

Toxigenic mycobiota associated with baby foods locally produced in Uganda with special reference to aflatoxins

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Abstract. Five baby food products locally produced in Uganda were bought from different shops and supermarkets at the stage of consumption and investigated for contamination by different toxigenic fungi and aflatoxins. These foods, each contained at least one or more cereal flour in their composition. The dilution plate method and three selective isolation media namely: pentachloronitrobenzene rose Bengal yeast extract sucrose agar (PRYES), peptone-pentachloronitrobenzene agar (peptone-PCNB), and *Aspergillus flavus/parasiticus* agar (AFPA) were used for enumeration and isolation of toxigenic fungi. PRYES plates revealed high level of contamination of the foods by *Penicillium*, with three species being nephrotoxicogenic (*P. viridicatum*, *P. verrucosum* and *P. citrinum*). On the other hand, on peptone-PCNB, 9 species of *Fusarium* were recovered in high frequency and counts. Of these *F. verticillioides* followed by *F. solani* were the most prevalent while *F. proliferatum* and *F. tricinctum* had more propagules. In addition, aflatoxigenic aspergilli were isolated on AFPA from the majority of samples of all the products investigated in this study. Many other fungi were also isolated on the three media, among which *Aspergillus flavus*, *A. niger*, *Cladosporium* and yeasts were prevalent. Regarding aflatoxins, all samples analyzed of the five products were found contaminated. Fortunately, the levels detected (0-10 ppb and 10-20 ppb) were below or in the current tolerance level of 20 ppb accepted in foodstuffs. The contaminated foods constitute a health hazard for human consumption and must be examined at regular intervals in order to assess their hygienic quality.

Key words: toxigenic fungi, nephrotoxicogenic *Penicillium*, *Fusarium*, aflatoxigenic aspergilli, aflatoxins, baby foods, Uganda.

Introduction

Fungi are widely distributed in nature and are common contaminants of agricultural commodities, foods and feeds (Gourama and Bullerman 1995, Ismail 2001). A variety of baby foods rich in carbohydrates and proteins are being produced from cereal and leguminous seeds/grains. The manufacture of these foods involves mixing the cereal flour with other ingredients such as powders of soybeans, dried fish, vegetables or fruits. If such foods (or their ingredients) are contaminated with either fungi or their mycotoxins, they become unpalatable and unsafe for consumption (Zohri *et al.* 1995, Munimbazi and Bullerman 1996). The presence of fungal toxins in foods, particularly in those for babies, is of great concern since they have carcinogenic, immunosuppressive, teratogenic, or nephrotoxic effects (based on the toxin type present) (Pitt and Hocking 2009).

In Uganda, knowledge about fungi contaminating foods is still limited, however a number of studies in the last ten years have been conducted on a variety of foods such as coffee beans (Tugume 1998), peanuts and coconuts (Ismail 2001), corn (Ismail *et al.* 2003), rice (Taligoola *et al.* 2004, 2010), passion fruits and

juice (Ismail 2006), cereal baby foods imported into Uganda (Ismail *et al.* 2008, 2010). So, this work was designed to survey the toxigenic fungi associated with baby foods locally manufactured in Uganda, and to assess the level of aflatoxins in these foods.

Material and methods

Fifty samples of five baby food products manufactured locally (10 packets each) were randomly collected from different shops from five towns of Uganda (Kampala, Jinja, Mbarara, Masaka and Mbale). Most of these products are made in these towns. Each of these foods contained at least one or more cereal flour. The names and components of these products are shown in Table (1).

Determination of moisture content

Three sub-samples of 50 g each were taken from each food sample and put in aluminium foil dish. These were dried in an oven at 110 °C for 24 hours, and reweighed (Magan and Lacey 1985, Pitt and Hocking 2009). The moisture content of each sample was expressed as the average percentage of the weight loss of the three replicates.

Table 1: Baby food products manufactured in Uganda, ingredients, producing companies and their mean moisture contents.

No	Product	Ingredients	Producing company	Mean moisture content
1	Baby soya	Maize, soya bean flour, carrot flour	East African Basic Food, Ltd	11.47±0.23
2	Kayebe	Maize flour, powdered Enkejeje (<i>Haplochromis</i> fish), soya bean flour	Kayebe Sauce Packers, Ltd	11.14±0.38
3	Mwebaza rice porridge	Rice flour	Ebenezer Packers, Ltd	11.9±0.23
4	Jacinta millet flour	Millet flour	Mahimba Company, Ltd	11.6±0.09
5	Mukuza	Maize flour, Sorghum flour, soya bean flour	Hodeco, Ltd	11.52±0.09

Mycological analysis

All food samples were analyzed mycologically on three selective media: (1) Pentachloronitrobenzene- rose Bengal yeast extract sucrose agar (PRYES) for isolation of nephrotoxicogenic *Penicillium* species (Frisvad 1983), (2) Peptone-PCNB (Nash and Snyder 1962) for *Fusarium* species, and (3) *Aspergillus flavus/parasiticus* agar, AFPA (Pitt *et al.* 1983). Isolation plates were set in replicates of four for each isolation medium for each sample. Plates were incubated at room temperature (25-27 °C) in day and night cycle of light conditions for 7-10 days for PRYES and Peptone-PCNB plates while the plates of AFPA were incubated for 42 hours at 30 °C (Pitt *et al.* 1983, Pitt and Hocking 2009). The reverse of AFPA plates was examined for a bright yellow/orange coloration (Pitt and Hocking 2009). The growing fungi on all media were enumerated, isolated and identified.

Identification of fungi

The identification of different fungal groups was mainly on the basis of their macroscopic and microscopic features using the methods and keys described by Raper and Fennell (1965) for *Aspergillus* and its teleomorphs; Booth (1971) and Leslie & Summerell (2006) for species of *Fusarium*; Pitt (1979) for species of *Penicillium*; Ellis (1971), Domsch *et al.* (1980), Moubasher (1993), and Pitt & Hocking (2009) for other fungi.

Aflatoxin analysis

A semiquantitative method for the determination of the total aflatoxins in food samples was applied, whereby a commercial immunological test kit, aflascan (from Rhône-Diagnostics and Technologies Ltd., Glasgow, Scotland) was used. The procedure outlined in the aflascan and also described by Ismail *et al.*

(2003, 2008) was followed. Thirteen out of the fifty mycologically investigated samples were randomly chosen for aflatoxin analysis: 3 from each of Kayebe, Mwebaza rice Porridge, Mukuza and 2 from each of Baby soya and Jacinta millet flour. All contents of two packets were thoroughly mixed then a 50 g sub-sample was blended with 4 g of sodium chloride (NaCl) and 250 ml of 60 % methanol. The extract was diluted with 250 ml of distilled water and then filtered using Whatman filter paper No. 4. Twenty five to fifty ml of the filtrate was collected; 10 ml of the filtrate was then passed through the immunoaffinity column so that the aflatoxins were adsorbed by the antibodies. The column was then washed twice with distilled water. Pure methanol was passed into the column to release the aflatoxins after which it was collected in a test tube. Water and chloroform were added (1 ml each) to the aflatoxin eluate and shaken gently, then left to stand. The bottom chloroform layer was pipetted out and then passed through a florisil tip. The florisil tip was then viewed under 366 nm UV light and the fluorescence of the toxins was compared with a standard comparator card (a component of the aflascan) with semi-quantitative values to obtain the range of total aflatoxin contamination in the extracts in ppb.

Statistical analysis: Analysis of variance (ANOVA) was used to analyze data (Steel and Torrie 1980).

Results and discussions

Nephrotoxicogenic *Penicillium* species and other fungi recovered on PRYES

The data of Table (2) reveal that the local baby food products tested were highly contaminated with *Penicillium* species. *Penicillium* was isolated from 82 % of the samples, accounting for 16.51 % of the total fungal propagules. In this respect, lower numbers of samples of imported baby foods (46 %) were found contaminated with penicillia

(Ismail *et al.* 2010). Nine species in addition to other unidentified species were isolated from the 5 local baby food products investigated on PRYES. Jacinta millet flour had the highest contamination level with *Penicillium* (58.03 % of the total propagules). Moderate contamination levels were recorded in Mwebaza rice porridge (17.51 %), Baby soya (11.28 %) and Kayebe (10.52 %) while the least was registered in Mukuza (1.12 %) (Table 3).

Three nephrotoxicogenic *Penicillium* species were isolated. *P. viridicatum* and *P. verrucosum* were recovered in rare frequency (4 % of the samples), and each accounting for a minor proportion of the total propagules. *P. verrucosum*, a producer of ochratoxin A and citrinin, was isolated from Baby soya while *P. viridicatum*, a producer of xanthomegnin and viomellein, from both Baby soya and Mwebaza rice porridge (one sample each). *P. viridicatum* was one of the chief species isolated from stored corn (Mislivec and Tuite 1970), from starches intended for human consumption (Suarez *et al.* 1981) and from the baby foods imported to Uganda (Ismail *et al.* 2010). *P. verrucosum* was absent in stored as well as pre-harvest corn from Valencia, Spain (Jimenez *et al.* 1985) while it was found in colonized barley and produced ochratoxin A (Ramakrishna *et al.* 1996).

P. citrinum, a producer of citrinin and a nephrotoxicogenic species but did not produce the yellow or brown colour on the reverse of the Petri dish, was the most commonly encountered species occurring in 52 % of the samples accounting for 83.39 % of the total *Penicillium* and 13.77 % of the total count of fungi (Table 2). Its highest contamination level was recorded in Jacinta millet flour (56.86 % of the total propagules). Low contamination levels were obtained from Baby soya (7.67 %), Kayebe (5.84 %), Mwebaza rice porridge (3.28 %) while the least was recorded in Mukuza (0.22 %) (Table 3). Earlier studies reported *P. citrinum* in moderate frequency from baby foods imported to Uganda (Ismail *et al.* 2010), sorghum and maize grains in Egypt (Moubasher *et al.* 1972, El-Kady *et al.* 1982) and in Uganda (Ismail *et al.* 2003). It was also isolated from millet grains and flour in Turkey (Aran and Eke 1987), soya beans (El-Kady and Youssef 1993), dried fish (Wheeler *et al.* 1986), and milled rice (Taligoola *et al.* 2004, Makun *et al.* 2007) in insignificant levels.

The remaining 6 *Penicillium* species were rarely encountered each from 1 or 2 products, and these were *P. chermesinum*, *P. pinophilum*, *P. oxalicum*, *P. expansum*, *P. chrysogenum*, and *P. corylophilum*. They collectively accounted

for about 14.5 % of *Penicillium* and 2.4 % of total fungi. Earlier findings reported *P. expansum* to be less common on cereals such as maize (Mislivec and Tuite 1970) and rice (Makun *et al.* 2007). *P. pinophilum* was found in maize from Thailand (Pitt *et al.* 1994). Contrary to our findings, *P. chrysogenum* was reported commonly from cereal grains such as rice (Kurata *et al.* 1968), maize (Mislivec and tuite 1970, Moubasher *et al.* 1972), flour (Graves and Hesseltine 1966), and maize based snack foods (Zohri *et al.* 1995). Other unidentified *Penicillium* species were also isolated.

A total of other 42 species belonging to 16 genera in addition to some other unidentified were also isolated. Of these, *Aspergillus* (16 species), *Fusarium* (8) and *Cladosporium* (3) were the most predominant genera isolated from the 5 products investigated (Table 2). *Aspergillus* was recovered from 96 % of the samples, accounting for 23.26 % of the total counts. It was most heavily isolated from all samples of Kayebe and Baby soya followed by Mwebaza rice porridge, Jacinta and Mukuza (9 samples each). *A. flavus* and *A. niger* were the most common species each occurring in 58 % of the samples, accounting for 16.45 % and 1.14 % of the total counts respectively. *A. oryzae* was moderately isolated in 28 % of samples from 3 products. The remaining 13 species were infrequently isolated (Table 2).

Fusarium was recorded in 56 % of the samples accounting for 14.58 % of the total count of fungi. However, *F. verticillioioides* was the most common species, recovered in high frequency from Baby soya, Kayebe and Jacinta millet flour, while moderately from Mwebaza rice porridge but not from Mukuza (Table 3). Despite its recovery in low frequency (14 % of the samples), *F. tricinctum* exhibited the highest number of propagules (9.40 % of the total count of fungi). However, it was recovered from Mwebaza rice porridge and Mukuza.

Cladosporium was recovered in 52 % of the samples accounting for 2.63 % of the total counts. Mukuza was the most heavily contaminated product, followed by Mwebaza rice porridge, Baby soya and Jacinta millet flour while Kayebe was the least.

Yeasts (35.02 % of the total propagules) and *Rhizopus stolonifer* (2.86 %) were isolated in moderate frequency while the remaining fungi were less frequent (Table 2).

Incidence of *Fusarium* species on Peptone-PCNB

Fusarium (9 species) was recovered from the 5 products investigated. It was isolated in high frequency (94 % of the samples), accounting for 67.32 % of the total counts (Table 2). Jacinta (87.37 % of the total counts) followed by kayebe (86.61 %) and Mwebaza rice porridge (79.61 %) had high contamination levels with *Fusarium* that contaminated all samples of these products. Moderate contamination level was recorded in Mukuza (37.36 %) while the least was registered in Baby soya (10.75 %) (Table 3). The high incidence of *Fusarium* on the local baby foods may be attributed to the fact that the cereals were milled such that the flours create a larger surface area, which harbors more spores. Secondly, some of the ingredients like millet, maize, sorghum and soybeans are dried on the ground where they become predisposed to contamination with soil fungi.

F. verticillioides was the most prevalent species, occurring in 60 % of the samples, contaminating 4 out of the 5 products investigated and accounting for 13.94 % of *Fusarium* propagules and 9.38 % of the total fungal propagules (Table 2). *F. verticillioides* is known to produce fumonisin B mycotoxin, a possible cause of human oesophageal cancer (Sydenham *et al.* 1990, 1993, Maheshwar *et al.* 2009). *F. verticillioides* was found mostly contaminating kayebe with 15.03 % of the total counts followed by Jacinta millet flour (12.23 %), then Mwebaza rice porridge (8.45 %) and the least was recorded in Baby soya. The high incidence of *F. verticillioides* on Kayebe, a product of maize is in agreement with earlier findings of its prevalence on maize kernels in Canada (Neish *et al.* 1983, Mills 1989), Egypt (Moubasher *et al.* 1972), South Africa (Marasas *et al.* 1979) and Uganda (Ismail *et al.* 2003). This fungus was also reported earlier on sorghum (Osman *et al.* 1988), rice (Pitt *et al.* 1994, Taligoola *et al.* 2004, Maheshwar *et al.* 2009), and soya beans (El-Kady and Youssef 1993, Pitt *et al.* 1994).

F. solani was the second most common species moderately isolated from 30 % of the samples and accounting for 1.04 % of the total *Fusarium* propagules. It was only found in low levels of contamination in Jacinta millet flour, Mwebaza rice porridge and Baby soya (Table 3). Earlier studies indicated low frequency of *F. solani* on rice (Makun *et al.* 2007, Taligoola *et al.* 2004, 2010), moderate frequency on soybeans (Domsch *et al.* 1980) and sorghum (Onyike and Nelson 1992), while others

indicated high frequencies on maize (Logrieco *et al.* 1995, Ismail *et al.* 2003).

Other 5 *Fusarium* species were encountered in low frequency. These included *F. fusarioides*, *F. lateritium*, *F. proliferatum*, *F. oxysporum* and *F. tricinctum*. Despite their low frequency, *F. proliferatum* and *F. tricinctum* had high contamination levels (48.93 % and 20.61 % of *Fusarium* propagules and 32.94 % and 13.88 % of the total fungi respectively). The occurrence of *F. tricinctum* in high incidences on Mwebaza rice porridge is a contrast to what Pitt *et al.* (1994) had found. The occurrence of *F. proliferatum* in high frequency on kayebe, a product of maize, is in agreement with earlier findings on maize and sorghum in Nigeria (Onyike and Nelson 1992), Argentina (Sydenham *et al.* 1993) and Uganda (Ismail *et al.* 2003). The absence of *F. proliferatum* on Mwebaza rice porridge is inconsistent with its high incidence from paddy rice (Pitt *et al.* 1994). However, the prevalence of *F. verticillioides*, *F. lateritium*, *F. proliferatum*, *F. solani* and *F. oxysporum* is consistent with the findings of Ismail *et al.* (2008, 2010) on cereal baby foods imported to Uganda.

The remaining two species were rarely encountered, each from one product: *F. poae* (from Mukuza) and *F. udum* (Mwebaza rice porridge). The absence of *F. poae* on Baby soya, a product of maize and soybeans is not consistent with findings where *F. poae* has been reported in high frequency occurrence from soybeans in the USA (Abbas and Bosch 1990) and maize (Flannigan 1969, Neish *et al.* 1982).

The Anova test of significance revealed that there were statistically significant differences between the total counts of the different *Fusarium* species recovered from the different 5 food products on Peptone-PCNB medium. Therefore, the type of baby food product may be selective to the type and total count of each *Fusarium* species recovered on Peptone-PCNB.

A total of other 24 fungal species belonging to 14 genera in addition to some others unidentified, were also isolated on this medium. Of these, *Aspergillus* (10 species) and *Cladosporium* (2) were the most common. They occurred in 58 % and 50 % of the samples, accounting for 5.45 % and 4.52 % of the total counts respectively. *A. flavus* and *C. sphaerospermum* were the most frequent. Yeasts were moderately isolated from 38 % of samples from all products investigated. The remaining 12 fungal species were infrequently isolated (Table 2).

Table 2. Incidence of nephrotoxic *Penicillium*, fusaria and aflatoxicogenic aspergilli and other fungi on PRYES, Peptone-PCNB and AFPA agar media.

Medium Fungi	PRYES					Peptone-PCNB					AFPA				
	TC	% TC	% F	OR	Source	TC	% TC	% F	OR	Source	TC	% TC	% F	OR	Source
Penicillium	26800	16.51	82	H	1,2,3,4	525	0.5	16	L	1,2,5	15200	13.90	32	M	1,2
<i>P. chermesinum</i> Biourge	200	0.12	4	R	2										
<i>P. chrysogenum</i> Thom	1075	0.66	12	R	1,2										
<i>P. citrinum</i> Thom	22350	13.77	52	H	1,2,3,4,5	275	0.26	12	R	1,2,5	14650	13.4	20	L	2
<i>P. corylophilum</i> Dierckx	550	0.34	12	R	2,5	250	0.24	8	R	1,2	200	0.18	8	R	2
<i>P. expansum</i> Link	125	0.08	4	R	1,2										
<i>P. pinophilum</i> Hedgcock	1525	0.94	6	R	2,3										
<i>P. oxalicum</i> Currie & Thom	400	0.25	6	R	4						25	0.02	2	R	5
<i>P. verrucosum</i> Dierckx	100	0.06	4	R	1										
<i>P. viridicatum</i> Westling	50	0.03	4	R	1,3										
<i>Penicillium</i> spp.	425	0.26	4	R	3						325	0.13	12	R	1
Fusarium	23700	14.58	56	H	1,2,3,4,5	70835	67.32	94	H	1,2,3,4,5	34775	31.8	52	H	1,2,3,4,5
<i>F. dimerum</i> Penzig	50	0.03	2	R	3										
<i>F. fusarioides</i> (Fragoso & Ciferii) C. Booth	650	0.40	6	R	4	2525	2.40	18	L	4					
<i>F. lateritium</i> Nees	3975	2.44	6	R	4	3275	3.12	18	L	2,4					
<i>F. poae</i> (Peck) Wollenw.	50	0.03	4	R	5	2475	2.35	6	R	5	750	0.7	2	R	5
<i>F. proliferatum</i> (Matsushima) Nirenberg						34660	32.94	10	R	2,4	1450	1.3	6	R	2
<i>F. semitectum</i> Berk. & Rav.											50	0.04	2	R	1
<i>F. solani</i> (Martius) Saccardo	250	0.15	6	R	3	1100	1.04	30	M	1,3,4	4400	4.0	8	R	2,5
<i>F. oxysporum</i> Schlechtendal						2300	2.19	24	L	1,3,4					
<i>F. tricinctum</i> (Corda) Saccardo	15275	9.40	14	L	5	14600	13.88	24	L	3,5	19350	17.7	26	M	4,5
<i>F. udum</i> Butler						25	0.02	2	R	3					
<i>F. verticillioides</i> (Saccardo) Nirenberg	3425	2.12	42	M	1,2,3,4	9875	9.38	60	H	1,2,3,4	8275	7.6	24	L	2,3,4
<i>Fusarium</i> spp.	25	0.02	2	R	5						500	0.45	10	R	1,5
Aflatoxicogenic aspergilli											34800	31.80	90	H	1,2,3,4,5
Other fungi	135610	83.50	100	H	1,2,3,4,5	34380	32.67	100	H	1,2,3,4,5	73775	67.42	100	H	1,2,3,4,5
<i>Acromonium strictum</i> W. Gams						25	0.02	2	R	5					
<i>Aspergillus</i>	37810	23.26	96	H	1,2,3,4,5	5735	5.45	58	H	1,2,3,4,5	35650	32.60	92	H	1,2,3,4,5
<i>A. aegyptiacus</i> Moubasher & Moustafa	125	0.08	10	R	1,3										
<i>A. candidus</i> Link	2775	1.72	16	L	1,3,5	125	0.12	6	R	1,3					
<i>A. flavus</i> Link	26740	16.45	58	H	1,2,3,4	3835	3.64	30	M	1,2,3,4,5					

<i>A. fresenii</i> Subram.	150	0.93	8	R	1	25	0.02	2	R	1					
<i>A. fumigates</i> Fresenius	225	0.14	14	L	1,2,3,	50	0.05	4	R	1,3	25	0.02	2	R	3
<i>A. niger</i> van Tieghem	1850	1.14	58	H	1,2,3,4,5	700	0.67	22	L	1,2,3	175	0.16	10	R	1,5
<i>A. ochraceus</i> Wilhelm	450	0.28	18	L	1,2,3,4	500	0.47	12	R	2,3,4	250	0.20	12	R	1,2,3
<i>A. oryzae</i> (Ahlburg) Cohn	1875	1.15	28	M	1,2,4	100	0.09	6	R	1,2					
<i>A. parasiticus</i> Speare	250	0.15	8	R	1,2										
<i>A. penicillioides</i> Spegazzini	225	0.14	8	R	1,2,3						100	0.09	6	R	5
<i>A. sydowii</i> (Bainier & Sartory) Thom & Church	100	0.06	4	R	2,3										
<i>A. tamarii</i> Kita						350	0.33	4	R	2,3					
<i>A. terreus</i> Thom	50	0.03	4	R	1,3	50	0.05	2	R	1					
<i>A. versicolor</i> (Vuillemin) Tiraboschi	275	0.17	18	L	1,3,5										
<i>A. wentii</i> Wehmer	895	0.55	14	L	1,2,5	25	0.02	2	R	2					
<i>Aspergillus</i> spp.	50	0.02	2	R	5						300	0.31	8	R	1,3
<i>Cladosporium</i>	4275	2.63	52	H	1,2,3,4,5	4750	4.52	50	H	1,2,3,4,5	5050	4.60	22	L	1,2,3,4,5
<i>C. cladosporioides</i> (Fresenius) de Vries	200	0.12	12	R	1	175	0.17	10	M	1,2,3,5	2225	2.0	10	R	1,2,3,4,5
<i>C. herbarum</i> (Persoon) Link	100	0.06	6	R	1	4575	4.35	44	M	1,2,3,4,5					
<i>C. sphaerospermum</i> Penzig	3975	2.44	46	M	1,2,3,4,5						2825	2.6	24	L	1,2,3,5
<i>Cochliobolus lunatus</i> Nelson & Haasis						75	0.07	4	R	1,3					
<i>Emericella nidulans</i> (Eidam) Vuillemin	225	0.14	4	R	1,3	25	0.02	2	R	1					
<i>Epicoccum nigrum</i> Link						25	0.02	2	R	5					
<i>Eurotium</i>	725	0.44	20	L	1,2										
<i>E. amstelodami</i> Mangin	225	0.14	6	R	1,2										
<i>E. herbariorum</i> (Wiggers: Fries) Link	100	0.66	2	R	2										
<i>E. cristatum</i> (Raper & Fennell) Malloch & Cain	375	0.23	18	L	1,2										
<i>E. repens</i> de Bary	25	0.02	2	R	1										
<i>Eupenicillium</i> sp.						25	0.02	2	R	1					
<i>Fennellia</i>											125	0.1	6	R	1,3,5
<i>F. flavipes</i> Wiley & Simmons											100	0.09	4	R	1,3
<i>F. nivea</i> (Wiley & Simmons) Samson											25	0.02	2	R	5
<i>Geotrichum candidum</i> Link	100	0.06	14	R	5										
<i>Lasiodiplodia theobromae</i> (Pat.) Griffon & Maubl.	25	.0.02	2	R	5						25	0.02	2	R	5
<i>Microdochium nivale</i> (Fries) Samuels	750	0.46	2	R	4	50	0.05	2	R	1	50	0.04	2	R	1

& Hallett															
<i>Mucor</i>	300	0.18	12	R	1,2,3						1400	1.3	14	L	1,2,3,4
<i>M. cicinelloides</i> van Tieghem	300	0.18	12	R	1,2,3										
<i>M. plumbeus</i> Bonord.											500	0.45	4	R	1,4
<i>M. racemosus</i> Fresenius											900	0.8	10	R	2,4
<i>Nigrospora oryzae</i> (Berkeley & Broome) Petch											1275	1.2	12	R	4,5
<i>Neurospora crassa</i> Shear & Dodge	500	0.31	6	R	3,5	75	0.07	2	R	5	300	0.27	6	R	5
<i>Paecilomyces variotii</i> (Thom) Samson	225	0.14	6	R	1										
<i>Pestalotiopsis guepinii</i> (Desm.) Stey.											25	0.02	2	R	5
<i>Phoma</i> sp.	75	0.04	2	R	4	50	0.05	2	R	2					
<i>Rhizopus</i>	4650	2.86	26	M	4,5						3575	3.26	16	L	1,3,4
<i>R. oryzae</i> Went & Prinsen-Geerligs											75	0.06	2	R	3
<i>R. stolonifer</i> (Ehrenberg) Vuillemin	4650	2.86	26	M	4,5						3500	3.2	14	L	1,4
<i>Scopulariopsis candida</i> (Gueguen) Vuill.						175	0.17	4	R	1					
<i>Scytalidium lignicola</i> Pesante	75	0.04	4	R	1										
<i>Talaromyces</i> sp.						75	0.07	2	R	1					
<i>Trichoderma harzianum</i> Rifai	375	0.23	4	R	2										
<i>Wallemia sebi</i> (Fries) von Arx	25	0.08	4	R	1,3										
Unidentified fungi	4750	2.92	10	R	3,5	25	0.05	4	R	3	100	0.09	6	R	5
Total yeasts	57100	35.12	34	M	1,2,3,4,5	22745	21.63	38	M	1,2,3,4,5	11825	10.80	26	M	1,2,3
<i>Rhodotorula mucilaginosa</i> (A. Jorgensen) F. C. Harrison	275	0.17	12	R	2,3,4	4050	3.85	20	L	1,2,3,4	2450	2.2	6	R	2
White yeasts	56825	35.02	24	L	1,2,3,4,5	18220	17.32	22	L	2,3,4,5	9375	8.6	24	L	1,2,3,5
Yellow yeasts						475	0.45	4	R	3					
Gross total counts	162585	100	100	H	1,2,3,4,5	105215	100	100	H	1,2,3,4,5	109425	100	100	H	1,2,3,4,5

TC = Total counts (calculated /g baby food product in 50 samples).

% TC = Percentage total counts (calculated per total fungal counts).

% F = percentage frequency (calculated per 50 samples investigated).

OR = occurrence remarks; High (H) = 50 % - 100 %, Moderate (M) = 25 % - 49 %, Low (L) = 13 % - 24 %, Rare (R) = less than 13 %.

Source: 1 = Baby soya, 2 = Kayebe, 3 = Mwebaza rice porridge, 4 = Jacinta millet flour, 5 = Mukuza.

Aflatoxigenic aspergilli recovered on AFPA

Aflatoxigenic *Aspergillus* species were found contaminating the majority of samples (90 %) of the 5 products (Table 2). Contrarily, considerably lower percentage of samples (34 %) of food products imported to Uganda was contaminated with aflatoxigenic aspergilli (Ismail *et al.* 2008). They accounted for 97.62 % of total *Aspergillus* propagules and 31.8 % of the total fungal propagules. The most heavily contaminated product was kayebe (57.72 % of the total propagules in ten samples). This was followed by Mwebaza rice porridge (19.8 5, 10 samples), Baby soya (14.30 %, 6), Jacinta millet flour (11.53 %, 10) while the least was Mukuza (5 %, 9) (Table 3). The high incidence of aflatoxigenic aspergilli on Kayebe, a product of maize, soybeans and fish, is in agreement with an earlier report in Egypt on cornsnacks, a product of maize flour (Zohri *et al.* 1995). Similarly, aflatoxigenic species were detected from corn and peanuts but not from soybeans in Uganda (Sebunya and Yourtee 1990, Ismail 2001, Ismail *et al.* 2003), on maize and sorghum grains in Ethiopia (Abate and Gashe 1985) and from foods intended for human consumption in Spain (Suarez *et al.* (1981). Also, soybeans were found to be contaminated with aflatoxigenic fungi and to contain aflatoxins by Shotwell and Stubblefield (1972). All cereals are a common source of *A. flavus* and maize and maize products are a particular problem (Shotwell 1977, Zohri *et al.* 1995, Ismail *et al.* 2003). *A. flavus* has been isolated from paddy and milled rice (Reddy *et al.* 2009, Taligoola *et al.* 2004, 2010) and pearl millet (Mishra and Daradhiyar 1991). Mukuza, whose main component is sorghum flour, was contaminated with aflatoxigenic fungi and this is in agreement with the findings of Anandam (1970) and Abate and Gashe (1985) on sorghum grains. In this respect, Calvo and Guarro (1977) stressed on the possible dangers caused by fungi contaminating baby foods.

Spoilage by *A. flavus* or aflatoxin production in small cereal grains was said to be a result of poor handling (Jarchovsaka *et al.* 1980). Hesseltine *et al.* (1975) observed that there is a positive correlation between the prevalence of the *Aspergillus flavus* group and the occurrence of aflatoxins in grains. Similarly, Munimbazi and Bullerman (1996) found that 37 out of 95 isolates of *A. flavus* and all isolates of *A. parasiticus* produced aflatoxins.

Other four *Aspergillus* species were also isolated but less frequently. These were *A. niger*, *A. fumigatus*, *A. ochraceus*, and *A.*

penicillioides. *A. ochraceus*, a well known for production of ochratoxin, was detected on Baby soya, Kayebe and Mwebaza rice porridge. Hesseltine (1974) isolated *A. ochraceus* from a variety of cereals. *A. ochraceus* and *A. penicillioides* were also isolated from cereals and cereal products (Pitt and Hocking 2009, Ismail *et al.* 2003, 2008, 2010, Taligoola *et al.* 2004, 2010). *A. ochraceus*, *A. wentii*, *A. flavus*, *A. niger* and *A. parasiticus* were found to be predominant fungi (Munimbazi and Bullerman 1996), but *A. fumigatus* was less common (Wheeler *et al.* 1986) on dried fish.

Other 23 species belonging to 13 genera were also isolated on AFPA. Of these, *Fusarium* was the most frequent, occurring in 52 % of the samples, accounting for 31.8 % of the total count of fungi (Table 2). It was found most heavily contaminating Jacinta millet flour, while the least contaminated were Kayebe and Baby soya (Table 3). *Penicillium* (3 species) was the second most common genus on AFPA, occurring moderately in 32 % of the samples accounting for 29.20 % of the total count. The highest level of contamination with *Penicillium* was recorded in Kayebe (29.2 %) followed by Baby soya (5.5 %). However, it was infrequently encountered in the other three products. The remaining genera and species were less common (Table 2).

Incidence of aflatoxins in food products investigated

All the 13 samples analysed of the five products were contaminated with aflatoxins (Table 4). Two levels of contamination were recorded (0-10 ppb in 9 samples and 10-20 ppb in 4 samples). Lower percentages of samples were found contaminated with aflatoxins in staple cereals from Ethiopia (Ayalew *et al.* 2006), baby foods imported to Uganda (Ismail *et al.* 2008), and in rice from India (Reddy *et al.* 2009), and from Uganda and Pakistan (Taligoola *et al.* 2010). Mwebaza rice porridge and Kayebe were the most heavily contaminated with aflatoxins and aflatoxigenic aspergilli. Baby soya, Jacinta millet flour and Mukuza had the lowest level of aflatoxin. The high aflatoxin contamination level in Mwebaza rice porridge is consistent with the findings that *A. flavus* was obtained on milled rice (Pitt *et al.* 1994, Taligoola *et al.* 2004, 2010). Similarly, Suarez *et al.* (1981) in their study of fungi associated with starches intended for human consumption (rice inclusive) found that out of the 21 strains of *A. flavus* tested only one did not prove toxic.

Kayebe, Baby soya and Mukuza were all products of maize. All their samples were contaminated with aflatoxins. This is in agreement with earlier reports where aflatoxins were found in corn snacks, a product of maize, in Egypt (Zohri *et al.* 1995) and on maize (Lillehoj *et al.* 1978, Ismail *et al.* 2003) and on cereal baby foods imported into Uganda (Ismail *et al.* 2008). Kayebe had a higher aflatoxin contamination level than Baby soya. The fact that Kayebe with milled dried fish as one of the components had a higher aflatoxin contamination level, is in agreement with an earlier report where aflatoxins were obtained from an African fish product (Johnsyn and Lahai 1992). Similarly, *A. flavus* was commonly isolated from dried fish in Sierra Leone (Johnsyn and Lahai 1992) and Nigeria (Dyaolu and Adebajo 1994). The low level of aflatoxins in Baby soya which had a high content of soyabean flour is consistent with another report where soya bean flour was found to be aflatoxins-free (Shotwell *et al.* 1975). This leads to a conclusion that soybeans may have a natural resistance to invasion by the aflatoxin-producing fungi. Mukuza also had lower aflatoxin contamination levels than Kayebe. The occurrence of aflatoxins on Mukuza (a product of sorghum) is

in agreement with the observation of Anandam (1970) who detected aflatoxins on sorghum. All Jacinta millet flour samples analyzed were also contaminated with both aflatoxins and aflatoxigenic aspergilli.

The high occurrence of *A. flavus* in the local baby products may be attributed to the poor storage and use of insect-damaged and broken grains. Lillehoj (1976) observed that *A. flavus* was more associated with insect-damaged grains, while aflatoxin contamination was found in poorly stored cereals (Nwokolo and Okonwo 1979). Moreover, the presence of aflatoxigenic species indicated the potential for mycotoxins contamination (Zohri *et al.* 1995, Ismail *et al.* 2003, 2008, Taligoola *et al.* 2010).

Conclusions: The present results revealed that more than 50% of samples of baby food products investigated were heavily contaminated with *Penicillium* of which 3 species are nephrotoxicogenic, with species of *Aspergillus* and *Fusarium* of which some are well-known as toxicogenic. Moreover, all samples analysed for aflatoxins were positive but having levels below or in the current tolerance level of 20 ppb accepted in foodstuffs.

Table 3: Incidence of nephrotoxicogenic *Penicillium*, *Fusarium* and aflatoxigenic aspergilli of baby food products manufactured in Uganda.

Food product	Baby soya		Kayebe		Mwebaza rice porridge		Jacinta millet flour		Mukuza	
	%TC	%F	%TC	%F	%TC	%F	%TC	%F	%TC	%F
On PRYES										
<i>Penicillium</i>	11.28	100	10.52	100	17.51	70	58.03	60	1.12	80
<i>P. chermisinum</i>			0.61	20						
<i>P. chrysogenum</i>	0.9	20	3.15	40						
<i>P. citrinum</i>	7.67	70	5.84	80	3.28	30	56.86	60	0.22	20
<i>P. corylophilum</i>			0.31	10					0.57	50
<i>P. expansum</i>	0.45	10	0.31	10						
<i>P. pinophilum</i>			0.31	10	12.69	20				
<i>P. oxalicum</i>							1.16	30		
<i>P. verrucosum</i>	1.81	20								
<i>P. viridicatum</i>	0.45	10			0.22	10				
<i>Penicillium</i> sp.					1.56	10			0.32	10
Other fungi	88.27	100	89.48	100	82.49	100	41.97	100	98.88	100
On Peptone-PCNB										
<i>Fusarium</i>	10.75	90	86.61	100	79.61	100	87.37	100	37.36	80
<i>F. fusarioides</i>							13.42	90		
<i>F. lateritium</i>			3.65	30			9.57	90		
<i>F. poae</i>									9.2	30
<i>F. proliferatum</i>			67.92	70			38.33	60		
<i>F. solani</i>	3.28	60			1.88	40	3.32	50		
<i>F. oxysporum</i>	0.3	10			2.81	50	10.4	60		
<i>F. tricinctum</i>					65.72	50			28.2	70
<i>F. udum</i>					0.23	10				
<i>F. verticillioides</i>	7.16	90	15.03	80	8.45	80	12.23	50		
Other fungi	89.25	100	13.39	100	20.89	100	12.62	100	62.63	100

On AFPA										
Aflatoxigenic aspergilli	14.3	60	57.72	100	19.8	100	11.53	100	5.0	90
Other aspergilli	4.6	30	0.3	30	12.1	30			0.9	50
Other fungi	81.1	100	42.0	100	68.1	100	88.47	100	94.0	100

• %TC: percentage total counts, calculated per total fungi in 10 samples of each food product investigated.

• %F: percentage frequency (out of 10 samples investigated)

Table 4: Incidence of total aflatoxins and aflatoxigenic aspergilli in baby food products investigated.

Product	Sample No.	Range of aflatoxins (in ppb)	% isolates of aflatoxigenic aspergilli
Baby soya	1	0 – 10	9.72
	4	0 – 10	14.29
Kayebe	14	10 – 20	54.46
	19	10 – 20	88.76
	20	0 – 10	82.63
Mwebaza rice porridge	21	10 – 20	50.00
	25	10 – 20	20.00
	27	0 – 10	25.00
Jacinta millet flour	31	0 – 10	14.81
	38	0 – 10	22.45
Mukuza	44	0 – 10	16.66
	46	0 – 10	24.00
	49	0 – 10	5.03

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