Microbiological And Mycotoxins Evaluation Of Cereals - Based Baby Food Samples Sold In Nigeria Market

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Abstract: Occurrence of microbial contaminants and mycotoxin level of cereal-based baby foods (flours) sold in Nigeria and its health impact on infants was evaluated. Random sampling of processed cereal based foods and infant formulae were done in different stores and markets in the cities of Ibadan and Uyo metropolis. The total heterotrophic count ranged from 1.0 - 7.5x10³cfu/g, Total Coliform count (1.0 - 2.0 x 10³cfu/g) and 1.0 x 10³cfu/g for Salmonella-Shigella count, Total fungi count ranged from 1.0-4.0x10³ cfu/g. The microbial contamination was not above the maximum limits $(10^3 cfu/g)$ by ICMSF and USFDA. The bacteria isolated from the samples include: Proteus mirabilis, Proteus penneri, Bacillus subtilis, Bacillus licheniformis and Salmonella sp. B. subtilis had the highest frequency of occurrence (33.33%). The fungi isolated were Aspergillus niger, A. fumigatus, A. terreus, A. flavus, A. glaucus, R.stolonifer, Penicillium sp. Fusarium semithectum, Furasium proliferatum and F. sacchari. *Esacchari* had the highest frequency of occurrence (28.57%). Afloatoxin: AFB₁ AFB₂, AFG₁, AFG₂ AFM₁ and AFM₂ concentration ranged from $0.89 - 4.67 \,\mu\text{g/kg}$, $0.00 - 2.47 \,\mu\text{g/kg}$, $0.00 - 0.21 \,\mu\text{g/kg}$, $0.00 - 0.07 \,\mu\text{g/kg}$, $0.41 - 0.07 \,\mu\text{g/kg}$ $13.34\mu g/kg$ and $0.00 - 4.15\mu g/kg$. Samples FGWMC and FRMC had the highest concentration. Aflatoxins B₂ G₁, G_2 and M_2 were not detected in some of the samples. Ochratoxin (OTA) concentration ranged from $0.07 - 1.45 \mu g/kg$, the highest concentration was recorded in sample FGWMC. OTB and OTC were not detected in the samples. Fumonisin: FB₁ and FB₂ concentration ranged from $1.08 - 6.43 \ \mu g/kg$ and $0.00 - 0.44 \ \mu g/kg$ the highest was recorded in sample FRMC and MWC. Patulin concentration ranged from $0.03 - 0.21 \mu g/kg$, sample FRMC had the highest.

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1. INTRODUCTION

Breast milk has been proven to be the perfect food for the infant during the first six months of life. It contains all the nutrients and immunological factors an infant requires to maintain optimal health and growth. Furthermore, breast milk also protects infants against the two leading causes of infant mortality, upper respiratory infections and diarrhea (UNICEF, 1999). However, at the age of six months and above when the child's birth weight is expected to have doubled nutritious complementary foods (weaning foods) are therefore introduced which typically covers the period from six to twenty four months of age in most developing countries (WHO, 2010).

Infants are a vulnerable part of the population due to, in part, their physiology, a fairly restricted diet and a higher consumption relative to their body. Therefore, the significance and potential health risk of any contaminant in foods consumed by infants is increased and diligent attention must be paid to this particular area. Cereals and milk are an important source of nutrition in infant diet and are among the first solid foods eaten. The presence of chemical contaminants in the human diet, and especially in the diet of vulnerable populations such as infants, is of great concern (Shephard *et al.*, 2006). Among the most important chemical contaminants found in foodstuffs are natural toxins such as mycotoxins and they are of primary concern when considering chronic health risks (EC, 2006).

Mycotoxins, particularly aflatoxins (AFTs) and ochratoxin A (OTA) pose a significant threat to human health. Aflatoxins are potent carcinogens and, in association with hepatitis B virus, are responsible for many thousands of human deaths per annum, mostly in non-industrialized tropical countries (EC, 2006).

Ochratoxin A is a probable human carcinogen, and it was reported to cause urinary tract cancer and kidney damage in people from Eastern Europe. Exposure to OTA seems to be the biggest hazard correlated to microscopic fungi for the European consumers of cereals (Cowan, 1985).

EC Regulation 1881/2006 sets a limit of 0.25 μ g kg-1 (dry product) for aflatoxin M₁ (AFM₁) for infant formulae and follow-on formulae, including infant milk and follow-on milk, and a limit of 0.10 μ g kg-1 for aflatoxin B1 (AFB₁) and 0.50 μ g kg-1 for OTA for processing cereal-based foods and baby foods for infants and young children (Holt *et al.*, 1994).

This research work aim of assessing the microbial quality and mycotoxins level of baby and infant foods formulae sold in Nigeria market. Results will be compared with the maximum levels established in the EU and the available literature

1. MATERIALS AND METHODS

2.1 Microbiological analysis

Ten grams of the baby food samples were taken for microbiological analysis. Standard pour plates were prepared from 10 - fold dilutions into a nutrient agar medium for total heterotrophic bacteria counts, MacConkey agar was used for total Coliform counts, Salmonella/Shigella agar for total Salmonella/Shigella counts, Thiosulphate citrate bile salt sucrose agar for total Vibrio counts, MRS agar for lactic acid bacteria count, Yeast extract agar for the total yeast count and Sabouraud dextrose agar with chloramphenicol (250mg/100ml) for total fungal counts. The bacterial plates were incubated at 37°C for 24-48 hours, while fungal plates were incubated at room temperature (28 \pm 2°C) for 3-5 days. Colonies were selected randomly and were characterized using morphological and biochemical test such as Gram stain, spore stain, motility, catalase, oxidize, coagulase, indole, MR-VP and Urease and sugar fermentation tests. Bacterial isolates were identified with reference to Cowan and Steel's Manual for the identification of Medical Bacteria (Sampson et al., 1984) and Bergev's Manual of Determinative Bacteriology (AOAC, 1998). Fungal isolates were identified based on their morphological and cultural characteristics as recommended by (Sampson et al., 1984).

2.2 Mycotoxin analysis

Aflatoxins detection and quantification

All aflatoxins analyses were performed by extracting the aflatoxins from the samples with chloroform according to the AOAC method (1998). The extract was concentrated and stored in dark bottles in the freezer prior to detection and quantitative determination. Thin layer chromatography of toxins extracted from the samples and aflatoxin standards of known concentration were performed on silica gel DG 254. Of the extracted samples 5, 10 and 15 μ l were spotted on three different points on a ruled base line of the Tin Layer Chromatography (TLC) plates. Also 5, 10 and 15 μ l of the aflatoxin standard were spotted on another three points near spotted points of the previous sample extract.

The plates were developed first with diethyl ether and then with chloroform: acetone (9:1v/v). Aflatoxin was identified on the basis of co-migration with aflatoxin standards (Fluka) and by their characteristic fluorescent color under ultraviolet (UV) illumination at 360nm and upon exposure to sulfuric acid (50:50v/v). The fluorescent spots of aflatoxin B₁, AFB₂, AFG₁, AFG₂, AFM₁ and AFM₂ were scraped off the TLC and eluted by chloroform: methanol (9:1 v/v). The solvent was evaporated under nitrogen to dryness

and the residue was dissolved in methanol. The concentration of the aflatoxins in solution was estimated using a spectrophotometer (Cecil Instrument CE 505) at a wavelength of 360nm respectively.

2.3 Confirmatory tests for aflatoxin

Three different derivatives were prepared by treating portions of the isolated toxin or the aflatoxin standard with formic acid thionyl chloride, acetic acid-thionyl chloride and tri-fluoroacetic acid. The test was done according to the method of Stoloff and Friedman (Stolof 1976)

2.4 Ochratoxins detection and quantification

About 1ml of chloroform and 0.2ml of the reconstituted extract was spotted on a precoated 20 x 20cm TLC plate along with ochratoxin standards of known concentration. The spotted TLC plate was developed in an equilibrated tank containing Toluene: acetylated: 90% formic acid (5:4:1v/v/v). The developed TLC was air-dried at an ambient temperature (28±20C) and ochratoxins was identified on the basis of co-migration with ochratoxin standards (Fluka) and by their characteristic fluorescent color under ultraviolet (UV) illumination at 366nm and upon exposure to sulfuric acid (50:50v/v). Preparative TLC plates (0.5um thick) were employed for the quantification. 0.8ml stored extracts was applied to the plate as a band rather than a spot to chromatography the maximum amount of samples at the same time. The preparative TLC plates were developed in an equilibrium tank as in ochratoxin extraction. The solvent front was allowed to rise to about $\frac{3}{4}$ of the total length of the plate; the plate was examined under the UV light. The area containing toxin of interest was scraped off, eluted with chloroform and filtered with Whatman No 1 filter paper. The extract was evaporated to dryness over a hot water bath and reconstituted with 3ml of chloroform. The 3ml reconstituted solution and ochratoxin standard of 10ug/ml concentration (Sigma and Aldrich, St Louis, MO USA) was used to read the absorbance on an Ultraviolet Spectrophotometer (Cecil Instrument CE505) at wavelength of 366nm. Ochratoxin concentration in µg/kg was calculated.

2.5 Fumonisin determination and quantification

The fumonisin was extracted from the food samples using 85% acetonitrile and 20g alumina was added. The mixture was filtered using Whatman No 1 filter paper. Standard of known concentration of Fumonisin B_1 and B_2 was prepared. An aliquot of the sample extract was prepared and 2ml of Dimethyl -formamide (DMF) was added to different concentration of the known standard and aliquot of the sample extract. The absorbance was taken after color development with 95% DMF at a wavelength of 560nm. Fumonisin B1 and FB2 in $\mu g/kg$ was calculated.

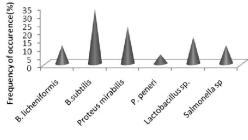
2.6 Statistical Analysis:

The Duncan multiple range test was used to compare significant differences between the means Duncan (1956).

1. RESULTS

The microbial load of the baby food samples is shown in Table 1. The total heterotrophic count ranged from $1.0 - 8.5 \times 10^3$ cfu/g in which sample NMSB had the highest. Total Coliform count ranged from $1.0 - 2.0 \times 10^3$ cfu/g and 1.0×10^3 cfu/g for *Salmonella-Shigella* count and LAB count ranged from 1.0×10^3 , Total fungi count ranged from $1.0-6.0 \times 10^3$ cfu/g. There was no observable growth on TCBS agar and Yeast extract agar respectively.

Frequency of occurrence of the bacteria and fungi isolated from the baby food samples are shown in Figure 1 and 2. The bacteria isolated from the baby food samples include: *Proteus mirabilis, Proteus penneri, Bacillus subtilis, Bacillus licheniformis* and *Salmonella* sp. *B. subtilis* had the highest frequency of occurrence (33.33%). The fungi isolate were *Aspergillus niger, A. fumigatus, A. terreus, A. flavus, A. glaucus, R. stolonifer, Penicillium* sp. *Fusarium semithectum, Furasium proliferatum* and *F. sacchari. F. sacchari* had the highest frequency of occurrence (28.57%).



Isolate

Figure 1: Frequency of occurrence (%) of bacteria isolated from the infant baby food samples

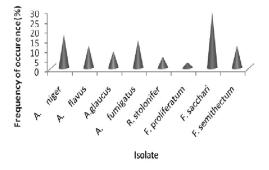


Figure 2: Frequency of occurrence (%) of Fungi isolated from the Infant baby food samples

The concentration of aflatoxins level in the infant formula samples is shown in Table 2a-c. The detected minimum and maximum level were $0.89 - 7.29 \ \mu g$ AFB₁ kg⁻¹, $0.06 - 2.75 \ \mu g$ AFB₂ kg⁻¹, $0.03 - 0.24 \ \mu g$ AFG₁/kg⁻¹, $0.02 - 0.06 \ \mu g$ AFG₂ kg⁻¹ respectively. This was very high since some of the samples contained AFBs above the maximum limit.

About 18 samples had AFBs above the maximum limit (0.10 / kg) acceptable level for infant formulae and baby foods as shown in Table 2a-c. Most of the samples are not contaminated with AFB₂, AFG1 and AFG₂ respectively.

The detected levels of AFB_1 were at 0.89 µg kg⁻¹ in minimum and 7.29 µg kg⁻¹ at maximum.

AFM₁ and AFM₂ minimum and maximum were $0.33 - 14.28 \mu g/kg$ and $0.26 - 5.94 \mu g/kg$ respectively. The level in some samples was higher than the acceptable limit (0.025 $\mu g/kg$)

About 98% and 20% of the samples were contaminated with FB₁ and FB₂ respectively. The detected levels of FB₁ and FB₂ were at $1.08\mu g/kg$ and $0.28\mu g/kg$ in minimum, $6.43\mu g/kg$ and $0.44\mu g/kg$ with a maximum. The highest was recorded in the MWC.

About 69% of the baby food samples were contaminated with Patulin. The detected minimum and maximum Patulin concentration in the samples was 0.07 μ g/kg and 0.21 μ g/kg in which sample CMF had the highest as shown in Table 3.

The detected minimum and maximum OTA level in the samples was 0.07 μ g/kg and 2.28 μ g/kg. The highest concentration was recorded in sample NSMS. About 98% of the samples were found contaminated with OTA and the level in some samples exceeds the permitted level (Table 4).

4.0 DISCUSSION

The microbial contamination of the samples was not above the maximum limits (10^3cfu/g) by ICMSF (1982) and USFDA, (1991). The presence of isolated bacteria species in baby Food sample is of particular interest because of their involvement in different infant (Elegbede, 1998). Most of the microorganisms isolated are potential pathogens. *Proteus mirabilis* and *P. penneri* has been reported as causative agent of opportunistic infection in humans and urinary tract infection, wound infection, pneumonia and septicemia and this calls for concern (Prescott *et al.*, 2005).

Salmonella sp have incriminated in enteritis (enteric fever) which include typhoid fever caused by S. *typhi* and paratyphoid fever caused by paratyphi A and B which are transmitted by water and food and has been implicated in a clinical case (Prescott *et al.*, 2005).

Bacillus subtilis are not considered as human pathogen; they can contaminate food but rarely causes food poisoning. They produce proteolytic enzyme *subtilisin*. Some strains of *B. subtilis* have been considered safe, and in some cases, use as probiotics in foods or in pharmaceutical preparations (Sanders et al., 2003). B. licheniformis are also common contaminant of dairy product and industrially produced baby food (Tatzel et al., 1994, Vuorio et al., 1998). The Bacillus subtilis and B. licheniformis isolated from the baby food samples might have been introduced into samples in a variety of ways, a major source of the contamination may be from the raw materials during food harvest, processing and handling operation since Bacillus species especially B. subtilis are inhabitants of soil where food crops are cultivated (Gordon et al., 1973). The presence of *Bacillus* species in baby food samples may also be due to their ability to form spores which are able to resist chemical and physical stress. This emphasizes the importance of quality control procedures at reducing Bacillus spore counts in baby food.

Occurrence of *Aspergillus* sp. and *Fusarium* sp. in the samples may be as a result of the fact that they attack cereals during pre and post harvest period and also during storage. *Aspergillus* sp. and *Fusarium sp* also cause cancer, oedema, leukaencephalomacia and other kinds of the diseases especially in animals. The incidence of *Aspergillus* sp. and *Fusarium* sp. can be checked by reducing the moisture content of cereals; they should be well dried to a level where these fungi species cannot grow. Also during storage the cereals should be well stored where mold cannot grow.

The detected levels of AFB₁ were at 0.89 μ g kg⁻¹ in minimum and 7.29 μ g kg⁻¹ at maximum. Using a similar approach by Beretta *et al.* (2002), the risk of AFB₁ intake from baby foods can be estimated. If a 5-month-old child with a body weight of 6.5 kg can consume 26 g of formula per portion, according to the manufacturer's suggestion, with the highest quantity of AFB₁ detected (7.29 μ g/kg), 29.16 μ g AFB₁/kg body weight would be taken at a time. AFB₁ is the most potent carcinogen known in mammals, the risk assessment of which is very well established.

 AFM_1 and AFM_2 minimum and maximum were $0.33 - 14.28\mu g/kg$ and $0.26 - 5.94\mu g/kg$ respectively. The level in some samples was higher than the acceptable limit (0.025 $\mu g/kg$)

With a similar calculation to AFB₁ mentioned above, a child with body weight of 6.5kg would uptake the maximum 57.12 μ g AFM₁/kg body weight at a time. Since AFM₁ is a gene-toxic carcinogen, the risks against infants and young children from AFM₁ exposure need careful consideration (Nakajima *et al.*, (2004).

The detected minimum and maximum OTA level in the samples was $0.07 \ \mu g/kg$ and $2.28 \mu g/kg$. According to European Commission the maximum level of OTA in baby foods and processed cereal –based baby foods for infants and young children was $0.50 \mu g/kg$. OTA limit by the Commission Regulation (EC, 2006) was considered as the guide in this study. About 98% of the samples were found contaminated with OTA and the levels in some samples exceed the permitted level (Table 4).

According to the instructions on the label, a baby consumes 25 g of formula per portion. If a 5-month old child with body weight of 6.5 kg and infant formula with the highest quantity of ochratoxin A found $(2.28\mu g/kg)$ were considered, the maximum portion intake would be 8.77 $\mu g/kg$ OTA per body weight (kg). The provisional tolerable weekly intake (PTWI) is 0.1 $\mu g/kg$ body weights for ochratoxin A (Herrman and Walker, 1999) so that there is a serious toxicological risk for a child that consumes a formula with OTA contamination above the permitted level. OTB and OTC were not detected in all the samples.

About 98% and 20% of the samples were contaminated with FB₁ and FB₂ respectively. The fumonisin level in all the samples was below the maximum limit recommended bv European Commission (EC, 2006). According to the instructions on the label, a baby consumes 25 g of formula per portion. If a 5-month old child with body weight of 6.5 kg and infant formula with the highest quantity of FB_1 and FB₂ found (6.43 and 0.44µg/kg) were considered. the maximum portion intake would be 24.738 μ g FB₁ kg⁻¹ and 1.6 μ g FB₂ kg⁻¹ per body weight (kg). It can be said that there is a mild toxicological risk of a child that consumes a formula with FB_1 and FB_2 contamination. However, the total daily mycotoxin intake with the other sources of mycotoxin could be an important risk to infants and young children. About 69% of the baby food samples were contaminated with Patulin.

The result revealed the microbiological quality and mycotoxins in infant baby food samples. Most of the samples under consideration were contaminated with AFB₁, AFB₂, AFG₁, AFG₂, AFM₁ AFM₂, OTA, FB₁, AFB₂ and Patulin. The presence of these microbial isolates and mycotoxins in the food samples can cause serious health problems in infants and babies such as poor growth, suppressed immune system, and cancer. An accurate prediction of the possible health impact of individual mycotoxins in foods for the vulnerable group is difficult; possible additive and synergistic effects of multiple mycotoxins make the task even more complex and the long-term effects are beyond foresight. Climate and environmental conditions during growth, harvest and storage have great influence on mycotoxin levels, which are probably also reflected in the levels in foodstuffs (Bennett and Klich, 2003; Creppy, 2002; Skaug, 1999).

Since it is difficult to remove the mycotoxin once formed, the best way of control is prevention. However, many measures have been to minimize the occurrence of mold such as alternative methods of soil cultivation, development of mold resistant species and drying and storage techniques (Creppy, 2002).

Microbiological examination and mycotoxins concentration in baby foods must be routinely monitored at every step of manufacturing and marketing. Manufacturing companies involved in the production of foods for infants and young children should give an extreme importance to microbial and mycotoxin content. Enough information and training to minimize health hazards and to form the public policies should be made available for pediatricians, health-care personnel and parents. In order to protect public health, it is essential to keep contaminants at levels toxicological acceptable. Ultimately, surveillance should be continuous, widespread and must be conducted by the government and related ministries as the quality of the end product depend on the precise controlling at every step of the production.

Table 1: Microbial Load of the Infant Baby Food Samples

SAMPLES CODE	count x 10^3 (cfi	Microbial					
	hic count	Coliform count	Vibrio count	Lactic acid bacteria count	Salmonella /Shigella count	Yeast count	Fungi count
NMSB(cereal+ soymilk)	8.5±0.028 ^a	NG	NG	NG	1.0±0.01 ^b	NG	1.0±0.005 ^e
CMM(cereal based + milk)	4.96±0.057 ^d	NG	NG	NG	1.0±0.01 ^b	NG	4.0±0.05 ^b
CRM(Rice +milk)	$5.0{\pm}0.05^{d}$	NG	NG	NG	1.7±0.001 ^a	NG	1.0±0.1 ^e
CMF (Cereal+mixed fruit +milk)	$7.0{\pm}0.005^{b}$	$2.0{\pm}0.02^{b}$	NG	NG	NG	NG	6.0±0.01 ^a
CWM(Wheat + milk)	2.0 ± 0.01^{j}	$1.0{\pm}0.01^{a}$	NG	1.0±0.01 ^a	$1.0{\pm}0.01^{b}$	NG	2.0 ± 0.02^{d}
FCRB(Rice + milk)	$6.5 \pm 0.057^{\circ}$	NG	NG	NG	NG	NG	2.0 ± 0.02^{d}
FCW(Cereal + wheat)	$2.2{\pm}0.058^{i}$	NG	NG	NG	NG	NG	1.0±0.02 ^e
FMCW(Milk + wheat)	4.0 ± 0.01^{f}	NG	NG	$1.0{\pm}0.01^{a}$	1.0 ± 0.01^{b}	NG	3.0±0.01 °
FGWM(Milk + wheat)	1.0 ± 0.06^{k}	1.0 ± 0.01^{a}	NG	NG	NG	NG	1.0 ± 0.015^{e}
MW(Milk - wheat)	$2.4{\pm}0.01^{h}$	NG	NG	NG	NG	NG	2.0 ± 0.01^{d}
GCP(Cereal based)	2.7 ± 0.006^{g}	NG	NG	NG	NG	NG	3.0±0.02 ^a
GFC Cereal based)	1.0 ± 0.05^{k}	1.0±0.01 ^a	NG	NG	NG	NG	1.0±0.01 °
GFB Cereal based)	1.0±0.01 ^k	NG	NG	NG	NG	NG	1.0 ± 0.01^{e}

Values are means of three triplicates. Mean values in rows with different superscript letters are significantly different ($P \ge 0.05$), NG- NO GROWTH

	Table 2a. Occurrence of Aflatoxins in the infant baby f	foods and infants Formulae
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SAMPLES CODE	AFLATOXINS (µg/kg)							
		AFE	3 1		AFI	B ₂		
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD		
NMSB(cereal + soymilk)	2.79	4.67	3.6475±0.84	0.00	1.98	0.4950±0.9		
CMM(cereal based + milk)	0.00	3.68	1.5675±1.86	0.00	0.00	0.0000		

CRM(Rice +milk)	0.00	3.46	1.7050±1.46	0.00	0.00	0.0000
CMF (Cereal +mixed fruit +milk)	0.00	4.26	1.8600±2.19	0.00	0.00	0.0000
CWM(Wheat + milk)	0.00	3.05	1.5050±1.73	0.00	0.00	0.0000
FCRB(Rice + milk)	4.05	4.67	4.3600±0.35	0.09	0.19	0.1533±0.05
FCW(Cereal + wheat)	1.67	1.99	1.9025±0.15	0.00	0.00	0.0000
FMCW(Milk + wheat)	0.00	3.76	1.8775±2.16	0.00	0.00	0.0000
FGWM(Milk + wheat)	0.00	7.29	3.6425±4.20	0.00	2.75	1.3050±1.51
MW(Milk - wheat)	3.97	5.17	4.5700±0.68	0.06	0.14	0.1025±0.04
GCP(Cereal based)	0.89	0.97	0.9400±0.03	0.00	0.00	0.0000
GFC (Cereal based)	0.00	0.00	0.000	0.00	0.00	0.0000
GFB (Cereal based)	0.00	0.00	0.000	0.00	0.00	0.0000

Table 2b. Occurrence of Aflatoxins in the infant baby foods and infants Formulae

SAMPLES CODE	AFLATOXINS (µg/kg)							
		A	FG ₁		AFG ₂			
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD		
NMSB(cereal + soymilk)	0.00	0.03	0.0075±0.01	0.05	0.08	0.006±001		
CMM(cereal based + milk)	0.00	0.00	0.0000	0.00	0.09	0.03 ± 0.51		
CRM(Rice +milk)	0.00	0.00	0.0000	0.00	0.00	0.0000		
CMF (Cereal +mixed fruit +milk)	0.00	0.00	0.0325 ± 0.06	0.00	0.00	0.000		
CWM(Wheat + milk)	0.00	0.00	0.0000	0.00	0.00	0.0000		
FCRB(Rice + milk)	0.18	0.22	0.2000 ± 0.018	0.00	0.00	0.00		
FCW(Cereal + wheat)	0.00	0.00	0.0000	0.00	0.00	0.0000		
FMCW(Milk + wheat)	0.00	0.00	0.0000	0.00	0.00	0.0000		
FGWM(Milk + wheat)	0.00	0.00	0.0000	0.00	0.00	0.0000		
MW(Milk - wheat)	0.06	0.24	0.1500 ± 0.09	0.00	0.00	0.00		
GCP(Cereal based)	0.00	0.00	0.0000	0.00	0.00	0.0000		
GFC Cereal based)	0.00	0.00	0.0000	0.00	0.00	0.0000		
GFB Cereal based)	0.00	0.00	0.0000	0.00	0.00	0.0000		

SAMPLES CODE			AFLATOXI	NS (µg/kg)			
	AFM ₁		AFM_2				
		Maximum Minimum	Mean±SD	Minimum	Maximum	Mean±SD	
NMSB(cereal + soymilk)	1.66	5.49	4.0425±1.65	0.00	3.26	0.0000	
CMM(cereal based + milk)	0.00	4.96	2.18 ± 2.56	2.68	4.13	0.0000	
CRM(Rice +milk)	0.00	4.96	2.9150±2.14	0.00	0.00	0.0000	
CMF (Cereal +mixed fruit +milk)	0.00	12.88	3.9375±6.11	0.26	5.94	0.0000	
CWM(Wheat + milk)	0.00	3.17	1.5825 ± 1.82	0.00	0.00	0.0000	
FCRB(Rice + milk)	12.75	13.34	13.0450±0.34	0.00	0.00	4.13±0.01	
FCW(Cereal + wheat)	0.45	0.69	0.570±0.138	0.00	0.00	0.0000	
FMCW(Milk + wheat)	0.00	4.26	2.13±2.45	0.00	0.00	0.0000	
FGWM(Milk + wheat)	0.00	8.36	4.18±4.82	0.00	0.00	0.0000	
MW(Milk - wheat)	4.84	14.28	9.56±5.45	0.00	0.00	0.00	
GCP(Cereal based)	0.33	0.41	0.370±0.04	0.00	0.00	0.0000	
GFC Cereal based)	0.00	0.00	0.0000	0.00	0.00	0.0000	
GFB Cereal based)	0.00	0.00	0.0000	0.00	0.00	0.0000	

Table 3. Occurrence of Fumonisins ($\mu g/kg$) in the infant baby foods and infants Formulae

SAMPLES CODE FUMONISIN (µg/kg)						
	FB_1			FB ₂		
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD
NMSB(cereal + soymilk)	1.09	4.12	2.5625±1.46	0.28	0.44	0.360±0.09
CMM(cereal based + milk)	0.00	4.67	1.8625±2.28	0.00	0.11	0.055±0.06
CRM(Rice +milk)	0.00	4.28	2.2500±1.84	0.00	0.00	0.00
CMF (Cereal + mixed fruit + milk)	0.00	5.08	2.4925±2.87	0.00	0.00	0.00
CWM(Wheat + milk)	0.00	6.18	0.3450±0.69	0.00	0.00	0.00
FCRB(Rice + milk)	5.33	2.15	5.7550±0.60	0.00	0.00	0.360±0.09
FCW(Cereal + wheat)	1.87	5.47	2.0100±0.16	0.00	0.00	0.00
FMCW(Milk + wheat)	5.37	3.78	5.4200±0.05	0.00	0.00	0.00
FGWM(Milk + wheat)	0.00	6.43	1.8900±2.18	0.00	0.00	0.00
MW(Milk - wheat)	4.25	1.23	5.3400±1.25	0.00	0.00	0.0550±0.06
GCP(Cereal based) GFC Cereal based) GFB Cereal based)	1.08 0.00 0.00		1.1550±0.08 0.000 0.000	0.00 0.00 0.00	0.00 0.00 0.00	0.00 0.00 0.00

SAMPLES CODE	OCHRATOXINS					PATUI	PATULIN(µg/kg)			
	(µg/kg	μg/kg)					DAT			
	OTA			OTB	OTC	PAT				
	Minimum	Maximum	Mean ±SD	CONC.	CONC.	Minimum	Maximum	Mean±SD		
NMSB(cereal + soymilk)	0.18	2.28	0.7750±1.00	0.00	0.00	0.00	0.03	0.00		
CMM(cereal based + milk)	0.00	0.51	0.1850±0.24	0.00	0.00	0.00	0.05	0.007±0.015		
CRM(Rice + milk)	0.00	0.56	0.2750±0.25	0.00	0.00	0.00	0.14	0.012±0.025		
CMF (Cereal + mixed fruit + milk)	0.00	0.47	0.1500±0.22	0.00	0.00	0.16	0.21	0.057±0.069		
CWM(Wheat + milk)	0.00	0.49	0.1225±0.24	0.00	0.00	0.11	0.15	0.00		
FCRB(Rice + milk)	0.67	0.94	0.8050±0.15	0.00	0.00	0.07	0.19	0.185±0.028		
FCW(Cereal + wheat)	0.11	0.27	0.1900±0.09	0.00	0.00	0.00	0.00	0.00		
FMCW(Milk + wheat)	0.34	0.44	0.3900±0.05	0.00	0.00	0.00	0.00	0.130±0.023		
FGWM(Milk + wheat)	0.00	1.45	0.7250±0.83	0.00	0.00	0.00	0.00	0.00		
MW(Milk + wheat)	0.77	0.84	0.8050 ± 0.04	0.00	0.00	0.00	0.00	0.130±0.069		
GCP(Cereal based)	007	0.9	0.0800 ± 0.011	0.00	0.00	0.00	0.00	0.00		
GFC Cereal based)	0.00		0.00	0.00	0.00	0.00	0.00	0.00		
GFB Cereal based)	0.00		0.00	0.00	0.00	0.00	0.00	0.00		

Table 4 Occurrence of Ochratoxins (µg/kg) and Patulin (µg/kg) in the infant baby foods and infants Formulae

ABREVIATION

UNEP - Unite Nations Environmental Programme ICMSF- International Commission of Microbiological Specification for Food NAS – NRC - National Academy of Sciences -National Research Council. FNB - Food and Nutrition Board. FAO - Food and Agricultural Organization

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CONCLUSSION

Putting this results into consideration, it could be concluded that mycotoxin incidence in some samples selected from commonly consumed baby food in Nigeria, poses a serious public health problem at the moment.

REFERENCES

- 1. UNICEF <u>http://www.unicef.org/nutrition/index_breastfee</u> ding.html 1999.
- 2. WHO. Weaning Foods: Characteristics and Guidelines

http://www.wishh.org/nutrition/papers-publicati ons/weaning foods-nutrition perspective.pdf 2000

 Alvito PC, Eric A, Sizoo CM, Almeida M and van Egmond PH. Occurrence of Aflatoxins and Ochratoxin A in Baby Foods in Portugal. *Food Anal. Methods*, 2010; 3:22–30 DOI 10.1007/s12161-008-9064-x Shephard GS In: Barug D, Bhatnagar D, van Egmond HP, vander Kamp JW, van Osenbruggen WA, Visconti A. (eds) The mycotoxins factbook, food and feed topics.

Academic, The Netherlands. 2006. ISBN-10: 90-8686-006-0 and ISBN-13:978-90-8686-006-7

- 5. European Commission (2006a) Commission Regulation (EC) 401/ 2006 of 23 February 2006. Official Journal of the EuropeanCommunities L70/12-34
- European Commission Commission Regulation (EC) 401/ 2006 of 23 February 2006. Official Journal of the EuropeanCommunities L70/12-34. 2006a.
- Cowan ST Cowan and Steel's Manual for the Identification of Medicated Bacteria (3rd Edn). Cambridge University Press, London; 1985; 81-100.
- Holt JG, Krieg NR, Sneath PHA and Stanley JTW. Bergey's Manual of Determinative Bacteriology (9th edn). Williams and Wilkins, Tokyo. 1994.
- Sampson RA, Hocktra ES and Vampoerschol CA. Introduction to food-borne fungi, Onter Bereau Voor Shimei Culture. 1984;105-107.
- AOAC. Association of Official Analytical Chemists, Methods of Analysis of the Association of Official Chemists 3rd Edn. Washinghton DC. 1998.
- Stolof L and Friedman L. Information bearing Copeinhagen, Demark, pp: 175-81. on the hazard to man from aflatoxin ingestion. 25. Sage, L., S. Krivobok, E. Delbos, F. Seigle, Murands PAG BULL 1976;6: 21-32.
- 12. Duncan PB. New multiple range and multiple A production in grapes and musts from France". F-tests in Biometrics, 1956;**11**: 1-42
- International Commision of Microbiological Specification for Food,(ICMSF). Microorganisms in Food 1. Their significance and Methods of enumeration. 2nd Edn. University of Toronto Press, 1982;19 – 30.
- 14. USFDA. Sanitation of shellfish. Growing Areas and Seafood safety (F.E Ahmed) National Academic Press. Washington DC,1991
- **15.** Elegbede JA. Legumes. In: Nutritional Quality of Plant Foods, Osagie, A.U. and O.U. Eka

11/29/2012

(Eds.). Post Harvest Research Unit, Benin City Nigeria, 1998.

- 16. Prescott LM, Harley JP and Klein DA. Microbiology (6th edn.). McGraw –Hill companies, Inc., New York. 2005.
- 17. Sanders ME, Morelli L and Tompkins TA. Spore formers as human probiotics Bacillus, sporolactobacillus, and Brevi bacillus. *Comp. Rev. Food Sci. Food Safety* 2003;**2**: 101-110.
- Tatzel RW, Ludwig K, Schleifer H and Wall nofer PR. Identification of Bacillus Strains Isolated from milk and cream with classical and nucleic acid hybridization methods. *J. Dairy Res.* 1994;61: 529-535 (Medline).
- Vuorio RM, Andersson A, Johansson T, Honkanen busalski T and Salkinoja-salonen MS. Toxin producing Bacillus licheniformis in infant food. Poster presented at international conference on food borne pathogens, Detection and typing. The Hagne the Netherlands, 1998;20-21 April 2008.
- Gordon RE, Haynes WC and Hor –nay Pang C. The genns Bacillus, Sp. 3-14. In Agriculture Hand book no. 427, 1973. United States Department of Agriculture, Washington D. C.
- Beretta B, De Domenico R, Gaiaschi A, Ballabio C, Galli CL, Gigliotti C and Restani, P. Ochratoxin A in cereal-based baby foods: Occurrence and safety evaluation. *Food Addit. Contam.* 2002; 19: 70-75.
- 22. Nakajima M, Tabata S, Akiyama H, Itoh Y, Tanaka T, Sunagawa H, Tyonan T, Yoshizawa T and Kumagai S. Occurrence of aflatoxin M1 in domestic milk in Japan during the winter season. *Food Addit. Contam.* 2004;21: 472-478.
- Herrman JL and Walker R. Risk analysis of mycotoxins by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA), FNA/ANA 1999;23: 17-24. <u>http://www.fao.org/</u> waicent/faoinfo/economic/esn/jefca. htm.
- 24. Bennett JW and Klich M. Mycotoxins. Clin. Microbiol. Rev. 2003;16: 497-516.
- 25. Creppy EE. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol. Lett.* 2002;127: 19-28.
- 26. Skaug MA. Analysis of Norwegian milk and infant formulas for ochratoxin A. *Food Addit. Contam.* 1999;16: 75-78.