J. F., & Debevere, J. (2007). Modelling of the individual and combined effects of water activity and temperature on the radial growth of *Aspergillus flavus* and *A. parasiticus* on corn. *Food Microbiology*, *24*, 517–529.

Sameni, M., Dubecke, A., & Weber, J. F. F. (2014). Simultaneous Multi- Residue Determination of Mycotoxins in Foods Using LC-MS/MS. *Journal of Environmental & Analytical Toxicology*, *5*, 1–7.

Samson, R. A., Hong, S. B., & Frisvad, J. C. (2006). Old and new concepts of species differentiation in *Aspergillus*. *Medical Mycology*, *44*, 133–148.

Samson, R. A., & Varga, J. (2007). Aspergillus *Systematics in the Genomic era*. *Studies in Mycology* (Vol. 59). Utrecht, The Netherlands: CBS Fungal Biodiversity Centre.

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S.-B., Hubka, V., Klaassen, C. H. W., … Frisvad, J. C. (2014). Phylogeny,identification and nomenclature of the genus *Aspergillus*. *Studies in Mycology*, *78*, 141–173.

Sawane, A., & Sawane, M. (2014). Mycotoxigenicity of *Aspergillus*, *Penicillium* and *Fusarium* spp. isolated from stored rice. *International Journal of Current Microbiology and Applied Sciences*, *3*, 116–121.

Schmidt-Heydt, M., Abdel-Hadi, A., Magan, N., & Geisen, R. (2009). Complex regulation of the aflatoxin biosynthesis gene cluster of *Aspergillus flavus* in relation to various combinations of water activity and temperature. *International Journal of Food Microbiology*, *135*, 231–237.

Schmidt-Heydt, M., Rüfer, C. E., Abdel-Hadi, A., Magan, N., & Geisen, R. (2010). The production of aflatoxin B_1 or G_1 by *Aspergillus parasiticus* at various combinations of temperature and water activity is related to the ratio of *aflS* to *aflR* expression. *Mycotoxin Research*, *26*, 241–246.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences*, *109*, 6241–6246.

Schwartzbord, J. R., & Brown, D. L. (2015). Aflatoxin contamination in Haitian peanut products and maize and the safety of oil processed from contaminated peanuts. *Food Control*, *56*, 114–118.

Shapira, R., Paster, N., Eyal, O., Menasherov, M., Mett, A., & Salomon, R. (1996). Detection of aflatoxigenic molds in grains by PCR. *Applied and Environmental Microbiology*, *62*, 3270–3273.

Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, *2*, 21–25.

Singh, B., & Singh, U. (1991). Peanut as a source of protein for human foods. *Plant Foods for Human Nutrition*, *41*, 165–177.

Siti Aminah, I. (2017). *Screening of Toxigenic and Atoxigenic Aspergillus flavus Isolates Collected from Corn Fields in UPM, Serdang.* MSc Thesis, Universiti Putra Malaysia.

Slater, G. W. (1993). Theory of band broadening for DNA gel electrophoresis and sequencing. *Electrophoresis*, 1–7.

Soleimany, F., Jinap, S., Faridah, A., & Khatib, A. (2012). A UPLC-MS/MS for simultaneous determination of aflatoxins, ochratoxin A, zearalenone, DON, fumonisins, T-2 toxin and HT-2 toxin, in cereals. *Food Control*, *25*, 647–653.

Somashekar, D., Rati, E. R., & Chandrashekar, A. (2004). PCR-restriction fragment length analysis of *aflR* gene for differentiation and detection of *Aspergillus flavus* and *Aspergillus parasiticus* in maize. *International Journal of Food Microbiology*, *93*, 101–107.

Songsermsakul, P. (2015). Mycotoxins contamination of food in Thailand (2000-2010): Food safety concerns for the world food exporter. *International Food Research Journal*, *22*, 426–434.

Stroka, J. (2000). *Determination of Aflatoxins in Food and Feed with Simple and Optimised Methods*. Bergische Universität-Gesamthochschule, Wuppertal.

Suanthie, Y., Cousin, M. A., & Woloshuk, C. P. (2009). Multiplex real-time PCR for detection and quantification of mycotoxigenic *Aspergillus*, *Penicillium* and *Fusarium*. *Journal of Stored Products Research*, *45*, 139–145.

Sugitha-Konishi, Y., Sato, T., Saito, S., Nakajima, M., Tabata, S., Tabata, T., … Kumagai, S. (2010). Exposure to aflatoxins in Japan: risk assessment for aflatoxin B_1. *Food Additives & Contaminants: Part A*, *27*, 365–37241.

Sulaiman, M. R., Chye, F. Y., Hamid, A., & Yatim, A. M. (2007). The occurrence of aflatoxins in raw shelled peanut samples from three districts of Perak, Malaysia. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *6*, 2045–2052.

Takahashi, T., Chang, P. K., Matsushima, K., Yu, J., Abe, K., Bhatnagar, D., … Koyama, Y. (2002). Nonfunctionality of *Aspergillus sojae aflR* in a strain of *Aspergillus parasiticus* with a disrupted *aflR* gene. *Applied and Environmental Microbiology*, *68*, 3737–3743.

Tam, E. W. T., Chen, J. H. K., Lau, E. C. L., Ngan, A. H. Y., Fung, K. S. C., Lee, K. C., … Woo, P. C. Y. (2014). Misidentification of *Aspergillus nomius* and *Aspergillus tamarii* as *Aspergillus flavus*: Characterization by internal transcribed spacer, β-tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser des. *Journal of Clinical Microbiology*, *52*, 1153–1160.

Taniwaki, M. H., Pitt, J. I., & Magan, N. (2018). *Aspergillus* species and mycotoxins: occurrence and importance in major food commodities. *Current Opinion in Food Science*, *23*, 38–43.

Tassou, C. C., Natskoulis, P. I., Magan, N., & Panagou, E. Z. (2009). Effect of temperature and water activity on growth and ochratoxin A production boundaries of two *Aspergillus carbonarius* isolates on a simulated grape juice medium. *Journal of Applied Microbiology*, *107*, 257–268.

Tassou, C. C., Panagou, E. Z., Natskoulis, P., & Magan, N. (2007). Modelling the effect of temperature and water activity on the growth of two ochratoxigenic strains of *Aspergillus carbonarius* from Greek wine grapes. *Journal of Applied Microbiology*, *103*, 2267–2276.

Teh, L. Y., & Latiffah, Z. (2013). A new record of *Penicillium pimiteouiense* from beach soil in Malaysia. *Mycobiology*, *41*, 256–259.

Thom, C., & Raper, K. B. (1945). *A Manual of the Aspergilli*. Baltimore, USA: The Williams & Wilkins Company.

Tominaga, M., Lee, Y., Hayashi, R., Suzuki, Y., Yamada, O., Sakamoto, K., … Akita, O. (2006). Molecular analysis of an inactive aflatoxin biosynthesis gene cluster in *Aspergillus oryzae* RIB strains. *Applied and Environmental Microbiology*, *72*, 484–490.

Torres, A. M., Barros, G. G., Palacios, S. A., Chulze, S. N., & Battilani, P. (2014). Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International*, *62*, 11–19.

Tropical Climate Zone. Retrieved 17 May 2018 from https://www.tes.com/lessons/VLfVxIiBQqIvmw/copy-of-tropical-climate-zones

Vaamonde, G., Patriarca, A., Fernández Pinto, V., Comerio, R., & Degrossi, C. (2003). Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section *Flavi* from different substrates in Argentina. *International Journal of Food Microbiology*, *88*, 79–84.

Varga, J., Frisvad, J. C., & Samson, R. A. (2011). Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. *Studies in Mycology*, *69*, 57–80.

Vetter, T. R. (2017). Descriptive Statistics: Reporting the Answers to the 5 Basic Questions of Who, What, Why, When, Where, and a Sixth, so What? *Anesthesia and Analgesia*, *125*, 1797–1802.

Viaro, H. P., da Silva, J. J., de Souza Ferranti, L., Bordini, J. G., Massi, F. P., & Fungaro, M. H. P. (2017). The first report of *A. novoparasiticus, A. arachidicola* and *A. pseudocaelatus* in Brazilian corn kernels. *International Journal of Food Microbiology*, *243*, 46–51.

Villers, P. (2014). Aflatoxins and safe storage. *Frontiers in Microbiology*, *5*, 1–6.

Villers, P. (2017). Food Safety and aflatoxin Control. *Journal of Food Research*, *6*, 1–12.

Wagacha, J. M., Mutegi, C. K., Christie, M. E., Karanja, L. W., & Kimani, J. (2013a). Changes in fungal population and aflatoxin levels and assessment of major aflatoxin types in stored peanuts (*Arachis hypogaea* Linnaeus). *Journal of Food Research*, *2*, 10–23.

Wagacha, J. M., Mutegi, C., Karanja, L., Kimani, J., & Christie, M. E. (2013b). Fungal species isolated from peanuts in major Kenyan markets: Emphasis on *Aspergillus* section *Flavi*. *Crop Protection*, *52*, 1–9.

Wagacha, J. M., & Muthomi, J. W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*, *124*, 1–12.

Waliyar, F., Osiru, M., Ntare, B. R., Kumar, K. V. K., Sudini, H., Traore, A., & Diarra, B. (2015). Post-harvest management of aflatoxin contamination in groundnut. *World Mycotoxin Journal*, *8*, 245–252.

Ward, O. P., Qin, W. M., Dhanjoon, J., Ye, J., & Singh, A. (2005). Physiology and biotechnology of *Aspergillus*. *Advances in Applied Microbiology*, *58*, 1–75.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct

Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols. A Guide to Methods and Applications* (pp. 315–322). New York, USA: Academic Press.

Wicklow, D. T., Wilson, D. M., & Nelsen, T. C. (1993). Survival of *Aspergillus flavus* sclerotia and conidia buried in soil in Illinois or Georgia. *Phytopathology*, *83*, 1141–1147.

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

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

Yamashita, A., Yoshizawa, T., Aiura, Y., Sanchez, P. C., Dizon, E. I., Arim, R. H., & Arim, R. H. (1995). *Fusarium* mycotoxins (fumonisins, nivalenol, and zearalenone) and aflatoxins in corn from Southeast Asia. *Bioscience, Biotechnology and Biochemistry*, *59*, 1804–1807.

Yazdani, D., Zainal Abidin, M. A., Tan, Y. H., & Kamaruzaman, S. (2010). Evaluation of the detection techniques of toxigenic *Aspergillus* isolates. *African Journal of Biotechnology*, *9*, 7654–7659.

Yazid, S. N. E., Thanggavelu, H., Norlia, M., Selamat, J., & Samsudin, N. I. P. (2018). Formulation of maize- and peanut-based semi-synthetic growth media for the ecophysiological studies of aflatoxigenic *Aspergillus flavus* in maize and peanut agro-ecosystems. *International Journal of Food Microbiology*, *282*, 57–65.

Yin, Y., Lou, T., Yan, L., Michailides, T. J., & Ma, Z. (2009). Molecular characterization of toxigenic and atoxigenic *Aspergillus flavus* isolates, collected from peanut fields in China. *Journal of Applied Microbiology*, *107*, 1857–1865.

Younis, Y. M. H., & Malik, K. M. (2003). TLC and HPLC assays of aflatoxin contamination in Sudanese peanuts and peanut products. *Kuwait Journal of Science and Engineering*, *30*, 79–84.

Yu, J., Chang, P., Ehrlich, K. C., Cary, J. W., Bhatnagar, D., Cleveland, T. E., … Bennett, J. W. (2004). Clustered pathway genes in aflatoxin biosynthesis. *Applied and Environmental Microbiology*, *70*, 1253–1262.

Yuan, G. F., Liu, C. S., & Chen, C. C. (1995). Differentiation of *Aspergillus parasiticus* from *Aspergillus sojae* by Random Amplification of Polymorphic DNA. *Applied and Environmental Microbiology*, *61*, 2384–2387.

Yue, X., Sui, J., Niu, T., Liu, Y., & Liu, X. (2011). Modeling the effect of temperature and water activity on the growth rate and lag phase of *Aspergillus flavus* during rice drying. *Drying Technology*, *29*, 1306–1312.

Zakaria, L., Zain, N. M., Salleh, B., & Zakaria, M. (2009). Morphological and RAPD analysis of *Fusarium* species associated with root and stem rot of *Dendrobium* Orchid in Northern Peninsula Malaysia. *HAYATI Journal of Biosciences*, *16*, 64–68.

Zanon, M. S. A., Barros, G. G., & Chulze, S. N. (2016). Non-aflatoxigenic *Aspergillus flavus* as potential biocontrol agents to reduce aflatoxin contamination in peanuts harvested in Northern Argentina. *International Journal of Food Microbiology*,

231, 63–68.

Zhang, C., Selvaraj, J. N., Yang, Q., & Liu, Y. (2017). A survey of aflatoxin-producing *Aspergillus* sp. from peanut field soils in four agroecological zones of China. *Toxins*, *9*, 1–14.

Zorzete, P., Baquião, A. C., Atayde, D. D., Reis, T. A., Gonçalez, E., & Corrêa, B. (2013). Mycobiota, aflatoxins and cyclopiazonic acid in stored peanut cultivars. *Food Research International*, *52*, 380–386.

Zulkifli, N. A., & Zakaria, L. (2017). Morphological and molecular diversity of *Aspergillus* from corn grain used as livestock feed. *HAYATI Journal of Biosciences*, *24*, 26–34.