UNIVERSITI PUTRA MALAYSIA

METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF MULTI-MYCOTOXINS IN PALM KERNEL CAKE

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ITA 2015 10

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By

SIMAYI YIBADATIHAN

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

August 2015

DEDICATION

Dedicated to my honourable and beloved parents

Ismail Kadir and Hamirahan Rozi

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF MULTI-MYCOTOXINS IN PALM KERNEL CAKE

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SIMAYI YIBADATIHAN

August 2015

Chairman : Professor Jinap Selamat, PhD
Faculty : Institute of Tropical Agriculture

Palm kernel cake (PKC) has been widely used for animal feed production as being a useful source of protein and energy for livestock. However, PKC can easily be contaminated with mycotoxins during production and storage owing to its favourable environments for the fungus to grow. Public concerns about the consequential economic losses and health risks for animals and humans have accentuated the need for evaluation of mycotoxins in PKC. Furthermore, a method for determination of multi-mycotoxins in PKC has so far not been established. The European Commission has set maximum regulatory limits of 5-50, 50-250, 100-3000, 900-12000, 5000-60000, 250-2000 and 250-2000 ng g^{-1} for aflatoxin B1, ochratoxin A, zearalenone, deoxynivalenol, fumonisins, T-2 and HT-2 toxins, respectively intended for animal feeds. This research was therefore conducted to develop an LC-MS/MS method for simultaneous determination of the 11 mycotoxins (aflatoxins B2, G1, G2 and the regulated mycotoxins) in PKC. The LC-MS/MS method was developed through the investigation of different ionization process, collision energies, and different types of mobile phase. Then, the LC-MS/MS detection conditions including flow rate (0.15-0.3 ml min^{-1}), acid percentage in the mobile phase (0-0.7%), organic percentage at the beginning (5-25%) and at the end (70-90%) of the gradient mobile phase were optimized using central composite design (CCD). The best detection and separation of all the target mycotoxins were achieved using electro spray ionization (ESI) in both positive and negative ion-modes under the optimized LC-MS/MS detection parameters, which were 10% methanol at the beginning and 90% at the end of the gradient mobile phase with 0.2% formic acid and at the flow rate of 0.2 ml min^{-1}. The efficiency of different ratios of extraction solvents composed of acetonitrile or methanol with water (50:50-90:10, v:v) was also investigated, followed by the evaluation of formic acid (0.1-2%) effect on the recovery of mycotoxins. Purification approaches of no clean-up and clean-up with immunoaffinity column (IAC) were also compared. The highest recovery of target mycotoxins (88-110%) were obtained by using acetonitrile:water:formic acid (70:29:1, v:v:v) as the extraction solvent and no clean-up method. The optimized method was then validated through determination of linearity, limits of detection (LOD), limits of quantification (LOQ), accuracy, precision and matrix effect. The LODs and LOQs in mycotoxins standards and PKC samples ranged from 0.02 to 17.5 ng g^{-1} and from 0.06 to 58 ng g^{-1}, respectively. The mean recoveries of mycotoxins in PKC samples which

have been added with standard solution ranged between 81-112%. The intra-day and inter-day precision values were in the range of 1.41-14.35% and 1.72-16.97%, respectively. The validated method was successfully applied on 25 PKC samples obtained from feed industries. The study found that all of the samples were contaminated with at least 7 out of 11 mycotoxins being studied. A total 96% of the samples exceeded the maximum regulatory limits of 5 and 100 ng g^{-1} for aflatoxin B1 and zearalenone, respectively. In conclusion, the newly developed and optimized LC-MS/MS method is sensitive, efficient and reliable for the simultaneous determination of multi-mycotoxins, which can be ideal to be used for routine analysis in laboratories.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat keperluan untuk Ijazah Sarjana Sains

PEMBANGUNAN KAEDAH PENENTUAN MULTI-MIKOTOKSIN SECARA SERENTAK BAGI DALAM KEK ISIRUNG KELAPA SAWIT

By

SIMAYI YIBADATIHAN

Ogos 2015

Pengerusi : Professor Jinap Selamat, PhD
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Kek isirong kelapa sawit (PKC) telah digunakan secara meluas sebagai sumber protein dan tenaga untuk pengeluaran makanan haiwan ternakan. Walau bagaimanapun, PKC mudah dicemari dengan mikotoksin semasa pengeluaran dan penyimpanan kerana persekitaran yang menggalakkan pertumbuhan kulat. Kebimbangan awam mengenai kerugian ekonomi yang berbangkit dan risiko kesihatan haiwan dan manusia telah meningkatkan keperluan untuk penilaian mikotoksin dalam PKC. Tambahan pula, setakat ini kaedah untuk menentukan pelbagai mikotoksin dalam PKC masih belum dibangunkan. Suruhanjaya Eropah telah menetapkan had peraturan maksimum untuk makanan haiwan, 5-50, 50-250, 100-3000, 900-12000, 5000-60000, 250-2000 dan 250-2000 ng g^{-1} untuk aflatoksin B1, ochratoxin A, zearalenone, deoxynivalenol, fumonisins, toksin T-2 dan HT-2, masing-masing. Oleh itu, kajian ini telah dijalankan untuk membangunkan kaedah LC-MS/MS bagi penentuan serentak 11 mikotoksin (aflatoksin B2, G1, G2 dan mikotoksin terkawal) dalam PKC. Kaedah LC-MS/MS telah dibangunkan melalui penyiasatan proses pengionan yang berbeza, tenaga perlanggaran, dan pelbagai jenis fasa bergerak. Kemudian, keadaan pengesanan LC-MS/MS termasuk kadar aliran (0.15-0.3 ml min^{-1}), peratusan asid dalam fasa bergerak (0-0.7%), peratusan organik pada awal (5-25%) dan akhir (70-90%) bagi fasa bergerak kecerunan telah dioptimumkan dengan menggunakan reka bentuk komposit pusat (CCD). Pengesanan dan pemisahan terbaik bagi semua mikotoksin sasaran telah dicapai dengan menggunakan semburan elektro pengionan (ESI) dalam kedua-dua ion-mod positif dan negatif pada parameter pengesanan LC-MS/MS yang telah dioptimumkan, iaitu 10% metanol pada permulaan dan 90% pada akhir fasa bergerak kecerunan dengan 0.2% asid formik dan pada kadar aliran 0.2 ml min^{-1}. Kecekapan nisbah pelarut pengekstrakan berbeza yang terdiri daripada asetonitril atau metanol dengan air (50:50-90:10, v:v) juga telah disiasat, diikuti dengan penilaian kesan asid formik (0.1-2%) ke atas pemulihan mikotoksin. Kaedah penyucian tanpa pembersihan dan pembersihan dengan lajur immunoaffiniti (IAC) juga telah dibandingkan. Pemulihan tertinggi mikotoksin sasaran (88-110%) telah diperolehi dengan menggunakan asetonitril:air:asid formik (70:29:1, v:v:v) sebagai pengekstrakan pelarut tanpa kaedah pembersihan. Kaedah yang dioptimumkan kemudian disahkan melalui penentuan kelinearan, had pengesanan (LOD), had kuantifikasi (LOQ), ketepatan, kejituan dan kesan matrik. Had LODs dan LOQs mikotoksin dalam larutan piawai dan

sampel PKC ialah di antara 0.02-17.5 ng g^{-1} dan 0.06-58 ng g^{-1}, masing-masing. Pemulihan purata mikotoksin dalam sampel PKC yang telah dimasukkan larutan piawai adalah antara 81-112%. Kejituan nilai-nilai antara hari dan mengikut hari adalah dalam lingkungan 1.41-14.35% dan 1.72-16.97%, masing-masing. Kaedah yang disahkan telah berjaya digunakan pada 25 sampel PKC yang diperolehi daripada industry makanan haiwan. Kajian mendapati bahawa semua sampel telah tercemar dengan sekurang-kurangnya 7 daripada 11 mikotoksin yang dikaji. Sebanyak 96% daripada sampel melebihi had maksimum yang dibenarkan, 5 dan 100 ng g^{-1} untuk aflatoksin B1 dan zearalenone, masing-masing. Kesimpulannya, kaedah LC-MS/MS yang baru dibangunkan dan dioptimumkan didapati sensitif, cekap dan berkesan untuk penentuan serentak berbilang mikotoksin, yang sesuai untuk digunakan bagi analisis rutin di makmal.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my profound and devoted gratitude to my advisor, Prof. Dr. HJ. Jinap Selamat. She has given me constant support, advice and encouragement throughout my graduate study, not only in academic field, but also for my personal life and development. I really appreciate her patience, willingness to help me and great sense of humour, which make my research life in UPM really enjoyable.

I would also like to give my thousands of thanks to Associate Professor Dr. Nor Ainy Mahyudin for her untiring support and contribution during this research work and for serving as a supervisory committee member. A sincere gratitude also goes to Dr. Seyed Hamed Mirhosseini for his kind assistance and valuable guidance on the statistical analysis of this study.

My gratitude also goes out to the Malaysian government for the valuable financial aid provided which greatly contributed towards the successful completion of my thesis. A special word of thanks goes to the University Putra Malaysia for providing the opportunity and facilities to undertake this research work.

I would like to take this opportunity to express my warm thanks to all my lab mates, the technicians and laboratorial staffs at the Department of Food Science, Faculty of Food science and technology for their generous help, support and assistance.

Last but not least, my genuine heartfelt thanks go out to my beloved parents, husband and other family members for their encouragements, supports, patience, tolerance and understanding.

I certify that a Thesis Examination Committee has met on 7 August 2015 to conduct the final examination of Yibadatihan Simayi on her thesis entitled "Method development for simultaneous determination of multi-mycotoxins in palm kernel cake" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AA	acetic acid
ACN	Acetonitrile
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFs	Aflatoxins
ANOVA	Analysis of Variance
AOZ	Aflatoxin, Ochratoxin A and Zearalenone
AOFZDT2	Aflatoxin, Ochratoxin A, Fumonisins, Zearalenone, Deoxynivalenol and T-2
APCI	Atmospheric pressure chemical ionization
BEA	Beauvericin
CCD	Central composite design
CV	Coefficient of variance
CRM	certified reference materials
DON	Deoxynivalenol
DAS	Diacetoxyscirpenol
EC	European Commission
EFSA	European Food Safety Authority
EI	Electron ionization
ESI	Electrospray ionization
FA1	Fumonisin A1
FA2	Fumonisin A2
FB1	Fumonisin B1

FB2	Fumonisin B2
FB3	Fumonisin B3
FB4	Fumonisin B4
FBs	Fumonisins
FDA	Food and Drug Administration
G	Gram
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
HT-2	HT-2 toxin
IAC	Immunoaffinity column
Kg	Kilogram
Kv	Kilovolts
L	Liter
LC	Liquid chromatography
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLS	Liquid-liquid Separation
LOD	Limit of detection
LOQ	Limit of quantification
ME	matrix effect
MeOH	Methanol
Min	Minute
Ml	Milliliter
Mm	Millimeter
MPLs	maximum permitted levels
MPOB	Malaysian Palm Oil Board

m/z	Mass/charge
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MRM	multiple reaction monitoring
Ng	Nanogram
NIV	nivalenol
OTA	Ochratoxin A
OTs	ochratoxins
OVAT	one variable at a time
PKC	Palm kernel cake
Rpm	Revolutions per minute
PW	Peak width
RASFF	Rapid Alert System for Food and Feed
RSD	Relative standard deviation
RSM	Response surface method
R^2	Correlation
SD	Standard deviation
S/N	Signal/noise
SPE	Solid-Phase Extraction
T-2	T-2 toxin
V	Volume
ZEA	Zearalenone
µg	Microgram
µl	Microliter

CHAPTER 1

INTRODUCTION

1.1 Background of study

Risk assessment, monitoring and prevention of contaminants in animal feeds have been crucial issues related with animal health, feed supply, national and international trade. Mycotoxins are one of these contaminants, which are recognized as prevalently toxic compounds produced as secondary metabolites by various fungi and excreted into their substrates. Animal feeds and various feed ingredients used for compound feed production are the main substrates which in mycotoxin contamination frequently occur. Mycotoxins have attracted worldwide attention due to significant losses associated with their impact on animal health, as well as consequential economic implications (Poornima & Palanisamy, 2013). When mycotoxins ingested by animals above a certain concentration, they cause a toxic response referred to as mycotoxicosis (Binder, 2007). The symptoms elicited by mycotoxins consumption range from reduced animal productivity and immune suppression, increased susceptibility to disease and parasites to overt disease and death (Streit et al, 2012). The economic impact of mycotoxins are loss of animal life, increased health care and veterinary care costs, reduced livestock production, loss of forage crops, disposal of contaminated feeds, regulatory coasts and investment in research to reduce severity of the mycotoxin problem (Saleemi, 2010; Zain, 2011).

To address these adverse effects of mycotoxin contaminants in feeds, national and international institutions and organizations, such as the European Committees (EC), the US Food and Drug Administration (FDA) have set regulatory maximum levels for some mycotoxins in feedstuffs. The regulation strategies need to be strict to protect animals from mycotoxins exposure, and to minimize economic losses. Therefore, the analytical methods for the determination of mycotoxins in feedstuffs should be accurate and should provide reliable data. These requirements have accentuating the need for development of more sensitive and accurate analytical methods for mycotoxins analysis.

Since the discovery of the mycotoxins, several methods have been developed for identification and quantification purpose. Among the existing methods, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method has gained more popularity than the others due to the advantages of high selectivity and sensitivity, as well as time and solvent-saving separation process without any pre- or post-column derivatization. Moreover, mycotoxigenic fungi are usually capable of producing more than one mycotoxin and feed raw materials are commonly contminated with various fungal species at a time (Striet et al, 2012). As a consequence, the co-occurrence of mycotoxins in various food and feedstuffs has been becoming a common issue. Since a vast amount of samples need to be tested in the industries, rapid and sensitive simultaneous determination methods have become more desirable approach. The high sensitivity and improved specificity of LC-MS/MS methods enable many different classes of mycotoxins to be detected simultaneously with high accuracy.

Another important and critical step in simultaneous mycotoxin analysis is sample preparation, including sample extraction and sample clean-up. To improve the sample extraction efficiency, different approaches have been developed, such as liquid solid extraction (Sulyok et al, 2006; Khayoon et al, 2010), liquid-liquid extraction (Song et al, 2013). The same considerations have also been put on the purification step by applying different clean-up techniques such as immunoaffinity column (IAC), liquid-liquid separation, and solid phase extraction. Moreover, there have been also some reports on the successful application of so called "No clean-up" method during sample preparation (Zöllner & Mayer-Helm, 2006; Herebian et al, 2009; Verga et al, 2013). With the aid of highly sensitive instruments like LC-MS/MS they have been able to inject crude sample extracts to the system. Thus, simple and efficient sample extraction methods can be developed without applying further clean-up steps during sample preparation instead of using the costly and labour demanding extraction methods.

The problem of increasing the scope of animal production to provide the much-needed animal protein has become pronounced in the face of the ever-increasing human population. To reduce the cost of feeding for livestock, which competes directly with human beings for the same feedstuffs, attempts have been made to use alternative sources of protein and energy (Adesehinwa, 2007). Palm kernel cake (PKC) is an important oil palm by-product. It does not form food for man or have other industrial uses besides its usage for animal feeding (Kperegbeyi & Ikperite, 2011). PKC has been used widely as a filler to increase the bulkiness of feed while providing some protein, energy, minerals and vitamins (Alimon and Hair-Bejo, 1995; Adesehinwa, 2007). Thus, PKC has been used as one of the alternative feed resources to spare the conventional feedstuffs such as maize, soya bean etc. Consequently, compared to other feedstuffs its nutritional value, low pricing and long-term availability make PKC more competitive in international feed markets.

1.2 Problem statement

Mycotoxins that may occur in feeding stuffs are produced mainly by microscopic filamentous fungi species of *Fusarium*, *Aspergillus*, *Penicillium* (Woloshuk and Shim, 2013). They originate predominantly not only in the field when crops or forage are growing, but also during their storage. Particularly, any failure to comply with the good manufacturing practices usually lead to increased mycotoxin production (Zachariasova et al, 2014). Most of the mycotoxins are relatively stable compounds, when they are present in the input materials, their concentrations are hardly reduced during the animal feed production steps including fermentation, pelleting, and extruding (Teller et al, 2012; Vaclavikova et al, 2013). Thus, the regular consumption of mycotoxins contaminated feeds would increase the accumulation of the mycotoxins in animal's body and result in various health problems.

PKC mostly produced in tropical countries which have hot and humid climate conditions throughout the year. Like other animal feeds it is also subject to increased levels of fungal and mycotoxin contamination. On top of that the unhygienic production and storage conditions with uncontrolled temperature and relative humidity can exacerbate the formation of mycotoxins. The usage of mycotoxins contaminated PKC in animal feed production would infect the entire animal feed and would cause

more toxic effects with the combination of other possible mycotoxins in the compound feeds, and lead to economic losses in the form of decreased productivity. They would also affect humans due to the carryover of these hazardous contaminants via animal products such as meat, milk and eggs. Therefore, it is essential to evaluate the mycotoxins in PKC for monitoring and prevention of their potential hazard to animal and human health, as well as to the economy.

Studies on risk assessment of mycotoxins in various animal feeds and feed ingredients continue to rise throughout the world. However this is not the case for PKC, duo to the limited data available on the incidence of mycotoxins and associated health disorders. Thus, the evaluation of mycotoxin contamination of this commodity has been overlooked while its usage for animal feeds has been increased remarkably. Owing to the importance of PKC in animal feed production and its high possibility of mycotoxin contamination, as well as the adverse effects of mycotoxins this study was conducted to generate data for the future planning on feed safety issues and to accentuate monitoring of mycological contamination of PKC.

Furthermore, there have been some analytical methods developed to determine the contamination levels of mycotoxins in various commodities, such as cereals (Rahmani et al, 2013), peanuts (Arzandeh et al, 2011; Khorasgan et al, 2013), peppers (Jalili et al, 2012), nutmeg (Kong et al, 2013), agro-food (Li et al, 2013), soybean-based food and feed (Piotrowska et al, 2013), milk (Sørensen & Elbæk, 2005; Muscarella et al, 2007), wines (Tamura et al, 2012) and animal feed (Ates et al, 2012; Kolosova & Strika, 2012). There have been only a limited number of reports on the frequency and levels of AFB1 in PKC (Coker et al, 2000; Reiter et al, 2011; Kolapo et al, 2012; Pranowo et al, 2013). However, no method has been developed for the determination of multi-mycotoxins in PKC. The reported single mycotoxin methods on the determination of AFB1 in PKC are not applicable for the multi-mycotoxin analysis, as they are costly, time consuming and not sensitive enough to determine the multi-mycotoxins simultaneously until trace levels. Besides, the other existing multi-mycotoxin methods have limited applicability to the samples other than the one type of sample, duo to the different composition and matrix effect of different types of samples. In addition, the industries have required the development of more sensitive and efficient (in time and cost) multi-mycotoxin methods to enforce the strict regulations and to cope with the increasing demand of production.

1.3 Objectives

The following objectives have been sought out in this study:

1. To develop and optimize an LC-MS/MS method for simultaneous determination of multi-mycotoxins.
2. To optimize the sample preparation procedure for simultaneous extraction of multi-mycotoxins in PKC.
3. To validate and verify the newly developed LC-MS/MS method for quantification of multi-mycotoxins in PKC.

REFERENCES

Åberg, A.T., Solyakov, A., Bondesson, U. 2013. Development and in-house validation of an LC-MS/MS method for the quantification of the mycotoxins deoxynivalenol, zearalenone, T-2 and HT-2 toxin, ochratoxin A and fumonisin B1 and B2 in vegetable animal feed. Food Additives and Contaminants Part A 30: 541-549.

Abia, W.A., Simo, G.N., Warth, B., Sulyok, M., Krska, R., Tchana, A., Moundipa, P.F. 2013. Determination of multiple mycotoxins levels in poultry feeds from Cameroon. Japanese Journal of Veterinary Research 61: 33-39.

Adejumo, T.O., Hettwer. U., Karlovsky. P. 2007. Survey of maize from south-western Nigeria for zearalenone, a- and b-zearalenols, fumonisin B1 and enniatins produced by fusarium species. Food Additives and Contaminants 24: 993-1000.

Adesehinwa, A.O.K. 2007. Utilization of palm kernel cake as a replacement for maize in diets of growing pigs: Effects on performance, serum metabolites, nutrient digestibility and cost of feed conversion. Bulgarian Journal of Agricultural Science 13: 593-600.

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., Mirhosseini, H. 2012. Ochratoxin A quantification: Newly developed HPLC conditions. Food Control 23: 113-119.

Afsah-Hejri, L., Jinap, S., Hajeb, P., Radu, S., Shakibazadeh, S.H. 2013.A Review on mycotoxins in food and feed: Malaysia case study. Comprehensive Reviews in Food Science and Food Safety 12: 629-651.

Ahmad, M., Ahmad, M.M., Hamid, R., Abdin, M.Z., Javed, S. 2013. Use of response surface methodology to study the effect of media composition on aflatoxin production by Aspergillus flavus. Mycotoxin Research 29: 39-45.

Akande, M.M., Abubakar, T.A., Adegbola, S.E, Bogoro. 2006. Nutritional and health implications of mycotoxins in animal feed: A review. Pakistan journal of nutrition 5 (5): 398-403.

Alimon, A.R. and Hair-Bejo, M. 1995. Feeding systems based on oil palm by-products in Malaysia. In: Ho, Y.W., Vidyadaran, M.K. and Sanchez, M.D. (eds.) Proc. 1st Symposium on Integration of Livestock to Oil Palm production. Malaysian Society of Animal Production 105-115.

Alimon, A.R. 2004. The nutritive value of palm kernel cake for animal feed. Palm Oil Developments. 40:12-16.

Al-Taher, F., Banaszewski, K., Jackson, L., Zweigenbaum, J., Ryu, D., Cappozzo, J. 2013. Rapid method for the determination of multiple mycotoxins in wines and

beers by LC-MS/MS using a stable isotope dilution assay. Journal of Agricultural and Food Chemistry 61: 2378-2384.

Amate, C.F., Unterluggauer, H., Fischer, R.J., Fernández-Alba, A.R., Masselter, S. 2010. Development and validation of a LC–MS/MS method for the simultaneous determination of aflatoxins, dyes and pesticides in spices. Analytical and Bioanalytical Chemistry 397: 93-107.

Amri, N.I. 2013. Characteristics of Malaysian palm kernel and its products. Journal of Oil Palm Research 25: 245-252.

Andrade, P.D., Gomes da Silva, J.L., Caldas, E.D. 2013. Simultaneous analysis of aflatoxinsB1, B2, G1, G2, M1 and ochratoxin A in breast milk by high-performance liquidchromatography/fluorescence after liquid-liquid extraction with low temperature purification (LLE-LTP). Journal of Chromatography A 1304, 23: 61-68.

Anjum, M.A., Sahota. A.W., Akram, M., Ali, I. 2011. Prevalence of mycotoxins in poultry feeds and feed ingredients in Punjab (Pakistan). Journal of Animal & Plant Sciences 21: 117-120.

Ao, X., Zhou, T.X., Meng, Q.W., Lee, J.H., Jang, H.D., Cho, J.H., Kim, I.H. 2011. Effects of a carbohydrase cocktail supplementation on the growth performance, nutrient digestibility, blood profiles and meat quality in finishing pigs fed palm kernel meal. Livestock Science 137: 238-243.

Arroyo-Manzanares, N., Huertas-Pérez, J.F., García-Campaña, A.M., Gámiz-Gracia, L. 2014. Simple methodology for the determination of mycotoxins in pseudocereals, spelt and rice. Food Control 36: 94-101.

Arzandeh, S., Selamat, J., Lioe, H., 2010. Aflatoxin in raw peanut kernels marketed in Malaysia. Journal of Food and Drug Analysis. 18: 44-50.

Ates, E., Mittendorf, K., Stroka, J., Senyuva, H. 2012. Determination of fusarium mycotoxins in wheat, maize and animal feed using on-line clean-up with high resolution mass spectrometry. Food Additives and Contaminants: Part A 30:156-165.

Banerjee, S. and Mazumdar, S. 2012. Electrospray ionization mass spectrometry: A technique to access the information beyond the molecular weight of the analyte. International Journal of Analytical Chemistry 2012: 1-40.

Berthiller, F., Schuhmacher, R., Buttinger, G., Krska, R. 2005.Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, 1062: 209-216.

Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76: 965-977.

Bhat, R., Rai, R.V., Karim, A. 2010. Mycotoxins in food and feed: present status and future concerns. Comprehensive Reviews in Food Science and Food Safety 9: 57-81.

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

Biselli, S. and Hummert, C. 2005. Development of a multicomponent method for fusarium toxins using LC-MS/MS and its application during a survey for the content of T-2 toxin and deoxynivalenol in various feed and food samples. Food Additives and Contaminants 22: 752-760.

Biselli, S. and Hummert, C. 2007. Development of a multicomponent method for fusarium toxins using LCMS/MS and its application during a survey for the content of T-2 toxin and deoxynivalenol in various feed and food samples. Food Additives and Contaminants Part A 22: 752-760.

Blesa, J., Moltó, J., Akhdari, S.E., Mañes, J., Zinedine, A. 2014. Simultaneous determination of fusarium mycotoxins in wheat grain from Morocco by liquid chromatography coupled to triple quadrupole mass spectrometry. Food Control 46: 1-5.

Borhan, A.N.A, Venugopal, R., Amiruddin, N. and Simeh, M.A. 2005. Palm kernel cake marketing: constraints and prospects. Oil Palm Industry Economic Journal 5: 37-46.

Bottalico, A. 1998. Fusarium Diseases of Cereals: Species complex and related mycotoxin profiles in European. Journal of Plant Pathology 80: 85-103.

Box, G.E.P. and Wilson, K.B. 1951 On the experimental attainment of optimum conditions. Journal of the Royal Statistical Society Series B 13: 1-45.

Boyd, R.K., Basic, C., Bethem, R.A. 2008. Trace quantitative analysis by mass spectrometry. Chichester : John Wiley & Sons Ltd.

Bryden, W. L., 2012.Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Animal Feed Science and Technology 173: 134-158.

Burmistrova, N.A., Rusanova, T.Y., Yurasov, N.A., Goryacheva, I.Y., Saeger, S.D. 2014. Multi-detection of mycotoxins by membrane based flow-through immunoassay. Food Control 46: 462-469.

Capriotti, A.L., Foglia, P., Gubbiotti, R., Roccia, C., Samperi, R., Laganà, A. 2010. Development and validation of a liquid chromatography/atmospheric pressure photoionization-tandem mass spectrometric method for the analysis of mycotoxins subjected to commission regulation (EC) No. 1881/2006 in cereals. Journal of Chromatography A, 1217: 6044-6051.

Castegnaro, M., Tozlovanu, M., Wild, C., Molinie, A., Sylla, A., Pfohl-Leszkowicz, A. 2006. Advantages and drawbacks of immunoaffinity columns in analysis of

mycotoxins in food. Molecular Nutrition & Food Research 50: 480-7.

Cavaliere, C., Foglia, P., Pastorini, E., Samperi, R., Lagana, A. 2005. Development of a method for analysis of major Fusarium mycotoxins in corn meal using liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry19: 2085-2093.

Cavaliere, C., Foglia, P., Guarino, C., Motto, M., Nazzari, M., Samperi, R., Lagana, A., Berardo, N. 2007. Mycotoxins produced by Fusarium genus in maize: determination by screening and confirmatory methods based on liquid chromatography tandem mass spectrometry. Food Chemistry 105: 700-710.

Cigić, I.K. and Prosen, H. 2009. An Overview of conventional and emerging analytical methods for the determination of mycotoxins. International Journal of Molecular Sciences 10: 62-115.

Coker, R.D., Nagler, M.J., Defize, P.R., Derksen, G.B., Buchholz, H., Putzka, H.A., Hoogland, H.p., Roos, A.H., Boenke, A. 2000. Sampling plans for the delermination of aflatoxin B I in large shipments of animal foodstuffs. J AOAC hrt 83: 1251-1258.

De Souza, M.L.M, Sulyok, M., Freitas-Silva, O., Costa, S.S., Brabet, C., Junior, M.M., B., Vargas, E.A., Krska, R., Schuhmacher, R. 2013. Co-occurrence of mycotoxins in maize and poultry feeds from brazil by liquid chromatography/tandem mass spectrometry. The Scientific World Journal 2013:1-9.

Desmarchelier, A., Oberson, J.M., Tella, P., Gremaud, E., Seefelder, W., and Mother, P. 2010. Development and comparison of two multi-residue methods for the analysis of 17 mycotoxins in cereals by liquid chromatography electrospray ionization tandemmass spectrometry. Journal of Agricultural and Food Chemistry 58: 7510-7 519.

Döll, S. and Dänicke, S. 2011. The Fusarium toxins deoxynivalenol (DON) and zearalenone (ZON)in animal feeding. Preventive Veterinary Medicine 102: 132-145.

Driffield, M., Hird, S.J., MacDonald, J., 2003. The occurrence of a range of mycotoxins in animal offal food products by HPLC-MS/MS. Aspects of Applied Biology 68:205-210.

Duarte, S.C., Lino, C.M., Pena, A. 2011. Ochratoxin A in feed of food-producing animals: An undesirable mycotoxin with health and performance effects. Veterinary Microbiology 154: 1-13.

European Commission Directive, 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Official Journal of the European Communities, L 140/10.

European Commission Directive, 657/2002 of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European union, L221/8: 1-29.

European Commission Regulation, 2006/401/EC, Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.Official Journal of the European Communities 2006, L70/12: 20-23.

European Commission Recommendation, 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Official Journal of the European Union, L 229/7.

EC Commission Recommendation. 2013/165/EU of 27 March 2013 on the presence of T-2 andHT-2 toxin in cereals and cereal products. Official Journal of the European Union, L 91/1.

European mycotoxins awareness network. Sample preparation techniques for the determination of mycotoxins. Retrieved 22 August, 2014 from http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=61

EFSA (European Food Safety Authority). 2004a. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to aflatoxin B1as undesirable substance in animal feed. EFSA Journal, 39: 1-27.

EFSA (European Food Safety Authority). 2004b. Opinion of the scientific panel on contaminants in food chain on a request from the commission related to ochratoxin A(OTA) as undesirable substance in animal feed. EFSA Journal, 101: 1-36.

EFSA (European Food Safety Authority). 2004c. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to deoxynivalenol (DON) as undesirable substance in animal feed. EFSA Journal 73: 1-41.

EFSA (European Food Safety Authority). 2005. Opinion of the scientific panel on contaminants in food chain on a request from the commission related to fumonisins as undesirable substances in animal feed. EFSA Journal 235: 1-32.

EFSA (European Food Safety Authority). 2006. Opinion of the scientific panel on contaminants in the food chain on a request from the commission regulated to ochratoxin A in food. EFSA Journal 365: 1-56.

EFSA (European Food Safety Authority). 2007. Opinion of the scientific panel on contaminantsin the food chain on a request from the commission regulated to the potential increase of consumer health risk by a possible increase of the maximum levels for afltoxins in almonds, hazelnuts and pistachios and derived products. EFSA Journal 446: 1–127.

EFSA (European Food Safety Authority). 2011. Scientific Opinion on the risks for public health related to the presence of zearalenone in food. EFSA Journal9:1-124.

EFSA (European Food Safety Authority). 2013. Scientific report of EFSA: Deoxynivalenol in food and feed: occurrence and exposure. EFSA Journal

11: 1-56.

Fazekas, B., Tar, A., Kovács, M. 2005. Aflatoxin and ochratoxin A content of spices in Hungary. Food Additives and contaminants 22:856-63.

FDA (Food and Drug Administration) Mycotoxin Regulatory Guidance. 2011. A Guide for Grain Elevators, Feed Manufacturers, Grain Processors and Exporters. National Grain and Feed Association.

Fellinger, A., 2006. Worldwide mycotoxin regulations and analytical challenges. World Grain Summit: Foods and Beverages, September 17-20, San Francisco, California,USA.

Ferracane, R., Tafuri, A., Logieco, A., Galvano, F., Balzano, D., Ritieni, A. 2007. Simultaneous determination of aflatoxin B1 and ochratoxin A and their natural occurrence in virgin olive oil. Food Additives and contaminants24:173-80.

Foroud, N.A. and Eudes, F. 2009. Trichothecenes in cereal grains. International Journal of Molecular Sciences 10: 147-173.

Frenich, A.G., Vidal, J.L.M., Romero-Gonza´lez, R. Aguilera-Luiz, M.d.M. 2009. Simple and high-throughput method for the multimycotoxin analysis in cereals and related foods by ultra-high performance liquid chromatography/ tandem mass spectrometry. Food Chemistry 117: 705-712.

Göbel, R. and Lusky, K. 2004. Simultaneous determination of aflatoxins, ochratoxin A, and zearalenone in grains by new immunoaffinity column/liquid chromatography. Journal of AOAC International 87: 411-416.

Gong, X., Wang, H., Zhang, Y., Sun, J., Dong, J., Lin, L., Yu, J., Feng, Z., Li, K. 2012. Determination of 15 mycotoxins in foods and feeds using high performance liquid chromatography-tandem mass spectrometry with gel permeation chromatography combined QuEChERS purification. Journal of Chromatography & Separation Techniques 3:1-7.

Griessler, K., Rodrigues, I., Handl, J., and Hofstetter, U. Occurrence of mycotoxins in SouthernEurope. World Mycotoxin J. 2010; 3: 301–309

He, X., Xi, C., Tang, B., Wang, G., Chen, D., Peng, T., Mua, Z. 2014. Simultaneous determination of 30 hormones illegally added to anti-ageing functional foods using UPLC-MS/ MS coupled with SPE clean-up. Food Additives & Contaminants: Part A 31: 1625-1638.

Herebian, D.1., Zühlke, S., Lamshöft, M., Spiteller, M. 2009.Multi-mycotoxin analysis in complex biological matrices using LC-ESI/MS: experimental study using triple stage quadrupole and LTQ-Orbitrap. Journal of Separation Science 32: 939-48.

Herzallah, S.M. 2009. Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. Food Chemistry 114: 1141-1146.

Huanga, Y., Yuan, Y., Zhou, Z., Liang, J., Chen, Z., Lia, G. 2014. Optimization and

evaluation of chelerythrine nanoparticles composed of magnetic multiwalled carbon nanotubes by response surface methodology. Applied Surface Science 292: 378-386.

Iqbal, S.Z., Asi, M.R., Jinap, S., Rashid, U. 2014. Detection of aflatoxins and zearalenone contamination in wheat derived products. Food Control 35: 223-226.

Jalili, M., Jinap, S. 2012. Natural occurrence of aflatoxins and ochratoxin A in commercial dried chili. Food Control 24:160-164.

Jia, W., Chu, X., Ling, Y., Huang, J., Changc, J. 2014. Multi-mycotoxin analysis in dairy products by liquid chromatography coupled to quadrupole orbitrap mass spectrometry. Journal of Chromatography A 1345: 107-114.

Josephs. R.D, Krska. R, Grasserbauer. M, Broekaert. J. A. C, Chromatogr. J. A. 1998. Determination of trichothecene mycotoxins in wheat by use of supercritical fluid Extraction and high-performance liquid chromatography with diode array detection or gas chromatography with electron capture detection. Journal of Chromatography A 795:297-304.

Kabak. B, 2009. The fate of mycotoxins during thermal food processing. Journal of Science and Food Agriculture 89:549-554.

Khayoon, W. S., Saad, B., Yan, C.B., Hashim, N.H., Mohamed Ali, A.S., Salleh, M.R., Salleh, B2010. Determination of aflatoxins in animal feeds by HPLC with Multifunctional column clean- up. Food Chemistry 118: 882-886.

Khorasgani, Z. N., Nakisa, A., Farshpira, N.R. 2013. The Occurrence of aflatoxins in peanuts in supermarkets in ahvaz, Iran. Journal of Food Research 2: 94-100.

Kokkonen, M.K., Jestoi, M.N.A. 2009. Multi-compound LC-MS/MS method for the screening of mycotoxins in grains. Food Analytical Methods 2: 128-140.

Kolapo, A.L., Oladimeji, G.R., Ifejika, A.I., Osakwe, O.E., Eyitayo, l.R., Oyelakin, O. A.2012. Aflatoxin, nutritive values and microbiological status of stored cakes of some selected Nigerian oil seeds. Global Journal of Science Frontier Research Agriculture & Biology 12: 13-22.

Kolosova, A., Stroka, J. 2012. Evaluation of the effect of mycotoxin binders in animal feed on the analytical performance of standardised methods for the determination of mycotoxins in feed. Food additives and contaminants Part A. 29:1959-71.

Kong, W.J., Liu, S.Y., Qiu, F., Xiao, X.H. Yang, M.H. 2013. Simultaneous multi-mycotoxin determination in nutmeg by ultrasound-assisted solid-liquid extraction and immunoaffinity column clean-up coupled with liquid chromatography and on-line post-column photochemical derivatization-fluorescence detection. Analyst 138: 2729-2739.

Kostiainen R. 1991. Identification of trichothecenes by thermospray, plasmaspray and dynamic fast-atom bombardment liquid chromatography-mass spectrometry. Journal of Chromatography B 562: 555-62.

Kperegbeyi, J.I. and Ikperite, S.E. 2011. The effectiveness of replacing maze with palm kernel cake in broilers' starter diets. Journal of Environmental Issues and Agriculture in Developing Countries 3: 145-149.

Krska, R., Welzig, E., Berthiller, F., Molinelli, A., Mizaikoff, B. 2005. Advances in A 22: 345–353.

Krska, R., Welzig, E., Boudra, H. 2007. Analysis of fusarium toxins in feed. Animal Feed Science and Technology 137: 241–264.

Krnjaja, V., Stojanović, L.J., Cmiljanić, R., Trenkovski, R., Tomašević, D. 2008. The presence of potentially toxigenic fungi in poultry feed. Biotechnology in Animal Husbandry 24:87-93.

Lacina, O.O., Zachariasova, M., Urbanova, J., Vaclavikova, M., Cajka, T., Hajslova, J. 2012. Critical assessment of extraction methods for the simultaneous determination of pesticide residues and mycotoxins in fruits, cereals, spices and oil seeds employing ultra-high performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 1262: 8-18.

Lanttanzio, V.M.T., Della Gatta, S., Godula, M., Visconti, A. 2011. Quantitative analysis of mycotoxinsin cereal foods by collision cell fragmentation-high-resolution mass spectrometry: performance and comparison with triple-stage quadrupole detection. Food Additives Contaminants: Part A 28:1424-1437.

Lanttanzio, V.M.T., Solfrizzo, M., Powers, S. Visconti, A. 2007. Simultaneous determination ofaflatoxins, ochratoxin A and Fusarium toxins in maize by liquid chromatography/tandemmass spectrometry after multitoxin immunoaffinity cleanup. Rapid Communications in Mass Spectrometry 21: 3253–3261.

Lattanzio, V.M.T., Solfrizzo, M., Visconti, A. 2008. Determination of trichothecenes in cereals and cereal-based products by liquid chromatography-tandem mass spectrometry. Food Additives and Contaminants 25: 320-330.

Lattanzio, V.M.T., Pascale, M., Visconti, A. 2009.Current analytical methods for trichothecene mycotoxins in cereals. Trends in Analytical Chemistry 28: 758–768.

Lerda, D. 2011. Maycotoxin fact sheet. Among hundreds of fungal metabolites a few fall into the category of mycotoxins and only a few considerable food safety hazards. 4th edition.

Li, X., Li, P., Zhang, Q., Li, R., Zhang, W., Zhang, Z., Ding, X., Tang, X. 2013. Multi-component immuneochromatographic assay for simultaneous detection of aflatoxin B1, ochratoxin A and zearalenone in agro-food. Biosensors and Bioelectronics 49:426-432.

Liao, C.D., Lin, H.Y., Chiueh, L.C., Shih, D.Y.C. 2011. Simultaneous quantification of aflatoxins, ochratoxin A and zearalenone in cereals by LC-MS/MS. Journal of Food and Drug Analysis 19: 259-268.

Lim, H.A., Ng, W.K., Lim, S.L., Ibrahim, C. 2008. Contamination of palm kernel meal

with aspergillus flavus affects its nutritive value in pelleted feed for tilapia, Oreochromis mossambicus. Aquaculture Research 32: 895-905.

Liu, D.D., Xu, H.M., Tian, B.C., Yuan, K., Pan, H., Ma, S.L., Yang, X.G., Pan. W.S. 2012. Fabrication of carve dilol nano suspensions through the anti-solvent precipitation-ultrasonication method for the improvement of dissolution rate and oral bioavailability. AAPS Pharmcectual Science and Technology 13: 295-304.

Malik, A.K., Blasco, C., Picó,Y. 2010. Liquid chromatography-mass spectrometry in food safety. Journal of Chromatography A 1217: 4018-4040.

Marin, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V. 2013.Mycotoxins: Occurrence, toxicology, and exposure assessment. Food and Chemical Toxicology 60: 218-237.

Marini, A.M., Daud, M.J., Noraini, S., Jame'ah, H. and Engku Azahan, E.A. 2005. Performance of locally isolated microorganism in degrading palm kernel cake (PKC) fibre and improving the nutritional value of fermented PKC. Journal of Tropical Agriculture and Food Science33: 311-319.

Martos, P.A., Thompson, W., Diaz, G.J. 2010. Multiresidue mycotoxin analysis in wheat, barley, oats, rye and maize grain by high-performance liquid chromatography-tandem mass spectrometry. World Mycotoxin Journal 3: 205-223.

Mason, R., Gunst, R., & Hess, J. 2003. Statistical design and analysis of experiments: with applications to engineering and science. London: John Wiley and Sons Inc.

Maul, R., Pielhau, R., Koch, M. 2014. Evaluation of an extraction method and spin column clean up procedure for Fusarium mycotoxins and their masked derivatives from grain matrix. Food Control 40: 151-156.

Mavungu, D.D., Monbaliu, S., Scippo, M.L., Maghuin-Rogister, G., Schneider, Y.J., Larondelle,Y., Callebaut, A., Robbens, J., Van Peteghem, C., De Saeger, S. 2009. LC-MS/MS multi-analyte method for mycotoxin determination in food supplements.Food Additives and Contaminants: Part A 26: 885-95.

Meneely, J.P., Ricci, F., Egmond, H.P., Elliott, C.T. 2011. Current methods of analysis for thedetermination of trichothecene mycotoxins in food. Trends in Analytical Chemistry 30: 192–203.

Milanez, T.V. and Valente-Soares, L.M. 2006. Gas chromatography - mass spectrometry determination of trichothecene mycotoxins in commercial Corn Harvested in the State of São Paulo, Brazil. Journal of the Brazilian Chemical 17: 412-416.

Milićević, D. R., Škrinjar, M., Baltić, T., 2010. Real and perceived risks for mycotoxins contamination in foods and feeds: challenges for food safety control. Toxins 2: 572–592.

Minervini, F., Debellis, L., Garbetta, A., De Girolamo, A., Schena, R., Portincasa, P., Visconti, A. 2014. Influence on functional parameters of intestinal tract induced by short-term exposure to fumonisins contaminated corn chyme samples. Food and

Chemical Toxicology 66, 166-172.

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

Mol, H.G., Plaza-Bolaños, P., Zomer, P., de Rijk, T.C., Stolker, A.A., Mulder, P.P. 2008. Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. Analytical Chemistry 80: 9450-9459.

Monbaliu, S., Van Poucke, C., Detavernier, C.L., Dumoulin, F.d.R., Van De Velde, M., Schoeters, E., Van Dyck, S., Averkieva, O., Van Peteghem, C., De Saeger, S. 2010. Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC–MS/MS method. Journal of Agricultural and Food Chemistry 58: 66-71.

Monbaliu, S., Van Poucke, C., Van Peteghem, C., Van Poucke, K., Heungens, K., De Saeger, S. 2009. Development of a multi-mycotoxin liquid chromatography/ tandem mass spectrometry method for sweet pepper analysis. Rapid Communications in Mass Spectrometry 23: 3-11.

Montgomery, D. 2001. Design and analysis of experiments. New York: John Wiley & Sons Inc.

MPOB (Malaysian Palm Oil Board). Retrieved on 22 of October 2014 from http://bepi.mpob.gov.my/index.php/statistics/production/118-production-2013/605-production-of-palm-kernel-2013.html.

Muniandy, N. 1989. The occurrence of aflatoxins in animal feedstuffs in Malaysia. Malaysian Journal of Veterinary Research 2:79-82.

Muscarella, M., Magro, S.L., Palermo, C., Centonze, D. 2007. Validation according to European Commission Decision 2002/657/EC of a confirmatory method for aflatoxin M1 in milk based on immunoaffinity columns and high performance liquid chromatography with fluorescence detection. Anal Chim Acta 594:257-264.

Ng, W.K. 2004. Researching the use of palm kernel cake in aquaculture feeds. Palm Oil Developments 41: 19-21.

Nguyen M.T., Tozlovanu M., Tran T.L., Pfohl-Leszkowicz A. 2007. Occurrence of aflatoxin B1,citrinin and ochratoxin a in rice in five provinces of central region in Vietnam. Food Chemistry 105: 42-47.

Njobeh, P.B., Dutton, M.F., Åberg, A.T., Haggblom, P. 2012. Estimation of multi-mycotoxins contamination in South African compound feeds. Toxins 4: 836-848.

Olsson, J., Börjesson, T., Lundstedt, T., Schnürer, J., Int. J. 2002. Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. Food Microbiol 72: 203-214.

Onji, Y., Aoki, Y., Tani, N., Umebayashi, K., Kitada, Y., Dohi, Y., 1998. Direct analysis of several Fusarium mycotoxins in cereals by capillary gas chromatography-mass spectrometry. Journal of Chromatography A 815: 59–65.

Patel, D., 2011. Matrix effect in a view of LC-MS/MS: An overview. International Journal of Pharma and Bio Sciences 2: 559-564.

Pietrasik, Z., & Li-Chan, E. 2002. Response surface methodology study on the effects of salt, microbial transglutaminase and heating temperature on pork batter gel properties. Food Research International 35: 387-396.

Pietruszka, K., Sell, B., Burek, O., Wiśniewska-Dmytrow, H. 2013.Simultaneous determination of multi-component mycotoxin in feeds by liquid chromatography-tandem mass spectrometry. Bulletin of the Veterinary Institute in Pulawy 57: 567-572.

Pittet, A. Modern methods and trends in mycotoxin analysis. Paper presented at the 117th annual conference of the society of food and environmental chemistry, Lausanne. September 2005.

Piotrowska, M., Śliżewska, K., Biernasiak, J. 2013. Mycotoxins in cereal and soybean-based food and feed. Agricultural and Biological Sciences 10: 187-230.

Pizzutti, I. R., Kok, A., Scholten, J., Righi, L.W., Cardoso, C.D., Rohers, G.N., Silva, R.C. 2014.Development, optimization and validation of a multi method for the determination of 36mycotoxins in wines by liquid chromatography-tandem massspectrometry. Talanta 129:352-363.

Poornima, K. and Palanisamy, A. 2013. Mycoflora and multitoxin analysis of poultry raw materials and biocontrol of mycoflora by medicinal plant extracts. International Journal of Pharma and Bio Sciences 2:265-271.

Pranowo, D., Nuryono., Agus, A., Weclhastri, S., Reiter, E.V., Razzazi-Fazeli, E., Zentek, J. 2013. A limited survey of aflatoxin contamination in Indonesian palm kernel cake and copra mear sampled from batches. Mycotoxin Research 29: 135-139.

Rahmani, A., Selamat, J., Soleimany, F. 2009.Qualitative and quantitative analysis of mycotoxins. Comprehensive review in food science and food 8: 202-251.

Rahmani, A., Selamat, J., Soleimany, F. 2010. Validation of the procedure for the simultaneous determination of aflatoxins ochratoxin A and zearalenone in cereals using HPLC-FLD. Food Additives and Contaminants 27: 1683-1693.

Rahmani, A., Jinap, S., Soleimany, F., Khatib, A., Tan, C.P. 2011a. Sample preparation optimization for the simultaneous determination of mycotoxins in cereals. European Food Research and Technology 232: 723-735.

Rahmani, A., Selamat, J., Soleimany, F.2011b. Optimization and validation of a HPLC method for simultaneous determination of aflatoxin B1, B2, G1, G2,ochratoxin A and zearalenone using an experimental design. Food Additives and Contaminants

28:902-912.

Rahmani, A., Jinap, S., Khatib, A., Tan, C.P. 2013. Simultaneous determination of aflatoxins, ochratoxin A, and zearalenone in cereals using a validated RP-HPLC method and phred derivatization system. Journal of Liquid Chromatography & Related Technologies 36: 600-617.

Rajeshkumar, S., Jobitha, G.G., Malarkodi, C., Kannan, C., Annadurai, G. 2013. Optimization of marine bacteria enterococcus sp. biomass growth by using response surface methodology. Journal of Environmental Nanotechnology 2: 20-27.

Rasmussen, R.R., Storm, I.M.L.D., Rasmussen, P.H., Smedsgaard, J., Nielsen, K.F. 2010. Multi-mycotoxin analysis of maize silage by LC-MS/MS. Analytical and Bioanalytical Chemistry 397: 765-776.

Razzazi-Fazeli, E., Böhm, J., Jarukamjorn, K., Zentek, J. 2003.Simultaneous determination ofmajor B-trichothecenes and the de-epoxy-metabolite of deoxynivalenol in pig urine and maize using high-performance liquid chromatography-mass spectrometry. Journal of Chromatography B 796: 21-33.

Reddy, K.R.N. and Salleh, B. 2010. A preliminary study on the occurrence of Aspergillus sppand aflatoxin B 1 in imported wheat and barley in Penang, Malaysia. Mycotoxin Research 26: 267-271.

Reddy, K. and Salleh, B. 2011. Co-occurrence of moulds and mycotoxins in corn grains Spectrometry used for animal feeds in Malaysia. Journal of Animal and Veterinary Spectrometry Advances 10: 668-673.

Reiter, E.V., Dutton, M.F., Agus, A., Nordkvist, E., Mwanza, M.F., Hjobeh P.B., Pranowo, D., Haeggblom, P., Razzazi-Fazeli, E., Zentek, J., Anderson, M.G. 201l. Uncertainty from sampling in measurernents of aflatoxins in animal feeding stuffs: application of the Eurachem/ClTAC guidclines. Analyst 136: 4059-1069.

Ren, Y., Zhang, Y., Shao, S., Cai, Z., Feng, L., Pan, H., Wang, Z. 2007. Simultaneous determination of multi-component mycotoxin contaminants in foods and feeds by ultra-performance liquid chromatography tandem mass spectrometry. Journal of Chromatography A 1143: 48-64.

Richard, L.J. 2007. Some major mycotoxins and their mycotoxicoses-An overview. spectrometry. International Journal of Food Microbiology 119: 3-10.

Rodríguez-Carrasco, Y., Berrada, H., Font, G., Mañes, J. 2012. Multi-mycotoxin analysis in wheat semolina using an acetonitrile-based extraction procedure and gas chromatography-tandem mass spectrometry. Journal of Chromatography A 1270: 28-40.

Rodrigues, I. and Naehrer, K.2012. Prevalence of mycotoxins in feedstuffs and feed surveyed worldwide in 2009 and 2010. Phytopathologia Mediterranea 51: 175-192.

Rodríguez-Carrasco, Y., Font, G., Mañes, J., Berrada, H. 2013. Determination of

mycotoxins in bee pollen by gas chromatography−tandem mass spectrometry. Journal of Agricultural and Food Chemistry 61: 1999-2005.

Royer, D., Humpf, H.U., Guy, P.A. 2004.Quantitative analysis of Fusarium mycotoxins inmaizeusing accelerated solvent extraction before liquid chromatography/ atmospheric pressure chemical ionization tandem mass spectrometry. Food Additives and Contaminants 21: 678-92.

Rubert, J., Soler, C., Manes, J. 2010. Optimization of matrix solid-phase dispersion method for simultaneous extraction of aflatoxins and OTA in cereals and its application to commercial samples. Talanta 82: 567-574.

Rundberget, T. and Wilkins, A.L. 2002. Determination of penicillium mycotoxins in foods andfeeds using liquid chromatography-mass spectrometry. Journal of Chromatography A 964: 189-97.

Saleemi, M.K., Khan, M.Z., Khan, A., Javed, I. 2010. Mycoflora of poultry feeds and mycotoxins producing potential of aspergillus species. Pakistan Journal of Botany 42: 427-434.

Schollenberger, M., Lauber, U., Jara, H. T., Suchy, S., Drochner, W., & Müller, H. M. 1998. Determination of eight trichothecenes by gas chromatography-mass spectrometry after sample clean-up by a two-stage solid-phase extraction. Journal of Chromatography A815: 123-132.

Schothorst, R.C. and Jekel, A.A. 2001. Determination of trichothecenes in wheat by capillary gas chromatography with flame ionisation detection. Food Chemistry 73: 111-117.

Scudamore, K.A., Hazel, C.M., Patel, S., Scriven, F. 2009. Deoxynivalenol and other Fusarium mycotoxins in bread, cake, and biscuits produced from UK-grown wheat under commercial and pilot scale conditions. Food Additives & Contaminants: Part A 26: 1191-1198.

Seo, D., Phat, C., Kim, D., Lee, C. 2013. Occurrence of fusarium mycotoxin fumonisin B1 and B2 in animal feeds in Korea. Mycotoxin Research 29:159-167.

Sharmila, A., Alimon, A.R., Azhar, K., Noor, H.M., Samsudin, A.A. 2014. Improving nutritional values of palm kernel cake (PKC) as poultry feeds: A Review. Malaysian Journal of Animal Science 17:1-18.

Škrbić, B., Godula, M., Đurišić-Mladenović, N., Živančev, J. 2011.Multi-mycotoxin analysis by UHPLC-HESI-MS/MS:A preliminary survey of Serbian wheat flour. Agronomy Research9: 461- 468.

Soleimany, F., Jinap, S., Abasb, F. 2012. Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. Food Chemistry 130:1055-1060.

Soleimany, F., Jinap, S., Rahmani, A., Khatib, A. 2011. Simultaneous detection of 12 mycotoxins in cereals using RP-HPLC-PDA-FLD with PHRED and a post-column derivatization system. Food Additives and Contaminants: Part A 28:494-501.

Solfrizzo, M., De Girolamo, A., Lattanzio, L.V.M.T., Visconti, A., Stroka, J., Alldrick, J., Egmond, H.P.V. 2013. Results of a proficiency test for multi-mycotoxin determinationin maize by using methods based on LC-MS/(MS).Quality Assurance and Safety of crops & foods 5: 15-48.

Song, S., Ediage, E.N., Wu, A., De Saeger, S. 2013. Development and application of salting-out assisted liquid/liquid extraction for multi-mycotoxin biomarkers analysis in pig urine with high performance liquid chromatography/tandem mass spectrometry. Journal of Chromatography A1292: 111-120.

Songsermsakul, P. and Razzazi-Fazeli, E. 2008. A review of recent trends in applications of liquid chromatography-mass spectrometry for determination of mycotoxins. Journal of Liquid Chromatography &Related Technologies 31: 1641-1686.

Spanjer, M.C., Rensen. P.M., Scholten, J.M. 2008. LC-MS/MS multi-method for Mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. Food Additives and Contaminants 25: 472-489.

Spanjer, M.C., Scholten, J.M., Kastrup, S., Jorissen, U., Schatzki, T.F., Toyofuku, N. 2006. Samplecomminution for mycotoxin analysis: Dry milling or slurry mixing? Food Additives and Contaminants 23: 73-83.

Stecher, G., Jarukamjorn, K., Zaborski, P., Bakry, R., Huck, C.W., Bonn, G.K. 2007. Evaluation of extraction methods for the simultaneous analysis of simple and macrocyclic trichothecenes. Talanta 73: 251-257.

Stevens, G.W., Lo, T.C., Baird, M.H.I. 2007. "Extraction, liquid-liquid", in Kirk-Othmer Encyclopedia of Chemical Technology. Accessed 25 July 2014.

Streit, E., Schatzmayr, G., Tassis, P., Tzika, E., Marin, D., Taranu, I., Tabuc, C., Nicolau, A., Aprodu, I., Puel, O., Oswald, I. 2012. Current situation of mycotoxin contamination and co-occurrence in animal feed-focus on Europe. Toxins 4:788-809.

Streit, E., Schwab, C., Sulyok, M., Naehrer, K., Krska, R., Schatzmayr, G. 2013. Multi-Mycotoxin screening reveals the occurrence of 139 different secondary metabolites in feed and feed ingredients. Toxins 5: 504-523.

Sulyok, M., Berthiller, F., Krska, R., Schuhmacher, R. 2006. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39mycotoxins in wheat and maize. Rapid Communications in Mass Spectrometry 20: 2649-2659.

Sulyok, M., Krska, R., Schuhmacher, R. 2007. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantifi cation of 87analytes and its application to semi-quantitative screening of moldyfood samples. Analytical and Bioanalytical Chemistry 389: 1505-1523.

Sulyok, M., Krska, R., Schuhmacher, R. 2010. Application of an LC-MS/MS based

multi-mycotoxin method for the semi-quantitative determination of mycotoxins occurring indifferent types of food infected by moulds. Food Chemistry 119: 408-416.

Sørensen, L.K., Elbæk, T.H. 2005. Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. Journal of Chromatography B 820: 183–196.

Tamura, M., Takahashi, A., Uyama, A., Mochizuki, N.A. 2012. Method for multiple Mycotoxins analysis in wines by solid phase extraction and multifunctional cartridge purification, and Ultra-High-performance liquid chromatography coupled to tandem mass spectrometry. Toxins 4: 476-486.

Tan. Q. L. P, Kieu. X. N. T, Kim. N. H. T and Hong. X. N. T, 2012. Application of response surface methodology (RSM) in condition optimization for essential oil production from Citrus latifolia. Emirates Journal of Food and Agriculture 24: 25-30.

Tanaka. H, Takino. M, Sugita-Konishi. Y and Tanaka. T.2006.Development of a liquid chromatography/time-of-flight mass spectrometric method for the simultaneous determination of trichothecenes, zearalenone and aflatoxins in foodstuffs. Rapid Communications in Mass Spectrometry 20: 1422-1428.

Tanaka, T., Yoneda, A., Inouea, S., Sugiura, Y., Ueno, Y. 2000.Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography-mass spectrometry. Journal of Chromatography A 882: 23–28.

Tang, Y.Y., Lin, H.Y., Chen, Y.C., Su, W.T., Wang, S.C., Chiueh, L.C., Shin, Y.C. 2012. Developmentof a quantitative multi-mycotoxin method in rice, maize, wheat and peanut using UPLC-MS/MS. Food Analitical Chemistry 6:727-736.

Teller, R.S., Schmidt, R.J., Whitlow, L.W., Kung Jr., L. 2012. Effect of physical damage to ears of corn before harvest and treatment with various additives on the concentration of mycotoxins, silage fermentation and aerobic stability of corn silage.Journal of Dairy Science 95:1428-1436.

Tsiplakou, E., Anagnostopoulos, C., Liapis, K., Haroutounian, S. A., Zervas, G. 2014. Determination of mycotoxins in feedstuffs and ruminant's milk using an easy and simple LC-MS/MS multi-residue method. Talanta130: 8-19.

Turcotte, A.M., Scott, P.M., Tague, B. 2013. Analysis of cocoa products for ochratoxin A andaflatoxins. Mycotoxin Research 29:193-201.

Turner, N.W., Subrahmanyam, S., Piletsky, S.A. 2009. Analytical methods for determination of mycotoxins: A review. Analytica Chimica Acta 632: 168-180.

Vaclavikova, M., Malachova, A., Veprikova, Z., Dzuman, Z., Zachariasova, M., Hajslova, J.2013.'Emerging' mycotoxins in cereals processing chains: changes of enniatins during beer and bread making. Food Chemistry 136: 170-757.

Varga, E., Glauner, T., Köppen, R., Mayer, K., Sulyok, M., Schuhmacher, R., Krska,

R., Berthiller, F. 2012. Stable isotope dilution assay for the accurate determination of mycotoxins in maize by UHPLC-MS/MS. Analytical and Bioanalytical Chemistry 402:2675-2686.

Varga. E, Malachova, A., Schwartz, H., Krska, R., Berthiller, F. 2013. Survey of Deoxynivalenol and its conjugates deoxynivalenol-3-glucoside and 3-acetyl-deoxynivalenol in374 beer samples. Food Additives & Contaminants: Part A 30: 137-146.

Ventura, M., Guille´n, D., Anaya, I., Broto-Puig, F., Lliberia, J.L., Agut, M., Comellas, L. 2006.Ultra-performance liquid chromatography/tandem mass spectrometry for the simultaneous analysis of aflatoxins B1, G1, B2, G2 and ochratoxin A in beer. Rapid Communications in Mass Spectrometry 20: 3199-3204.

Vishwanath, V., Sulyok, M., Labuda, R., Bicker, W., Krska, R. 2009.Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatography/ tandem mass spectrometry. Analytical and Bioanalytical Chemistry 395:1355-1372.

Wang, M.L., Yan, H.F., Fu, S.L., Zhang, F., Yao, J.T., Dai, H., Li, Y.J. 2012. Rapid screeningand confirmation of hormones in health foods by high performance liquid chromatography-ion trap time of flight tandem mass spectrometry. Chinese Journal of Chromatography30:980-985.

Wang, U.K., Shi, Y.B., Zou, Q., Sun, J.H., Chen, Z.F., Wang, H.A., Li, S.Q., Yan, Y.X. 2013. Development of a rapid and simultaneous immune chromatographic assay for the determination of zearalenone and fumonisin B1 in corn, wheat and feedstuff samples. Food Control 31:180-188.

Wang, Y., Chai, T., Lu, G., Quan, C., Duan, H., Yao, M., Zucker, B., Schlenker, G., 2008. Simultaneous detection of airborne aflatoxin ochratoxin and zearalenone in a poultry house by immunoaffinity clean-up and high-performance liquid chromatography. Environmental Research 107: 139-144.

Wei, R., Qiu, F., Kong, W., Wei, J., Yang, M., Luo, Z., Qin, J., Mad, X. 2013.Co-occurrence of aflatoxin B1, B2, G1, G2 and ochrotoxin A in Glycyrrhiza uralensis analyzed by HPLC-MS/MS. Food Control 32: 216-221.

Whitmire, M., Ammerman, J., de Lisio, P., Killmer, J., Kyle, D. 2011. LC-MS/ MS Bioanalysis method development, validation, and sample analysis: points to consider when conducting nonclinical and clinical studies in accordance with current regulatory guidance. Analytical & Bioanalytical Techniques 3: 1-10.

Woloshuk, C.P., Shim, W.B. 2013. Aflatoxins, fumonisins and trichothecenes: a convergence of knowledge. FEMS Microbiology Reviews 37: 94-109.

Wong, C.M.V.L., Lau, S.Y.L., Abdullah, N. and Elaine, R.D.T. 2010. Bioconversion of palm kernel cake (PKC) to value added feed using fibrolytic bacteria and fungi. Paper presented at the National Biotechnology Seminar, Putra World Trade Centre, Kuala Lumpur.

Yang, Y., Shao, B., Zhang, J., Wu,Y.N., Duan, H.J. 2009. Determination of the residues of 50anabolic hormones in muscle, milk and liver by very-high-pressure liquid chromatography-electrospray ionization tandem mass spectrometry. Journal of chromatography B.877:489-496.

Zachariasova, M., Lacina, O., Malachova, A., Kostelanska, M., Poustka, J., Godula, M., Hajslova, J. 2010. Novel approaches in analysis of fusarium mycotoxins in cereals employing ultra performance liquid chromatography coupled with high resolution mass spectrometry. Analytica Chimica Acta 662:51-61.

Zachariasova, M., Dzumana, Z., Veprikova, Z., Hajkova, K., Jiru, M., Vaclavikova, M., Zachariasova, A., Pospichalova, M., Florian, M., Hajslova, J. 2014. Occurrence of multiple mycotoxins in European feeding stuffs, assessment of dietary intake by farm animals. Animal Feed Science and Technology 193: 124-140.

Zahari, W.M. and Alimon, A.R. 2004. Use of palm kernel cake and oil palm by-products in feed compounds. Palm Oil Developments 40: 5-9.

Zain, M.E. 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15: 129-144.

Zhang, K., Wong, J.W., Krynitsky, A.J., Trucksess. M.W. 2014. Determining mycotoxins in baby foods and animal feeds using stable isotope dilution and liquid chromatography tandem mass spectrometry. Journal of Agricultural and Food Chemistry62: 8935-8943.

Zo¨ llner, P. and Mayer-Helm, B. 2006. Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionization mass spectrometry. Journal of Chromatography A 1136: 123-169.