Occurrence of aflatoxins in cornbread (arepas) traded in Medellin, Colombia.

Dora Luz Duque Múnera

Trabajo de investigación para optar al título de Magister en Microbiología

Asesora
Luz Elena Cano Restrepo, PhD
Docente escuela de Microbiología, Universidad de Antioquia. Medellín, Colombia.

Universidad de Antioquia
Escuela de Microbiología
Medellín
2012

TABLA DE CONTENIDO

Pàg.

LISTA DE TABLAS

LISTA DE FIGURAS

Pág.

ABSTRACT

The occurrence of Aflatoxins in “arepas” (cornbread) from different zones of Medellín (Colombia) was investigated using a competitive direct enzyme-linked immunosorbent assay (CD-ELISA). The *arepa* is a cornbread without yeast, made from degermed corn andis a staple food of the Colombian population, particularly in the region known as the “Coffee Axis”. Sixty-nine samples were collected in supermarkets and retail storesfrom 16 geographical locations of Medellin, Colombia. Samples were grouped into two categories: (1) those with a registered sanitary code and (2) those without. Total aflatoxins were detected in 48/69 (69.6%) of samples, at concentrations ranging from 1.7 to 33.7ng/g. Fifteen samples (21.7%) contained total aflatoxins concentrations above 10ng/g that exceeded the maximum limit permitted by Colombian legislation. No significant differences were found between the concentrations of aflatoxins in samples that had a sanitary registration code and those which lacked it. The highest aflatoxin concentrations were found in *arepas* collected from the central-west and south-western zones of Medellin, ranging up 33.7ng/g and 27.1ng/g, respectively. Given that these areas also show the highest risk of hepatic cancer in the city of Medellin it should be determined whether chronic exposure to aflatoxins through frequent consumption of contaminated *arepas* increases the risk of developing this condition. Based on our findings we recommend that health authorities implement aflatoxin monitoring programmes for corn and corn-based products.

KEY WORDS:
Arepa, Aflatoxins, corn-based foods.

1. INTRODUCTION

Aflatoxins are a type of mycotoxin produced mainly by *Aspergillus flavus* and *A. parasiticus*, two common fungal contaminants of corn, peanut sand other commodities. Although approximately 20 aflatoxins have been identified, four of them *i.e*., aflatoxins B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2) are the most prevalent isoforms of this family. They occur naturally and are significant contaminants of a wide variety of foods *(17, 28)*.The high resistance of aflatoxins to food processing has been shown in a number of studies *(4, 8, 23)*

Aflatoxin B1 has been shown to be a potent genotoxic agent in many tests and there is also evidence of their carcinogenicity to humans*(16)*.Aflatoxin B1 was classified as Group 1 (*carcinogenic to humans*) by The International Agency for Research on Cancer (IARC) *(15)*.AFB_1 and related compounds (AFB_2, AFG_1 and AFG_2) may be the cause of some toxic hepatitis and hepatocellular carcinomas (HCC) seen in various parts of Africa and the Far East*(18, 19, 32, 33)*. Epidemiological studies have shown associations between aflatoxins and Reye syndrome or Kwashiorkor *(11, 24, 29)*. There are evidences on the association between aflatoxins and both growth retardation and spermatotoxic effects in humans*(27)*.

In Central America, Mexico and parts of the Andean zone, corn is a staple food of the population, especially in rural areas where it is grown for domestic use. In Central and parts of South America, corn crops are highly susceptible to fungal contamination and mycotoxin production due to the high humidity and temperatures encountered in this region. High levels of contamination of corn and corn-products by aflatoxins in Latin America have been demonstrated in several studies*(26)*. Most countries on this region have specific aflatoxins regulations. Although the maximum permissible levels for aflatoxins in foods vary between countries with different regulations, levels of 5-20 ppb are allowed for human consumption. The maximum levels for aflatoxins in Colombian foods are 10 ppb*(13)*;however, this regulation is not an official rule. MERCOSUR has set standard levels for aflatoxins in a number foods*(20)*.

Aflatoxins have been reported in Colombian field corn and some other agricultural products *(1, 2, 5, 7)*. However, there are only two reports dealing with contamination levels of corn-based foods. Diaz G.J. et al *(7)*found aflatoxins B1 (2.0-103.3ng/g) in 12.8% of 109

samples of corn and corn- based foods. In another survey, Arcila & Martinez*(3)*found low levels of aflatoxins in corn and arepas, contamination being associated with deficient quality control of the grains used in preparation of arepas. In Colombia, arepa is a corn-based product that is a food staple mainly in the "*EjeCafetero*" or Coffee Axis, the main coffee-growing area of the country. These results indicate the need for more data about contamination of this product.

Colombian *arepas* area type of corn bread without yeast and are made from degermed corn. The moistened corn is crushed to obtain the endosperm and the hulls and germs removed by adding water to the mixture. The endosperm is then cooked and milled to prepare dough. Small portions of this dough are made into balls and then flattened for toasting on both sides*(10)*.The present paper reports the results of a pilot survey on the occurrence and levels of aflatoxins in commercially produced cornbread (arepas) sold in different geographical sections from Medellin, Colombia.

2. MATERIALS AND METHODS

2.1 Cornbread samples.

Sixty-nine samples from commercially prepared packets of different *arepa* brands were purchased from retail stores or local supermarkets in 16 geographical sections (communes) of Medellin, Antioquia, Colombia, between February and May 2011 (Figure 1). The samples provided a random selection of the popular regional brands taken from store shelves. Forty-seven samples were of brands registered by the local sanitary authority, while the others did not carry a sanitary registration code.

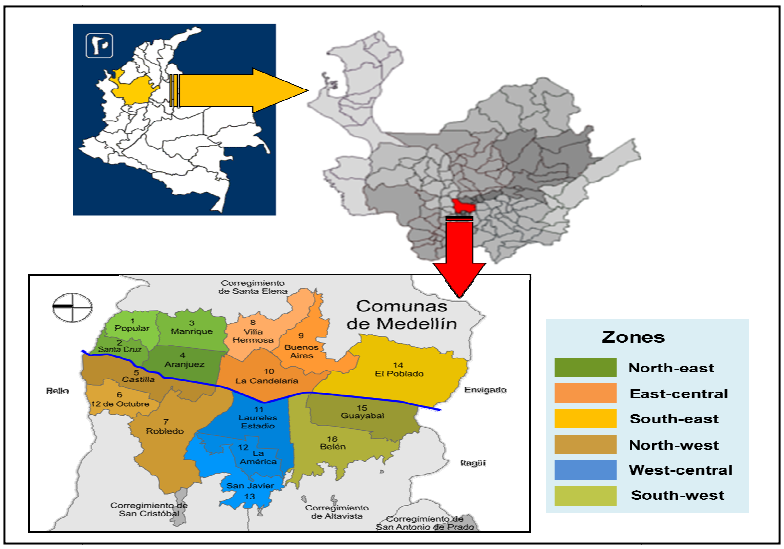

Figure 1. Geographical sections (communes) of Medellin, Colombia.

Care was taken in order to prevent the repetition of batches. The *arepas* used in this study were of the thin white cornbread type according to the FAO Latinfoods A027:Arepa designation *(9)*.Each sample consisted of five units of *arepa*, ground with a food processor to a uniform consistency prior to analysis.

2.2 Extraction and analysis.

Duplicate subsamples weighting 10g each were placed into 100-ml glass-stoppered Shott bottles. Methanol:water (7:3, vol:vol) extraction solvent (50 ml) was added to each flask which was then sealed and vigorously shaken for 3 min. The extract was filtered through Neogen filter syringe and a 5ml aliquot collected.

2.3 Aflatoxin determination.

Total aflatoxins levels were quantified using a CD-ELISA test kit (quantitative kit for aflatoxins, Veratox®, Neogen Corp., Lansing Mich.USDA-GIPSA 2008-011. AOAC License number 050901). Aflatoxin standards (0,5,15 and50ng/g, Veratox® aflatoxins test kit, Neogen Corp., Lansing Mich.) or sample extracts (100µl) were added to mixing wells containing 100 µl of peroxidase-conjugated aflatoxins. The sample or standard and conjugate solutions were mixed with a 12-channel micropipette, and 100 µl of this mixture transferred to the antibody-coated wells and incubated for 2 min at room temperature. Reagents were washed out from the antibody wells and 100 µl of an enzyme substrate added, mixed and incubated for 3 min. Development of the colour was halted by addition of a stop reagent. Aflatoxin concentrations were quantified by recording optical density readings at 650 nm using a Stat Fax microwell strip reader programmed with the Logit Log data regression program (Neogen Corporation).

2.4 Analytical quality control.

The assay was validated in–house for *arepa*by determination of spiking recovery, intra-assay variability, and linearity testing. Detection and quantification limits used in this method were providing by manufacturer, they were 1.4ng and 5ng of aflatoxins per g of food respectively. The Linearity test was studied by relating the responses (absorbance) with the analyte concentration. The model is given by the equation: $Logit\left(\frac{A}{A_0}\right) = a + b * \ln[C]$, where:$logit\left(\frac{A}{A_0}\right)$is the asymptote,$a$ is the intercept, b is the slope and $\ln[C]$is the dependent variable (concentration of interest$X$).The linear regression coefficient (R^2) of standard curve must be ≥ 0.98. Software Microsoft® Office Excel 2010 spreadsheet was used.The measurement Uncertainty (U) was estimated, it ranged from 2.53 to 8.59. The outcomes of the

uncertainty measurements were below the maximum standard uncertainty (U*f: 10.024*).The method is considered ‘Fitness-for-purpose’ approach according whit the Commission Regulation (EC) No 401/2006*(6)*.

2.5 Statistical analysis.

Statistical analyses were performed with SPSS 19.0 software. Differences between groups were calculated by non-parametric Mann-Whitney U-test for two independent samples, after evaluating homogeneity of variances with Levene’s test. The total number of values (n) used in the arepas was 69 (47 with sanitary registration codes and another 22 without). Differences between medians were considered significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Since no guaranteed aflatoxin-free cornbread sample was available, the concentration of naturally occurring aflatoxins was determined by the described method and found to be 7.6 ng/g. Astandard solution of aflatoxins was added to this control sample to obtain test samples spiked at three levels. Duplicate test portions of these spiked test samples were analyzed and recoveries were calculated after the values obtained for the control were subtracted from the levels found on the samples. The standard solution of aflatoxins for spiking samples was obtained from Micotox Ltda, Bogota, DC. It contained 0.4µg/mL of aflatoxins B1 + G1; 0.12µg/mL aflatoxins B2 + G2 in acetonitrile (Total aflatoxins 1.04µg/ml), one vial x 4 mL. From this vial was taken the volume needed to spike a control sample at three levels. They were processed after 24 h in order to ensure evaporation of the solvent. Recovery >80% was the quality criterion applied andmean recovery was 92.7% ± 5.8% (Table 1). Results are not corrected for recovery and showed the high degree of accuracy obtained using this system.

Table 1. Recovery of Aflatoxins AFB_1, AFG_1, AFB_2 and AFG_2 added to *arepas*

Total Aflatoxins			
Added, ng/g	Total found, ng/g	Net found, ng/g	Recovery %
0	7.6	0	-
0	8.2	0	-
5	12.3	4.4	88.0
5	12.1	4.2	84.0
20	27.2	19.3	96.5
20	26.8	18.9	94.5

40	46.2	38.3	95.8
40	46.8	38.9	97.2
		Average	92.7
		SD	5.4
		RSD%	5.8

Results from analyses of *arepas* from Medellin are shown in Table 2. Aflatoxins were detected in 48 samples (69.5% of the total analyzed) at concentrations which varied from 1.7 to 33.7ng/g. Among the positive samples 20 (29%) presented AFs at trace levels varying between 1.5ng/g and 4.9ng/g while 15 (21.7%) had concentrations above 10ng/g, exceeding the maximum limit permitted by Colombian legislation. No aflatoxins were detected in 21 samples (30.4%) (*i.e.*, concentration< 1.5ng/g).

Table 2. Total Aflatoxins levels on *Arepas*

Total Aflatoxins (ng/g)	Frequency	% Samples
< 1.5	21	30,4
1.5 – 4.9	20	29
5.0 – 10.0	13	18.8
10.1 – 20.0	11	15.9
>20.1	4	5.8
Total	69	100

Total aflatoxins ranged from 0 to 33.7ng/g. The mean quantity of AFs in *arepas* was 3.2 ng/g and the median was 0.6ng/g. No significant differences were found for concentrations of aflatoxins between samples having sanitary registration codes and those which did not carry them (Table 3). Moreover the highest aflatoxins concentrations were found in *arepas* collected from central-west and southwestern Medellin.

Table 3. Minimum (min), maximum (max), mean and standard deviation for Total Aflatoxinsin*arepas* with and without sanitary register code

White thin flat *arepas* (500g)						*Over positive samples AFs>5ng/g*				
	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD
With Sanitary register code										
Total Aflatoxinsng/g	0	33.7	6.2	3.4	8.1	5.1	33.7	13.6	11.8	8.8
Without Sanitary register code										
Total Aflatoxinsng/g	0	25.4	5.8	4.4	6.1	5.7	25.4	10.7	9.3	5.8

Medians for Total Aflatoxinas were not significant *P< 0.826*.

The lowest concentrations were in *arepas* from central-east and southeastern Medellin (Figure 2).

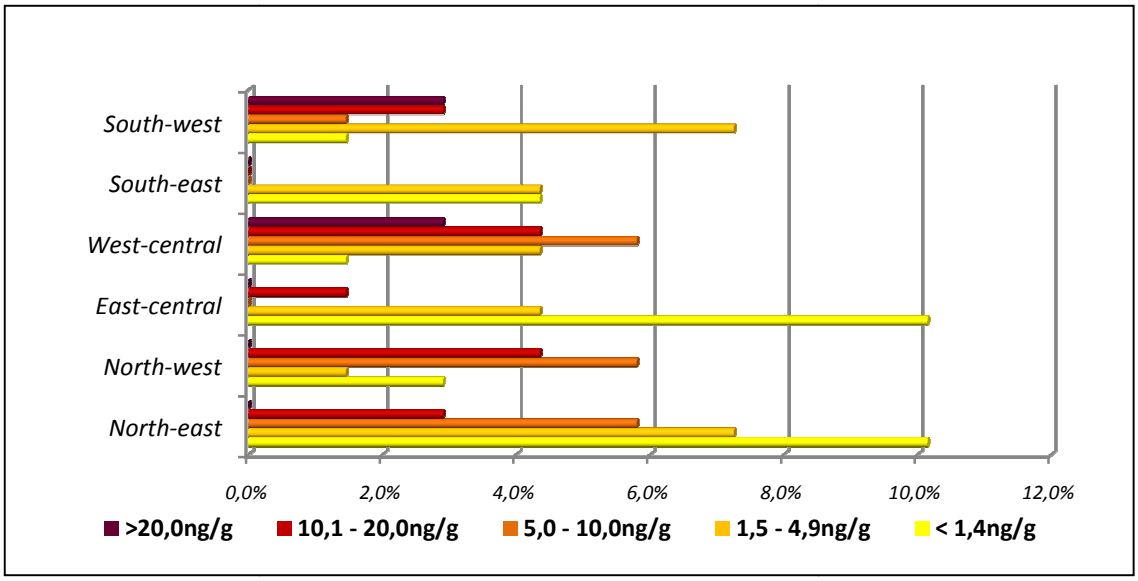

Figure 2. Distribution of Aflatoxins According Geographical Area.

The results obtained in the present trial are slightly different from the values obtained by Arcila & Martinez*(3)*, who observed that only 2% (2/276) of *arepa* samples were positive for AFs at levels >3ng/g. Diaz et al.*(7)*, found low occurrence of AFs in corn and Colombian corn-based foods (14/109), although the contamination levels found by them

were higher than those of the present study, concentrations ranging from 2.0-103.3ng/g (mean 17.3ng/g).

Aflatoxins have been reported to occur in corn-based thermally treated foods. Villa & Markaki*(30)* showed the presence of AFB1 in 56.3% of breakfast cereal samples from Athens market, Greece (mean 1.4 ng/g). Seven samples were found to be contaminated at levels higher than the EU limit (2 ng/g). In Spain, Ibañez-Vea M et al *(12)* showed lower levels of AFB_1 in corn-based breakfast cereal samples (<0.2µg/Kg) while Razzazi-Fazeli et al *(25)* found 2/11 (18%) corn snack samples from Indonesia to be contaminated. These snacks consisted of three industrial-produced and eight home-made snacks. The levels detected ranged from 6-12µg/Kg. Midio et al *(21)* found aflatoxins in 9.3% (30/322) of samples obtained from traditional Brazilian cooked food. Twenty-four of these samples contained aflatoxins levels above 20ng/g.

In Colombia, the permissible aflatoxins level in *arepas* is 10ng/g *(14)*, much higher that that set by the European Union which allows 4ng/g for total aflatoxins in transformed cereals. Based on this last value, 40.1% of the positives samples would be considered unsuitable for human consumption in the EU. Under Colombian national regulations only 27.5% of positive samples would be rejected, indicating deficient control practices for aflatoxins in companies that manufacture *arepas.*

Aflatoxin contamination of *arepas* from Medellin may pose a significant risk for human health in this region. The Coffee Axis and Colombian department of Antioquia had the highest rates of death by cancer in 2005. Liver cancer ranked fifth in importance for both men and women at 4.9/100000 and 6.9/100000 respectively *(22)*. In Medellin specifically the mortality rate by cancer increased from 113/100000 in 2005 to 118.6/100000 in 2006. Mortality by liver cancer was the third most frequent in the city (10.2%). With respect to cancer mortality rates per commune, highest values were recorded for Las Americas, La Candelaria, Laureles, Buenos Aires, Guayabal and Belen, while low risk was found for the communes of Popular, Doce de Octubre, Poblado, Villa Hermosa and Santa Cruz. Deaths from cancers of the liver and biliary tract were highest in the La Candelaria and Laureles communes *(31)*.

The results show that highest aflatoxins concentrations are present in *arepas* consumed by the populations that are currently experiencing the highest mortality rate due to hepatic cancer. However, a cause and effect relationship has not been demonstrated and additional studies are necessary in order to establish the possible consequences of

aflatoxins consumption by these populations. This should involve evaluating levels of human exposure to aflatoxins by means of biomarkers to demonstrate the real impact of exposure to these toxins and disease.

4. CONCLUSION

The results of this study indicate that may be a risk of human exposure to aflatoxins through the consumption of *arepas*, even after physical and thermal processing. There appears to be a need for adoption of selective grain quality practices by *arepa* manufacturers. Furthermore sanitary authorities should establish avoidance and monitoring programs for aflatoxins in corn and corn-based products in order to avoid consumption of these micotoxins by a population with a high risk from cancer*(31)*.

ACKNOWLEDGEMENTS

The authors are grateful to the *Proyecto Sostenibilidad Grupos A1*, Universidad de Antioquia for financial support; Microgest Representaciones (Medellin, Colombia) for supplying the Stat Fax microwell strip reader and reference material; and to Beatriz E. Sanchez for statistical analysis of the data.

REFERENCES

1. Acuña, C., G. Díaz, and M. Espitia. 2005. Aflatoxinas en maiz: Reporte de caso en la costa Atlanica Colombiana*.Rev. Med. Vet. Zoot.* 52:156:162.

2. Arango, M., and L. Marín. 1998. Aflatoxinas en salud humana*.Med. lab*. 8:161-9.

3. Arcila, M., and O. L. Martinez. 2006. Factores relacionados con la presencia de aflatoxinas en la fabricación de la arepa delgada de maíz blanco en dos industrias de Medellin y su área metropolitana. 2005*.In*, Facultad Nacional de Salud Publica, vol. Maestria en Salud Publica. Universidad de Antioquia, Medellin.

4. Bullerman, L. B., and A. Bianchini. 2007. Stability of mycotoxins during food processing*.Int J Food Microbiol*. 119:140-6.

5. Carpintero de Jimeno, M., N. Peña Beltrán, and A. López de Herrera. 1986. Identificación y estimación del nivel de contaminación de aflatoxina en algunos productos agrícolas de consumo humano y animal*.Instituto Colombiano Agropecuario*. Bogota.

6. Communities., T. C. o. t. E. 2006. Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs*.In* T.c.o.t.E. communities (ed.), vol. (EC) N° 401/2006. Official Journal of the European Union, Brussels.

7. Diaz, G. J., N. S. Perilla, and Y. Rojas. 2001. Occurrence of aflatoxins in selected colombian foods*.Mycotoxin Research*. 17:15-20-20.

8. Elias-Orozco, R., A. Castellanos-Nava, M. Gaytan-Martinez, J. D. Figueroa-Cardenas, and G. Loarca-Pina. 2002. Comparison of nixtamalization and extrusion processes for a reduction in aflatoxin content*.Food Addit Contam*. 19:878-85.

9. Food and Agriculture Organization (FAO). 2002. Tabla de Composición de Alimentos de América Latina. A027 Arepa. p. Arepa, maíz blanco, plana delgada. *In* FAO/LATINFOODS, Colombia.

10. Food and Agriculture Organization of the United Nations (FAO). 1993. Maize in human nutrition. Rome.

11. Hendrickse, R. G. 1991. Kwashiorkor: The hypothesis that Incriminates aflatoxins.*Pediatrics*. 88:376-379.

12. Ibáñez-Vea, M., R. Martínez, E. González-Peñas, E. Lizarraga, and A. López de Cerain. 2011. Co-occurrence of aflatoxins, ochratoxin A and zearalenone in breakfast cereals from Spanish market.*Food Control*. 22:1949-1955.

13. Instituto Colombiano de Normas Tecnicas y Certificacion (ICONTEC). 2006. Norma técnica Colombiana NTC 3581 Industrias Alimentarias. Nivel Máximo Permitido de Aflatoxinas en los Alimentos.*In* C.P.A. Diversos (ed.) Editorial Instituto Colombiano de Normas Técnicas y Certificación.

14. Instituto Colombiano de Normas Técnicas y Certificación (ICONTEC). 2007. Norma técnica Colombiana NTC 5372, industrias alimentarias. Arepas de maíz refrigeradas. Especificaciones de producto.*In* Comité Productos Alimenticios Diversos (ed.) Editorial Instituto Colombiano de Normas Técnicas y Certificación.

15. International Agency for Research on Cancer (IARC). 1993. Some Naturally Occurring Substances:Food Items and Constituents,Heterocyclic Aromatic Amines and Mycotoxins. p. 245. *In*, Monograph on the Evaluation of Carcinogenic Risk to Human, vol. 56. World Health Organization, International Agency for Research on Cancer, Lyon, France.

16. JECFA. 1998. Forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Safety Evaluation of Certain Food Additives and Contaminants.*In* Aflatoxins (ed.) World Health Organization, Geneva.

17. Jelinek, C. F., A. E. Pohland, and G. E. Wood. 1989. Worldwide occurrence of mycotoxins in foods and feeds--an update.*J Assoc Off Anal Chem*. 72:223-30.

18. Kensler, T. W., B. D. Roebuck, G. N. Wogan, and J. D. Groopman. 2011. Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. *Toxicol Sci*. 120 Suppl 1:S28-48.

19. Liu, Y., and F. Wu. 2010. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment.*Environ Health Perspect*. 118:818-24.

20. Mercosur. 2002. Reglamento técnico mercosur sobre límites máximos de aflatoxinas admisibles en leche, maní y maíz.*In*, vol. MERCOSUR/GMC/RES. Nº 25/02., Buenos Aires, Argentina.

21. Midio, A. F., R. R. Campos, and M. Sabino. 2001. Occurrence of aflatoxins B1, B2, G1 and G2 in cooked food components of whole meals marketed in fast food outlets of the city of Sao Paulo, SP, Brazil.*Food Addit Contam*. 18:445-8.

22. Ochoa-Jaramillo, F. L., and L. P. Montoya-Vélez. 2009. Cancer mortality in Colombia in 2005.*Rev. fac. med. unal*. 57:304-315.

23. Park, D. L. 2002. Effect of processing on aflatoxin.*Adv Exp Med Biol*. 504:173-9.

24. Peraica, M., B. Radic, A. Lucic, and M. Pavlovic. 1999. Toxic effects of mycotoxins in humans.*Bull World Health Organ*. 77:754-66.

25. Razzazi-Fazeli, E., C. Noviandi, S. Porasuphatana, A. Agus, and J. Böhm. 2004. A survey of aflatoxin B1 and total aflatoxin contamination in baby food, peanut and corn products sold at retail in Indonesia analysed by ELISA and HPLC.*Mycotoxin Research*. 20:51-58-58.

26. Resnik, S., M. L. Costarrica, and A. Pacin. 1995. Mycotoxins in Latin America and the Caribbean.*Food Control*. 6:19-28.

27. Shuaib, F. M., J. Ehiri, A. Abdullahi, J. H. Williams, and P. E. Jolly. 2010. Reproductive health effects of aflatoxins: a review of the literature. *Reprod Toxicol*. 29:262-70.

28. Sweeney, M. J., and A. D. Dobson. 1998. Mycotoxin production by Aspergillus, Fusarium and Penicillium species.*Int J Food Microbiol*. 43:141-58.

29. Tchana, A. N., P. F. Moundipa, and F. M. Tchouanguep. 2010. Aflatoxin contamination in food and body fluids in relation to malnutrition and cancer status in Cameroon.*Int J Environ Res Public Health*. 7:178-88.

30. Villa, P., and P. Markaki. 2009. Aflatoxin B1 and ochratoxin A in breakfast cereals from athens market: Occurrence and risk assessment.*Food Control*. 20:455-461.

31. Vélez, L. P. M., and F. L. O. Jaramillo. 2008. Epidemiología de la mortalidad por cáncer en Medellín, 2006.*Comité Científico*. 3:50.

32. Wild, C. P., and P. C. Turner. 2002. The toxicology of aflatoxins as a basis for public health decisions.*Mutagenesis*. 17:471-481.

33. Williams, J. H., T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. Jolly, and D. Aggarwal. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions.*Am J Clin Nutr*. 80:1106-22.