

TABLE OF CONTENTS	PAGE
Acknowledgement	i
Abstract	ii
Abstrak	v
List of Tables	xiv
List of Figures	xviii
List of Plates	xx
Abbreviations	xxi

Chapter 1.0 General Introduction

1.1 Basic concepts of water availability in food	1
1.1.1 Water activity (A_w)	1
1.1.2 Sorption effects	2
1.2 Factors affecting fungal growth and mycotoxin production in food	4
1.2.1 Water activity	4
1.2.2 Temperature	5
1.2.3 Interaction of temperature and water activity	6
1.2.4 pH	8
1.2.5 Oxygen (and carbon dioxide) tension	8
1.2.6 Type of substrate and nutritional factors	9
1.2.7 Consistency	10
1.2.8 Chemical treatment and presence of preservatives	11
1.2.9 Specific solute effects	12
1.3 Mycotoxins	12
1.3.1 Definition	12
1.3.2 History of mycotoxins	13
1.3.3 Structure and formation of mycotoxins	14
1.3.4 Mycotoxicoses	19
1.3.5 Toxicological impact of important mycotoxins	21
1.4 Mycotoxins produced by species of <i>Penicillium</i> and <i>Aspergillus</i> occurring on cereals	26
1.5 Present status of fungal contamination and mycotoxin situation in Malaysia	30
1.6 Scope and objectives of study	34

	PAGE
Chapter 2.0 Survey of fungal counts and the incidence of aflatoxin producing species in starch-based food and screening for aflatoxins in ordinary rice and wheatflour at the consumer level in Malaysia	
2.1 Introduction	37
2.2 Materials and methods	39
2.2.1 Sampling of starch-based food	39
2.2.2 Determination of fungal counts in starch-based food by dilution plating	40
2.2.3 Extraction and analysis of aflatoxins	41
2.3 Results	42
2.3.1 Total mycoflora count and aflatoxin producing colonies	42
2.3.2 Resolution of aflatoxin B ₁ , aflatoxin B ₂ , aflatoxin G ₁ – and aflatoxin G ₂	47
2.3.3 Aflatoxin contamination in rice and wheatflour samples	50
2.4 Discussion	54
Chapter 3.0 Water adsorption isotherms and fungal development on starch-based food stored at different levels of water activity	
3.1 Introduction	57
3.2 Materials and methods	59
3.2.1 Sampling and subsampling of starch-based food	59
3.2.2 Determination of the initial moisture content of starch-based food	59
3.2.3 Determination of the water adsorption isotherm of starch-based food	60
3.2.4 Fungal development in starch-based food stored at various levels of water activity	60
3.3 Results	61
3.3.1 The initial moisture content of starch-based food	61
3.3.2 Water adsorption isotherm of starch-based food	61
3.3.3 Time taken for the development of fungi in starch-based food stored at various levels of water activity at 25 °C	66

	PAGE
3.4 Discussion	68
 Chapter 4.0 The incidence of fungi in starch-based food stored at different levels of water activity and their role in biodeterioration	
4.1 Introduction	72
4.2 Materials and method	75
4.2.1 Fungal incidence on starch-based food stored at different levels of water activity	75
4.2.1.1 Incubation of starch-based food	75
4.2.1.2 Direct plating method	75
4.2.1.3 Dilution plating method	75
4.2.1.4 Media used for the enumeration of fungi	76
4.2.1.5 Incubation and analysis of plates	76
4.2.2 Participatory roles of isolates in biodeterioration of starch-based food	77
4.3 Results	78
4.4 Discussion	104
 Chapter 5.0 Descriptions of species isolated	
5.1 List of the species isolated from starch-based food	109
5.2 Descriptions of the species	110
5.3 Colony Plates	136
5.4 Light microscope and scanning electron microscope (SEM) plates of fungi from starch-based food	158
 Chapter 6.0 Keys	
6.0 Key to genera of fungi isolated from starch-based food	181
6.1 General key to starch-based food fungi	180
6.2 Microscopic key to the genera Mucorales	180
6.3 Key to <i>Aspergillus</i> species and teleomorphs	181
6.4 Key to <i>Penicillium</i> species and teleomorphs	182
 Chapter 7.0 Biochemical studies of starch-based food after storage at different levels of water activity	
7.1 Introduction	186

	PAGE
7.2 Materials and methods	192
7.2.1 Extraction and reversed phase HPLC methods for detection and quantitation of mycotoxins	192
7.2.1.1 Apparatus	192
7.2.1.2 Reagents	193
7.2.1.3 Sample preparation	193
7.2.1.4 Extraction and cleanup	194
7.2.1.5 Recovery and reproducibility studies	194
7.2.1.6 Mycotoxin standard solutions	195
7.2.1.7 HPLC analyses	196
7.2.2 The production of mycotoxins in starch-based food stored at different levels of water activity	197
7.2.2.1 Incubation of starch-based food	197
7.2.2.2 Analysis for mycotoxins	197
7.2.3 Toxicity of starch-based food extracts on the brine shrimp, <i>Artemia salina</i> L.	197
7.2.3.1 Breeding of the larvae	- 197
7.2.3.2 Method of testing	198
7.2.3.3 Analysis of bioassay data	199
7.3 Results	199
7.3.1 Resolution of seven mycotoxins by reversed-phase HPLC	199
7.3.2 Recovery and reproducibility studies	203
7.3.3 Quantitation of mycotoxins in starch-based food stored at different levels of water activity	205
7.3.4 Toxicity of starch-based food extracts on the brine shrimp, <i>Artemia salina</i> L.	214
7.4 Discussion	225
Chapter 8.0 General discussion	231
Chapter 9.0 References	243
Chapter 10.0 Appendices	
Appendix A: Culture media	265
Appendix B: Analytical methods	268
Appendix C: Newspaper reports on mycotoxin incident in Malaysia	274
Appendix D: Experimental data	282

LIST OF TABLES	PAGE
1.1 Polyketide-derived mycotoxins	16
1.2 A selection of trichothecenes and their sources	17
1.3 Potential mycotoxin production by important species of <i>Aspergillus</i> and teleomorphs occurring on cereals	28
1.4 Potential mycotoxin production by important species of <i>Penicillium</i> and teleomorph occurring on cereals	29
2.1 Total mycoflora counts (cfu per gram sample) and aflatoxins producing colonies on ordinary rice and glutinous rice samples collected from retail outlets	43
2.2 Total mycoflora counts (cfu per gram sample) and aflatoxins producing colonies on riceflour and glutinous riceflour samples collected from retail outlets	44
2.3 Total mycoflora counts (cfu per gram sample) and aflatoxins producing colonies on wheatflour and cornflour samples collected from retail outlets	46
2.4 Reproducibility of retention times of aflatoxins by HPLC with coefficient of variation	47
2.5 Peak height reproducibility in the separation of aflatoxins by HPLC	49
2.6 Detection limit and sensitivity of aflatoxins by HPLC	49
2.7 Distribution of aflatoxins in ordinary rice grains collected in Malaysia	50
2.8 Concentration of aflatoxins detected in positive ordinary rice samples	51
2.9 Distribution of aflatoxins in wheatflour collected in Malaysia	52
2.10 Concentration of aflatoxins detected in positive wheatflour samples	53
3.1 The initial moisture content of the composite samples of each starch-based food (% dry basis)	61
3.2 Equilibrium moisture content of starch-based food at different water activities at 25 °C	62

	PAGE
3.3 Days before visible appearance of fungi on six starch-based food stored at 25 °C	66
4.1 The incidence of fungi on composite samples of starch-based food before storage (Day 0) at different levels of water activity	79
4.2 The incidence of fungi on ordinary rice stored at different levels of A_w at 25 °C for 96 days	83
4.3 The incidence of fungi on glutinous rice stored at different levels of A_w at 25 °C for 96 days	85
4.4 The incidence of fungi on riceflour stored at different levels of A_w at 25 °C for 96 days	88
4.5 The incidence of fungi on glutinous riceflour stored at different levels of A_w at 25 °C for 96 days	90
4.6 The incidence of fungi on wheatflour stored at different levels of A_w at 25 °C for 96 days	93
4.7 The incidence of fungi on cornflour stored at different levels of A_w at 25 °C for 96 days	95
4.8 Fungal growth rates, amyolytic activity and changes they induced on starch-extract agar after 7 days of growth at 25 °C	102
7.1 Reproducibility of retention times for seven mycotoxins by HPLC with coefficient of variation	201
7.2 Peak height reproducibility in the separation of seven mycotoxins by HPLC	202
7.3 Detection limit and sensitivity of mycotoxins by HPLC	203
7.4 Recovery and reproducibility data for mycotoxins in spiked starch-based food	204
7.5 The incidence of mycotoxins ($\mu\text{g}\cdot\text{g}^{-1}$) in starch-based food stored at different levels of A_w at 25 °C	207
7.6 Computed probit analyses data (18 hrs) obtained for brine shrimp larvae, tested against ordinary rice extracts stored at different levels of A_w	218

	PAGE
7.7 Computed probit analyses data (18 hrs) obtained for brine shrimp larvae, tested against glutinous rice extracts stored at different levels of A_w	219
7.8 Computed probit analyses data (18 hrs) obtained for brine shrimp larvae, tested against riceflour extracts stored at different levels of A_w	220
7.9 Computed probit analyses data (18 hrs) obtained for brine shrimp larvae, tested against glutinous riceflour extracts stored at different levels of A_w	222
7.10 Computed probit analyses data (18 hrs) obtained for brine shrimp larvae, tested against wheatflour extracts stored at different levels of A_w	223
7.11 Computed probit analyses data (18 hrs) obtained for brine shrimp – larvae, tested against cornflour extracts stored at different levels of A_w	224
B3.1 Concentration of sodium hydroxide giving specified Vapour pressure at 25 °C	268
D2.1 Data for retention times of aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ obtained by six successive injections of single and mixed aflatoxin standards at 0.10 and 0.15 mgml ⁻¹ over 4 days detected by HPLC using acetonitrile : methanol : water (1 : 1 : 2)	282
D2.2 Data for peak heights reproducibility of aflatoxins obtained by six successive injections of mixed aflatoxins standards at 0.10 and 0.15 mgml ⁻¹ over 2 days detected by HPLC using acetonitrile : methanol : water (1 : 1 : 2)	282
D3.1 Equilibrium moisture content of starch-based food at different water activity at 25 °C	283
D3.2 Days before visible appearance of fungi on starch-based food stored at different levels of water activity at 25 °C	283
D4.1 Incidence of fungi on starch-based food at various level of water activities	284
D4.2 The percentage incidence (% of the total no. of plates fungal species occurred/total no. of plates) of dominant fungal species on starch-based food at each level of water activity over a period of 96 days at 25 °C	326

D4.3	Growth rate (mmday^{-1}) colony diameter during linear phase of growth at 25 °C	327
D7.1	Data for retention times of seven mycotoxins by reversed-phase HPLC obtained by seven successive injections of single and mixed mycotoxin standards over four days	328
D7.2	Data for peak heights of seven mycotoxins by reversed-phase HPLC obtained by seven successive injections of mixed mycotoxin standards ranging from 0.1 mgml^{-1} - 1.6 mgml^{-1} over two days	328

LIST OF FIGURES	PAGE
1.1 Relationship between moisture content and A_w value for foods showing typical hysteresis loop consisting of adsorption and desorption isotherms	3
1.2 Intermediates linking primary and secondary metabolism	15
1.3 The nonadrides	18
1.4 Primary and secondary mycotoxinoses	20
2.1 HPLC of a mixture of aflatoxins B ₁ , B ₂ , G ₁ and G ₂	48
3.1 Water adsorption isotherm of ordinary rice grains at 25 °C	63
3.2 Water adsorption isotherm of glutinous rice grains at 25 °C	63
3.3 Water adsorption isotherm of riceflour at 25 °C	64
3.4 Water adsorption isotherm of glutinous riceflour at 25 °C	64
3.5 Water adsorption isotherm of wheatflour at 25 °C	65
3.6 Water adsorption isotherm of cornflour at 25 °C	65
3.7 Relationship between water activity and the time before visible appearance of fungi on starch-based food at 25 °C	67
4.1 Changes in incidence of dominant species of fungi on ordinary rice stored at different levels of water activity at 25 °C	98
4.2 Changes in incidence of dominant species of fungi on glutinous rice stored at different levels of water activity at 25 °C	98
4.3 Changes in incidence of dominant species of fungi on riceflour stored at different levels of water activity at 25 °C	99
4.4 Changes in incidence of dominant species of fungi on glutinous riceflour stored at different levels of water activity at 25 °C	99
4.5 Changes in incidence of dominant species of fungi on wheatflour stored at different levels of water activity at 25 °C	100
4.6 Changes in incidence of dominant species of fungi on cornflour stored at different levels of water activity at 25 °C	100
7.1 HPLC of a mixture of seven mycotoxins	200

	PAGE
7.2 HPLC of starch-based food at day 0	206
7.3 HPLC of ordinary rice grains stored at 0.90 A_w at 25 °C for 96 days	209
7.4 HPLC of glutinous rice grains stored at 0.95 A_w at 25 °C for 26 days	211
7.5 HPLC of riceflour stored at 0.90 A_w at 25 °C for 54 days	212
7.6 HPLC of glutinous riceflour stored at 0.95 A_w at 25 °C for 54 days	213
7.7 HPLC of wheatflour stored at 0.85 A_w at 25 °C for 26 days	215
7.8 HPLC of cornflour stored at 0.90 A_w at 25 °C for 54 days	216
D7.3 Chromatograms of starch-based food stored at different levels of water activity at 25 °C	329

LIST OF PLATES	PAGE
4.1 Strongly amyolytic fungi isolated from starch-based food	103
4.2 Moderately amyolytic fungi isolated from starch-based food	103
4.3 Non-amyolytic fungi isolated from starch-based food	104
5.3 Colony plates	136
5.4 Light microscope and scanning electron microscope (SEM) plates of fungi from starch-based food	158

LIST OF ABBREVIATIONS

A_w	: water activity
ATA	: Alimentary Toxic Aleukia
BET	: Brunauer-Emmett Teller
cfu	: colony forming units
cfug^{-1}	: colony forming units per gram
CCL_4	: carbon tetrachloride
CDA	: Czapek-dox agar
CHCL_3	: chloroform
CO_2	: carbon dioxide
$^{\circ}\text{C}$: degree Celcius
ERH	: Equilibrium relative humidity
g	: grams
HPLC	: High Performance Liquid Chromatography
hrs	: hours
IARC	: International Agency for Research on Cancer
ICMSF	: International Commission on Microbiological Specification for Foods
KNO_3	: potassium nitrate
LC_{50}	: fifty percent lethal concentration
MARDI	: Malaysian Agricultural Research and Development Institute
MEA	: Malt extract agar
μgg^{-1}	: microgram per gram
μgkg^{-1}	: microgram per kilogram
μgml^{-1}	: microgram per millilitre
μl	: microlitre
μm	: micrometre
mins	: minutes
mgml^{-1}	: milligram per millilitre
mlmin^{-1}	: millilitre per minute
mm	: millimetre
mmday^{-1}	: millimetre per day
$(\text{NH}_4)\text{SO}_4$: ammonium sulphate
O_2	: oxygen
PDA	: Potato-dextrose agar
S.E.	: standard error
TFA	: Trifluoroacetic acid
U.S.A.	: United States of America
V_p	: vapour pressure of water in food
V_{pw}	: vapour pressure of pure water
WHO	: World Health Organisation