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

Cereal products are exposed to a wide variety of microorganisms from dust, water, diseased plant material, insects, soil, fertiliser and animal droppings during growth and drying in the field. During storage, the cereals and its products must be correctly ventilated to prevent moisture build up in pockets, to prevent fungal growth. During transport, processing and distribution they may become further contaminated. In a tropical developing country such as Malaysia, sufficient economic resources may not be available in controlling the postharvest formation of mycotoxins in storage.

In Malaysia food processors or companies had no set of microbiological specifications for starch-based raw materials incorporated into food products hence, no microbiological tests were carried out on the raw materials and are used as obtained in the market. The use of raw materials with high fungal contamination and incorporated into higher moisture foods are vulnerable to premature spoilage if the product was incorrectly processed or handled. Furthermore, these raw materials may be contaminated by mycotoxigenic fungi hence risk of mycotoxicosis among the consumers.

Aflatoxins is the most important mycotoxins which have an impact on human health in the tropics and on the economy of international trade in food and animal feeds throughout the world. Aflatoxins are produced by the fungal species *A. flavus* and *A. parasiticus* (Hocking, 1982) and recently *A. nomius* Kurtzman a rare species reported from soil and other sources in Thailand (Saito *et al.*, 1989) and United States (Kurtzman *et al.*, 1987) was also found to produce aflatoxins (Pitt, 1992). *A. flavus* produces only B aflatoxins (and cyclopiazonic acid), while

A. parasiticus produces both B and G aflatoxins, while cyclopiazonic acid is not produced. *A. nomius* like *A. parasiticus* produces both B and G aflatoxins (Pitt, 1992).

The ability to produce aflatoxins can vary considerably with growth conditions. Reports from other countries indicate a wide range in the proportion of aflatoxin producing strains from as low as 3 - 26 % in India, up to 94 % in the U.S.A. (Moreau and Moss, 1979). Studies by Hocking (1982) have shown that approximately 95 % of Australian isolates from peanuts are aflatoxigenic. Bryden *et al.*, (1975) found that 82 % of strains of *A. flavus* isolated from animal feeds in Australia were toxigenic. The total amount of aflatoxin and the ratios of B_1, B_2, G_1, and G_2 produced can vary markedly between isolates and also with growth conditions. Only about 40 % of *A. flavus* isolated from nature produces aflatoxin in the laboratory culture, but virtually all known isolates of *A. parasiticus* produces aflatoxins all the time (Pitt, 1992).

The ubiquity of potentially aflatoxigenic fungi and the large number of aricultural products in which the presence of aflatoxins has been detected naturally and more importantly the wide spectrum of toxicologic effects (Pestka and Bondy, 1990) induced by aflatoxins when ingested has led to the introduction of regulations in Malaysia (Chapter 1). It is necessary for certain foodstuffs be constantly monitored to ensure that they do not contain aflatoxins at levels capable of producing acute or chronic effects in man.

Hence, this survey was set out to determine the level of mycoflora count and the presence of aflatoxigenic species in starch-based raw materials that are commonly consumed directly or incorporated into food-products, rather than to

evaluate an enormous range of consumer items produced. Contamination of starch-based food with mycotoxigenic fungi is of concern because of the high proportion of cereal based products in the human diet. An estimate of the mycoflora count can be used to provide assurance that the food was prepared from good quality raw materials and that it will not serve as a major source of contamination of products to be manufactured from the dried raw material. Few reports are available on the aflatoxin contamination in starch-based food in Malaysia. Hence, a more systematic survey was described here to establish the current status of the aflatoxin problem in starch-based food most regularly consumed in Malaysia i.e. rice and wheatflour. These survey should provide an empirical basis to assist in the formulation of appropriate regulations and/or the modification of those already existing as well as to ensure that the regulations are being adhered to for the effective control of mycotoxin contamination.

2.2 MATERIALS AND METHODS

2.2.1 Sampling of starch-based food

Fifty gram samples of starch-based food consisting of ordinary rice, glutinous rice, riceflour, glutinous riceflour, wheatflour and cornflour were collected at random from retail outlets in Selangor. A total of 50 samples of each starch-based food were analysed for fungal counts according to the methods of Samson *et al.*, (1992). In addition, a survey of aflatoxins in a total of 84 samples of ordinary rice and 83 samples of wheatflour were examined from four regions in

West Malaysia. The Northern region consists of Perlis, Kedah, Penang and Perak. The Mid region consists of Selangor and Kuala Lumpur. The Southern region consists of Negeri Sembilan, Melaka and Johor while the East Coast region consists of Pahang, Terengganu and Kelantan. All samples were obtained at random from the following consumers: (1) Retail shops/supermarkets; (2) Restaurants and hotels/hostels and (3) Private homes. Samples of 50 - 100g were taken and sent to the laboratory.

2.2.2 Determination of fungal counts in starch-based food by dilution plating

Ten gram samples of each starch-based food were homogenised in a sterile Waring blender with 90 ml of sterile 0.1% peptone for 2 mins. Immediately after homogenisation 0.1- 0.3 ml aliquots were taken out using a sterile pippette and discharged into sterile plastic petri dishes containing solidified dichloran rose bengal chloramphenicol (DRBC) agar to obtain less than 50 colonies per plate. The composition of this media was given in Appendix A. This medium is effective for enumeration and isolation of storage fungi in food and it is recommended by the International Commission on Food Mycology (ICFM), as a general purpose isolation media (King *et al.*, 1979). The inoculum was spread using sterile bent glass tubing and incubated upright in the dark at 30 °C for 5 days. The results are expressed as the number of colony forming units (cfu) per gram of original sample. Potential aflatoxins producing colonies i.e. *A. flavus* or *A. parasiticus* were identified and counted.

2.2.3 Extraction and analysis of aflatoxins

Aflatoxins was extracted from 20 g powdered samples of ordinary rice and wheatflour using 200 ml of 20% H_2SO_4-KCL-acetonitrile (2+20+178) and shaken for 30 min on a rotary shaker at 250 rpm. The extract was filtered through fluted Whatman No. 1 filter paper and 150 ml aliquots of filtrate was transferred to a 600-ml beaker. 150 ml clarifying solution, ammonium sulphate (30%) and 3 g of diatomaceous earth was added to this aliquot and stirred with a glass rod. The extract was filtered through fluted Whatman No. 1 filter paper and 100 ml of the upper layer (acetonitrile) was transferred into a 250-ml separatory funnel. Fifty millilitre water was added and the aqueous acetonitrile layer was extracted with 50 ml chloroform by shaking for 5 mins. Extraction was repeated with 30 ml and then 10 ml chloroform.

The extracts were combined and evaporated to dryness in vacuo at 45 °C on a rotary evaporator. The residue were dissolved in 250 µl chloroform and chromatographed using reversed-phase HPLC. Aflatoxins were separated on an Econosil C18 (Alltech) reversed phase column and the elution solvent used was acetonitrile : methanol : water (1 : 1 : 2) as adapted from Jarvis *et al.*, (1981). The flow-rate used was 1 $mlmin^{-1}$ and detected by UV-Vis detector at a wavelength of 365 nm. Quantitation of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 were done on the basis of the standard curves obtained in Appendix B based on the peak height response. Initial runs of autoclaved rice and wheatflour samples showed no components having the same retention times as the aflatoxins, hence derivatizing the aflatoxins with trifluoroacetic acid was not required.

2.3 RESULTS

2.3.1 Total mycoflora count and aflatoxin producing colonies

Fifty samples each of six starch-based food obtained from retail outlets were examined for total fungal count and aflatoxigenic species on dichloran rose bengal chloramphenicol agar and the results are displayed in Tables 2.1 - 2.3.

In Table 2.1, among the ordinary rice samples, fungal colonies were detected on 30 samples ranging from 0 - 660 $cfug^{-1}$ sample and two samples were positive for aflatoxigenic colonies each containing 30 $cfug^{-1}$ sample. The average total fungal count and aflatoxigenic colonies on ordinary rice were 137 ± 189 and 1 ± 6 $cfug^{-1}$ respectively while the most common value among the positive samples of ordinary rice were 90 and 180 $cfug^{-1}$ sample. Out of 50 glutinous rice samples surveyed fungal colonies were detected on 37 samples ranging from 0 - 810 $cfug^{-1}$ sample and two samples were positive for aflatoxigenic colonies each containing 30 $cfug^{-1}$ sample. The average total fungal count and aflatoxigenic colonies on glutinous rice grains were 111 ± 160 and 1 ± 6 $cfug^{-1}$ respectively while the most common value among the positive samples of glutinous rice was 30 $cfug^{-1}$ sample.

Table 2.2 shows that fungal colonies were detected on 37 out of 50 samples of riceflour ranging from 0 - 1440 $cfug^{-1}$ sample and no aflatoxigenic colonies were detected on any samples. The average total fungal count were 225 ± 266 $cfug^{-1}$ sample and the most common value among the positive samples of riceflour was 90 $cfug^{-1}$ sample. Among the 50 glutinous riceflour samples examined, fungal colonies were detected on 24 samples ranging from 0 - 4140 $cfug^{-1}$ sample and one sample was positive for aflatoxigenic colonies. The average

Table 2.1 Total mycoflora counts (cfu per gram sample) and aflatoxins producing colonies on ordinary rice and glutinous rice samples collected from retail outlets

Ordinary rice samples	cfu per g sample	Aflatoxigenic colonies	Glutinous rice samples	cfu per g sample	Aflatoxigenic colonies
1	0	0	1	0	0
2	180	0	2	810	0
3	0	0	3	120	0
4	0	0	4	720	0
5	0	0	5	0	0
6	0	0	6	180	0
7	0	0	7	0	0
8	450	0	8	0	0
9	0	0	9	0	0
10	180	0	10	30	0
11	0	0	11	30	0
12	180	0	12	60	0
13	0	0	13	240	0
14	630	0	14	90	0
15	0	0	15	60	0
16	450	0	16	120	0
17	0	0	17	0	0
18	0	0	18	0	0
19	180	30	19	0	0
20	120	0	20	0	0
21	240	0	21	0	0
22	150	0	22	0	0
23	240	0	23	0	0
24	360	0	24	30	0
25	30	0	25	60	0
26	30	0	26	30	30
27	90	0	27	180	30
28	30	0	28	60	0
29	120	0	29	210	0
30	180	0	30	210	0
31	30	0	31	30	0
32	90	0	32	270	0
33	0	0	33	120	0
34	0	0	34	30	0
35	0	0	35	30	0
36	0	0	36	210	0
37	0	0	37	30	0
38	0	0	38	270	0
39	0	0	39	30	0
40	0	0	40	180	0
41	270	0	41	90	0
42	30	0	42	180	0
43	750	30	43	0	0
44	150	0	44	60	0
45	60	0	45	180	0
46	330	0	46	0	0
47	120	0	47	180	0
48	450	0	48	30	0
49	660	0	49	210	0
50	90	0	50	180	0
Ave ± S.E	137 ± 189	1 ± 6	Ave ± S.E	111 ± 160	1 ± 6

Table 2.2 Total mycoflora counts (cfu per gram sample) and aflatoxins producing colonies on riceflour and glutinous riceflour samples collected from retail outlets

Riceflour samples	cfu per g sample	Aflatoxigenic colonies	Glutinous riceflour samples	cfu per g sample	Aflatoxigenic colonies
1	630	0	1	0	0
2	180	0	2	90	0
3	180	0	3	180	90
4	720	0	4	0	0
5	450	0	5	2610	0
6	630	0	6	450	0
7	450	0	7	0	0
8	360	0	8	0	0
9	360	0	9	0	0
10	0	0	10	0	0
11	0	0	11	0	0
12	0	0	12	0	0
13	0	0	13	0	0
14	0	0	14	0	0
15	0	0	15	0	0
16	0	0	16	0	0
17	0	0	17	0	0
18	0	0	18	0	0
19	0	0	19	0	0
20	0	0	20	0	0
21	0	0	21	1350	0
22	360	0	22	4140	0
23	270	0	23	2520	0
24	90	0	24	1080	0
25	90	0	25	90	0
26	1440	0	26	180	0
27	720	0	27	270	0
28	180	0	28	990	0
29	450	0	29	270	0
30	90	0	30	90	0
31	270	0	31	90	0
32	180	0	32	180	0
33	540	0	33	0	0
34	270	0	34	0	0
35	180	0	35	450	0
36	90	0	36	270	0
37	180	0	37	180	0
38	270	0	38	90	0
39	180	0	39	0	0
40	270	0	40	0	0
41	90	0	41	0	0
42	90	0	42	360	0
43	90	0	43	0	0
44	90	0	44	0	0
45	90	0	45	270	0
46	90	0	46	0	0
47	180	0	47	180	0
48	0	0	48	90	0
49	180	0	49	0	0
50	270	0	50	0	0
Ave ± S.E	225 ± 266	0	Ave ± S.E	329 ± 781	2 ± 13

total fungal count and aflatoxigenic colonies detected were 329 $\pm$ 781 and 2 $\pm$ 13 $cfug^{-1}$ respectively and the most common value among the positive samples was 90 $cfug^{-1}$ glutinous riceflour sample.

In Table 2.3, fungal colonies were detected on all wheatflour samples surveyed ranging from 180 - 13,230 cfu g^{-1} sample and aflatoxigenic colonies were detected on 10 out of 50 samples ranging from 0 - 450 $cfug^{-1}$ sample. The average total fungal count and aflatoxigenic colonies were 1026 $\pm$ 1842 and 27 $\pm$ 73 $cfug^{-1}$ respectively while the most common value for total fungal count were 360 and 450 $cfug^{-1}$ wheatflour samples. On cornflour samples on the other hand, fungal colonies were detected on only 13 samples ranging from 0 - 3780 $cfug^{-1}$ sample with no aflatoxigenic colonies present. The average total fungal counts were 126 $\pm$ 551 $cfug^{-1}$ sample and the most common value among the positive samples was 90 $cfug^{-1}$ sample.

Table 2.3 Total mycoflora counts (cfu per gram sample) and aflatoxins producing colonies on wheatflour and cornflour samples collected from retail outlets

Wheatflour samples	Cfu per g sample	Aflatoxigenic colonies	Cornflour samples	Cfu per g sample	Aflatoxigenic colonies
1	1170	180	1	90	0
2	1530	0	2	0	0
3	2610	0	3	0	0
4	1260	0	4	0	0
5	1440	0	5	180	0
6	720	0	6	180	0
7	13230	0	7	0	0
8	1170	0	8	90	0
9	540	0	9	180	0
10	720	90	10	0	0
11	810	0	11	90	0
12	3060	0	12	0	0
13	360	0	13	0	0
14	1710	0	14	0	0
15	900	0	15	90	0
16	180	0	16	90	0
17	360	0	17	0	0
18	810	0	18	0	0
19	360	0	19	90	0
20	900	0	20	270	0
21	720	0	21	90	0
22	360	0	22	0	0
23	630	90	23	0	0
24	990	0	24	0	0
25	810	0	25	0	0
26	720	0	26	0	0
27	630	0	27	0	0
28	360	0	28	0	0
29	450	0	29	0	0
30	450	0	30	0	0
31	630	0	31	0	0
32	900	90	32	0	0
33	990	0	33	0	0
34	540	0	34	0	0
35	360	0	35	1080	0
36	720	0	36	0	0
37	270	0	37	0	0
38	630	90	38	0	0
39	450	0	39	0	0
40	540	0	40	0	0
41	270	0	41	0	0
42	630	0	42	0	0
43	450	0	43	0	0
44	450	90	44	3780	0
45	810	90	45	0	0
46	450	0	46	0	0
47	450	0	47	0	0
48	900	90	48	0	0
49	360	90	49	0	0
50	540	450	50	0	0
Ave ± S.E	1026±1842	27 ± 73	Ave ± S.E	126 ± 551	0

2.3.2 Resolution of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2

Aflatoxins can be separated readily on an Econosil-C18 reversed-phase column as shown in Fig. 2.1. The four aflatoxins were eluted in 19 mins at a flow-rate of 1 $mlmin^{-1}$. Six injections of individual and mixed aflatoxins over a 3-day period gave mean retention times as shown in Appendix D(2.1) and summarized in Table 2.4.

Table 2.4 Reproducibility of retention times of aflatoxins by HPLC with coefficient of variation

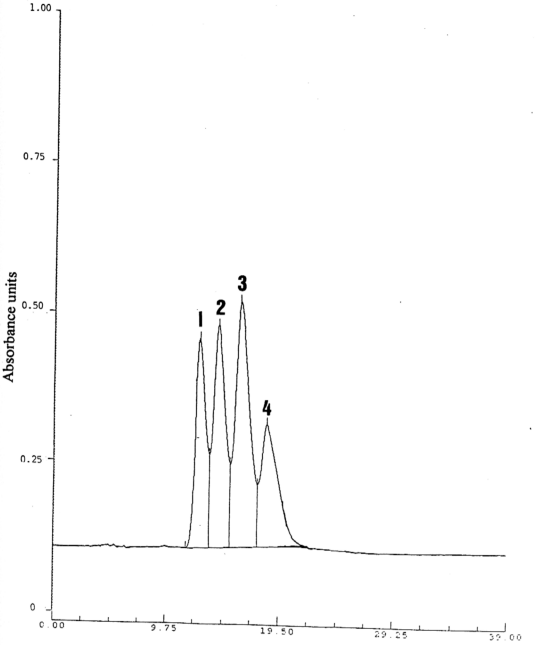
	Statistics*				
	Retention time (sec)		Mean retention (min)	Std deviation (sec)	Coeff of variation (%)
Aflatoxins	Range	Mean			
B_1	1108.2 - 1123.2	1113.6	18.56	5.24	0.5
B_2	970.8 - 989.4	980.4	16.34	7.56	0.8
G_1	853.2 - 883.8	864.0	14.40	11.18	1.3
G_2	757.2 - 771.0	763.2	12.72	4.85	0.6

*Based on six successive injections of 20 µl aliquots of individual and mixed aflatoxins standards.

Retention time of the aflatoxins were highly reproducible with the elution solvent and column used giving mean retention times, min, of 18.56 (aflatoxin B_1), 16.34 (aflatoxin B_2), 14.40 (aflatoxin G_1), and 12.72 (aflatoxin G_2) with coefficient of variation ranging from 0.5 - 1.3 %.

The data for the relationship between peak heights and the amount of aflatoxins injected over a concentration range was obtained and given in Appendix B for the construction of standard curves by the HPLC and used in the quantitation of aflatoxins in rice and wheatflour samples.

Fig. 2.1 HPLC of a mixture of aflatoxins B_1, B_2, G_1 and G_2 .



Aflatoxins B_1, B_2, G_1, and G_2 eluted from 10 μm Econosil (C_{18}) reversed-phase column by acetonitrile : methanol : water (1 : 1 : 2) at a flow-rate of 1 $mlmin^{-1}$ and detected by UV-Vis detector at a wavelength of 365 nm.

Peak 1 : 0.1 $mgml^{-1}$ aflatoxin G_2

Peak 2 : 0.1 $mgml^{-1}$ aflatoxin G_1

Peak 3 : 0.1 $mgml^{-1}$ aflatoxin B_2

Peak 4 : 0.1 $mgml^{-1}$ aflatoxin B_1

Precision was evaluated by injecting six 20 µl aliquots of mixed aflatoxin standards having a concentration of 0.1 $mgml^{-1}$ over a 2-day period. Reproducibility of the peak height measurements was good as shown in Appendix D(2.2), with coefficients of variation of 1.0 - 2.9 % representing the combined errors of HPLC resolution, injection and detection as summarized in Table 2.5.

Table 2.5 Peak height reproducibility in the separation of aflatoxins by HPLC

Aflatoxins	Statistics*			
	Peak height (mm)		Std. deviation (mm)	Coeff. of variation (%)
	Range	Mean		
B_1	43 - 47	45.2	1.33	2.9
B_2	134 - 139	136.8	1.72	1.4
G_1	121 - 125	122.8	1.60	1.3
G_2	114 - 117	115.8	1.17	1.0

*Based on six successive 20 µl injections of aflatoxin standards at detector range of 0.32.

Detection limits and sensitivity for the aflatoxins are shown in Table 2.6. The detection limits and peak ratios indicate that aflatoxin B_2 is most sensitive followed by aflatoxin G_1, aflatoxin G_2 and aflatoxin B_1. The minimum concentration of detection is 5 μgml^{-1} for all the aflatoxins at 0.32 detector range.

Table 2.6 Detection limit and sensitivity of aflatoxins by HPLC

Aflatoxins	Min. peak height (mm)	Min. conc. (μgml^{-1})	Sensitivity (peak ht (mm) / conc (μgml^{-1})	Peak ratios
B_1	2.0	5.0	0.4	1.00
B_2	4.0	5.0	1.0	2.00
G_1	3.5	5.0	0.8	1.75
G_2	3.0	5.0	0.6	1.50

2.3.3 Aflatoxin contamination in rice and wheatflour samples

Table 2.7 and 2.8 summarizes the distribution and amount of aflatoxins detected in 84 rice samples collected from different regions in West Malaysia. Table 2.7 indicate that 6% of the samples collected in West Malaysia were contaminated with aflatoxins and the highest occurrence was from the mid region (10.7 %), followed by the East Coast (6.2 %), and Southern region (4.3 %). No aflatoxin positive samples occurred in the Northern region. All the positive samples were collected from the private homes. Referring to Table 2.8, only aflatoxin G was present whereby 4 out of 5 samples have concentrations higher than the permitted level of 35 μgkg^{-1} i.e. ranging from 36.88 - 96.25 μgkg^{-1}. –

Table 2.7 Distributions of aflatoxins in ordinary rice grains collected in Malaysia

Region	Place collected*	No. of samples collected	No. of positive samples	% positive within places	% positive within region
Northern	A	6	0	0	0
	B	5	0	0	
	C	6	0	0	
Mid	A	4	0	0	10.7
	B	12	0	0	
	C	12	3	25.0	
Southern	A	-	-	-	4.3
	B	1	0	0	
	C	22	1	4.5	
East Coast	A	5	0	0	6.2
	B	1	0	0	
	C	10	1	10.0	
	Total	84	5	6.0	

* A : Retail shops/supermarkets

B : Restaurants and hotels/hostels

C : Private homes

Table 2.8 Concentration of aflatoxins detected in positive ordinary rice samples

Sample code (Region/Place collected*)	Aflatoxins (μgkg^{-1})				Total aflatoxins (μgkg^{-1})
	B_1	B_2	G_1	G_2	
R1 (Mid/C)	-	-	73.12	-	73.12
R4 (Mid/C)	-	-	-	36.88	36.88
R6 (Mid/C)	-	-	77.50	-	77.50
R39 (East Coast/C)	-	-	-	96.25	96.25
R58 (South/C)	-	-	-	3.69	3.69

* A : Retail shops/supermarkets

B : Restaurants and hotels/hostels

C : Private homes

Table 2.9 and 2.10 summarizes the distribution and amount of aflatoxins detected in 83 wheatflour samples collected in West Malaysia. From Table 2.9, 21.7 % of the samples collected were contaminated with aflatoxin and the highest occurrence was from samples collected in the Mid region (34.5 %) followed by the East Coast region (20.0 %), followed by the Northern region (12.5 %) and Southern region (8.7 %). As in rice samples, positive wheatflour samples were mostly collected from private homes.

Referring to Table 2.10, 11 out of 18 positive samples contained total aflatoxin concentration higher than 35 μgkg^{-1} ranging from 41.88 - 436.25 μgkg^{-1}. One sample contained aflatoxin B_1 only, 4 contained aflatoxin B_2 only, 3 contained aflatoxin G_1 only, 10 contained aflatoxin G_2 only and 1 sample contained both aflatoxin B_2 and aflatoxin G_2.

Table 2.9 Distribution of aflatoxins in wheatflour collected in Malaysia

Region	Place collected*	No. of samples collected	No. of positive samples	% positive within places	% positive within region
Northern	A	5	0	0	
	B	6	0	0	12.50
	C	5	2	40.0	
Mid	A	3	0	0	
	B	12	3	25.0	34.50
	C	14	7	50.0	
Southern	A	-	-	-	
	B	1	0	0	8.70
	C	22	2	9.1	
East Coast	A	4	2	50.0	
	B	1	0	0	20.0
	C	10	1	10.0	
Total		83	18	21.7	

* A : Retail shops/supermarkets

B : Restaurants and hotels/hostels

C : Private homes

Table 2.10 Concentration of aflatoxins detected in positive wheatflour samples

Sample Code (Region/Place collected *)	Aflatoxins (μgkg^{-1})				Total aflatoxins (μgkg^{-1})
	B_1	B_2	G_1	G_2	
WF 81 (Northern/C)	25.62	-	-	-	25.62
WF 89 (Northern/C)	-	252.50	-	30.00	282.50
WF 19 (Mid/B)	-	11.25	-	-	11.25
WF 20 (Mid/B)	-	-	-	111.25	111.25
WF 22 (Mid/B)	-	-	90.00	-	90.00
WF 1 (Mid/C)	-	-	-	27.50	27.50
WF 2 (Mid/C)	-	16.88	-	-	16.88
WF 3 (Mid/C)	-	-	-	126.88	126.88
WF 4 (Mid/C)	-	-	-	41.88	41.88
WF 5 (Mid/C)	-	20.00	-	-	20.00
WF 39 (Mid/C)	-	-	25.00	-	25.00
WF 45 (Mid/C)	-	-	-	16.25	16.25
WF 63 (Southern/B)	-	-	-	296.88	296.88
WF 58 (Southern/C)	-	-	-	250.62	250.62
WF 71 (Southern/C)	-	-	-	246.88	246.88
WF 38 (EastCoast/A)	-	-	-	98.12	98.12
WF 35 (East Coast/A)	-	-	289.38	-	289.38
WF 29 (East Coast/C)	-	-	-	436.25	436.25

* A : Retail shops/supermarkets
B : Restaurants and hotels/hostels
C : Private homes

2.4 DISCUSSION

Food manufacturers in Malaysia had no acceptance specifications for cereal products and flour used as raw materials for food products. In a survey carried out by Andrews (1992), nearly half of the Australian food manufacturers had strict acceptance specifications of less than 200 fungi per gram of cereal products and flour. A similar number specified less than 2000 per gram but three companies accepted 5×10^3 - 5×10^4 fungi per gram. The variation seen in acceptance specifications for cereal products and flour is a reflection on different grain pretreatments, different type of flours made, and different end uses. This survey supports the recommendation set by the International Commission on Microbiological Specifications for Foods (ICMSF) (1986), for fungal tolerances for flour and cereal products in the range 10^2 - 10^4 per gram but rejected products with > 10^5 fungi per gram.

Hence, based on the ICMSF tolerance level, the mycoflora count on starch-based food surveyed in this study are acceptable for food manufacturers. In the survey of fungal counts on ordinary rice and glutinous rice grains, all positive samples had total fungal count below 10^3 $cfug^{-1}$, while among the positive samples of riceflour, glutinous riceflour and cornflour, one, five and two samples respectively had more than 10^3 but less than 10^4 $cfug^{-1}$ sample. Wheatflour is of concern since fungal colonies were present on all samples screened at levels above 10^2 $cfug^{-1}$ with 8 samples having levels above 10^3 and one at above 10^4 $cfug^{-1}$. Out of 50 samples of starch-based food screened, aflatoxigenic colonies were absent on riceflour and cornflour but were present on 10 samples of wheatflour. Only one

sample of glutinous riceflour and two samples of ordinary rice and glutinous rice were positive for aflatoxigenic colonies.

The degree of mould contamination in stored grains and animal feeds is traditionally used as a measure of their quality. It is generally thought that feed or grains with low mould counts (10^1 - 10^3 spores/g) are of a higher quality and safer than those having higher mould counts (10^6 spores/g). However, Karunaratne and Bullerman, (1990) stated that field experience has shown that fungal contamination, regardless of level, can lead to toxicity problems. Following this, Sharma *et al.* (1980) and Karunaratne and Bullerman (1990), observed that final mycelial dry weights were comparable irrespective of the size of initial inoculum. They only differ on the initial rate of development whereby the rate was higher as the spore level was increased. More importantly, the inoculum level had a tremendous effect on aflatoxin production i.e. at 28 °C, unusually high amounts of aflatoxin B_1 (380 $\mu g g^{-1}$) were produced when 10^3 spores were inoculated into 50 g rice, while the lower and the higher spore levels produced comparatively lower levels of aflatoxin. At 35 °C, the lowest spore level (<10^1 spores) produced the highest amount of aflatoxin B_1. The higher spore levels at 35 °C either did not result in any aflatoxin formation or the amounts produced were negligible. Hence, even though inoculum levels is low germination of even one spore of aflatoxigenic species may result in a very high level of aflatoxin when environments are favourable for growth.

The HPLC method chosen in this survey gave a reliably good separation for identification and quantitation of aflatoxins B_1, B_2, G_1 and G_2 even though baseline separation were not achieved. The method was fast eluting in 19 mins.

Hence, it can be adopted in the screening of aflatoxins in large number of samples.

Of the two commodities surveyed, wheatflour revealed a higher incidence of aflatoxin contamination than rice grains even though the moisture content of rice is higher than wheatflour. This may be due to the presence of aflatoxin producing species occurring more often on wheatflour than on ordinary rice as shown by the survey on mycoflora count. Also, wheatflour may be in storage for a longer period than rice grains since rice being a staple food in the Malaysian population, is consumed faster after being purchased as opposed to wheatflour. This is supported by the higher incidence of aflatoxins from samples collected from private homes as opposed to restaurants and hotels/hostel i.e. where the rate of consumption is slower thus subjected to different environmental conditions during long-term storage. The percentage contamination of samples collected from retail shops or supermarkets were nil in rice grains and very low in wheatflour indicating that aflatoxin contamination occurs at the consumer level.

This survey reveals that aflatoxin contamination in wheatflour calls for concern rather than rice grains. However, because of the continuous consumption of rice in the diet, even a low level of contamination by aflatoxins may have adverse effects on human health. Hence, since food of low moisture content also has a tendency for aflatoxin contamination, it is important that the government take steps to ensure that food manufacturers be educated of an appropriate method of storage of raw materials used and that all food products sold in the market should have an expiry date. Further, it is possible to reduce fungal contamination through adequate education of the food storage habits of the consumers.