African Journal of Biotechnology Vol. 11(78), pp. 14354-14360, 27 September, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.324
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Occurrence of mycotoxins and associated mycoflora in peanut cake product (kulikuli) marketed in Benin

Euloge S. Adjou[1], Boniface Yehouenou[1], Codjo M. Sossou[2], Mohamed M. Soumanou[1]* and Comlan A. de Souza[3]

[1]Unit of Research in Enzyme Engineering and Food, Laboratory for Study and Research in Applied Chemistry, Polytechnic School of Abomey-Calavi, University of Abomey, 01 P.O.B: 2009, Cotonou, Benin.
[2]Direction of Quality Promotion and Packaging of Agricultural Products, Ministry of Agriculture, Livestock and Fisheries, P.O.B: 362 Cotonou, Benin.
[3]Laboratory of Microbiology and Quality Control of Foodstuffs, High School of Biological and Food Technology, University of Lome, P.O.B: 1515, Lome, Togo.

Accepted 8 May, 2012

The detection of spoilage fungi and mycotoxins contamination in peanut cake product, popularly called ''kulikuli'' was investigated in this study. Forty five major markets were sampled and peanut cake products were analyzed for aflatoxins and ochratoxin A contaminations, and associated mycoflora. Total coliform count ranged between 1.6 × 10^1 and 14.0 × 10^2 CFU g^{-1}, while the fungal count was between 1.0 to 8.1 × 10^2 CFU g^{-1}. Bacteria isolated from peanut cake product were *Eschericha coli*, *Klebsiella spp.* and *Clostridium spp.* The fungal isolates include *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. being the dominant microflora in decreasing frequency of occurrence. High concentrations of aflatoxins were detected. They were between 25.54 to 455.22 µg/kg for AFB_1, 33.94 to 491.20 µg/kg for AFB_2, 0.41 to 100.33 µg/kg for AFG_1 and 22.04 to 87.73 µg/kg for AFG_2. Ochratoxin A concentrations ranged between 0.3 and 2 µg/kg. The coexistence of aflatoxins and ochratoxin A (OTA) in peanut was also established. The results show that peanut cake product sold in market was highly contaminated and therefore unacceptable for human nutrition.

Key words: Peanut cake, fungi, aflatoxins, ochratoxin A, liquid chromatography mass spectrometry (LC/MS/MS).

INTRODUCTION

Peanut cake products are one of the most important foods in the diet of the population, especially in rural areas. It is made from peanut after oil extraction. Previously, peanuts were exported from Benin, but nowadays it is consumed locally and also used in the preparation of food (peanut cake products) and for oil extraction. Peanut is one of the most important staple feed for the majority of the Benin population (Elisha, 2004; Egal et al., 2005; Jolly et al., 2009; Honfo et al., 2010). Compared to other countries in western Africa such as Senegal and Nigeria, peanut is an important source of food and constitutes an inexpensive source of protein, fat, minerals and vitamins in the diets of rural populations, especially children. It is consumed in the boiled or roasted form, and also as peanut cake product (‘kulikuli’) (Bankole et al., 2005). However, the grain is vulnerable to contamination by mycotoxigenic fungi which include *Aspergillus*, *Fusarium* and *Penicillium*. Peanut contamination by fungi does not only reduce its quality but may also lead to mycotoxin production (Sultan and Magan, 2010).

Mycotoxins are toxic secondary metabolites produced by fungi in agricultural products that are susceptible to mould infestations (Wagacha and Muthomi, 2008;

*Corresponding author. E-mail: msoumanoufr@yahoo.fr.

Morenoa et al., 2009). Aflatoxins B1, B2, G1, and G2 are produced by some strains of *Aspergillus flavus, A. parasiticus* and *A. nomius* (Cotty and Bhatnagar, 1994; Wild, 1996; Cardwell and Cotty, 2002). Toxins are produced during production, harvest, transportation, storage and food processing (Murphy et al., 2006). Recently, additional new aflatoxin-producing species was isolated from peanuts in Argentina (*A. arachidicola* sp. nov. and *A. minisclerotigenes* sp. nov. (Pildain et al., 2008).

Mycotoxin attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Hell et al., 2000, 2003, 2008; Fandohan et al., 2005; Wagacha and Muthomi, 2008). Mycotoxins are known to be hazardous to the health of humans, in some cases directly causing illness and even death. Aflatoxins are implicated in liver cancer (JECFA, 1998; Wild and Hall, 2000). It has been reported to impair childhood growth in children from Benin and Togo (Gong et al., 2002). International Agency for Research on Cancer recognized aflatoxin B1 as a Class 1 human carcinogen (IARC, 1993) and about 4.5 billion people in developing countries are chronically exposed to the uncontrolled amounts of aflatoxins (Williams et al., 2003). In Benin, several studies on contamination of peanut with toxin have been left out (Egal et al., 2005; Jolly et al., 2009; Ediage et al., 2011). At the farm level, contaminated peanut may appear like normal kernels without any outward physical signs of fungal infection. Actually, there are a lot of advancements in analytical methods for aflatoxins; however, investigation methods by biomarker were also used in order to evaluate the level of contamination in the population (Gong et al., 2002). The aflatoxin contamination in peanut has been associated with drought combined with high temperature, as well as insect injury (Betran and Isakeit, 2003; Craufurd et al., 2006).

Poor harvesting practices, improper storage and less than optimal conditions during transport and marketing of peanut cake products can also contribute to fungal growth and proliferation of mycotoxins (Bankole et al., 2005; Wagacha and Muthomi, 2008; Mutegi et al., 2009). In food manufacturing, destruction of mycotoxins by conventional food processing is difficult because they are typically highly resistant (Murphy et al., 2006).

The present work aimed to determine the level of aflatoxins and associated fungi species in peanut cakes product marketed in Benin.

MATERIALS AND METHODS

Collection of samples

Samples of peanut cake product (kulikuli) were collected in the main markets of 14 localities (the major sales depot of kulikuli) in Benin. A total of 45 different markets were investigated, and samples were purchased from five different sellers in each market, and were mixed together to give a composite samples from each market which were used for the analysis. The samples were later ground in a blender and stored at 4°C until analysis. For each parameter, sample determinations were made in triplicate. Peanut cake products collected from markets were obtained through peanut processing. The processing of peanut to peanut cake product was performed as follows: peanuts were ground and pound until a smooth paste was formed; water was then added to the paste and mixed thoroughly, and this resulted in a separation of phases. The oily layer (upper phase) was scooped away. Enough water was added to remove most of the oil. The processed peanut was later shaped into balls and fried in oil, until a fairly brown color was obtained (Figure 1). Spices could also be added just before frying. These peanut balls (peanut cake product) were later kept at room temperature and marketed.

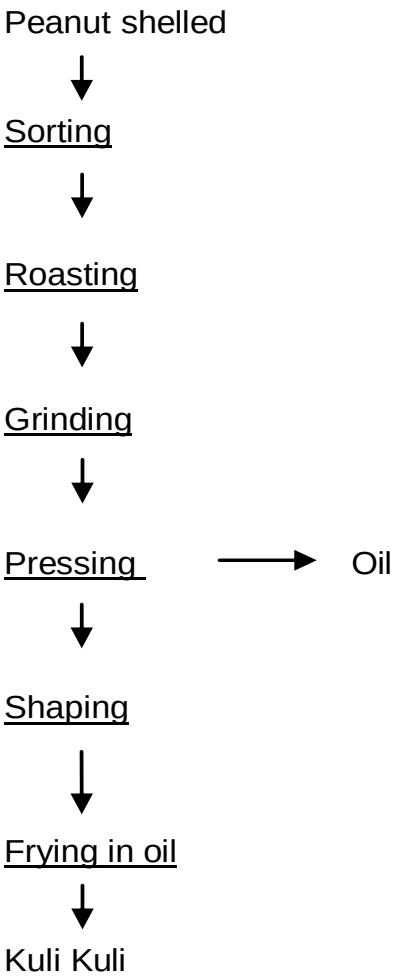

Figure 1. Processing of kulikuli marketed in Benin.

Microbiological analysis

For microbiological analysis, 25 g of each sample and 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated

at 30°C for 72 h. Desoxycholate was used for the total coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using Eosine methylene blue (EMB) medium. Tryptone sulfite neomicin agar was used for anaerobic sulfito-reducer (ASR) count, and tubes were incubated at 37°C for 24 h. After incubation, the number of colonies was tracked, using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, bearing in mind the factors of dilution. All media used for microbiological analysis were prepared as indicated by the manufacturer.

Fungal isolation and identification

The isolation of fungi from samples was performed using dilution plating method. 10 g of each peanut cake sample were added separately to 90 ml of sterile water containing, 0.1% peptone water. This was thoroughly mixed to obtain the 10^{-1} dilution. Further, 10-fold serial dilutions up to 10^{-4} were made. 1 ml volume of each dilution were separately placed in Petri dishes, over which, 10 to 15 ml of potato dextrose agar amended with 60 µg/ml chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days. Fungal isolates from PDA were sub-cultured on malt extract agar (MEA), and identification was carried out by using a taxonomic schemes primarily based on morphological characters, using the methods described by Singh et al. (1991).

Determination of aflatoxins in peanut by screening test

A screening test based on monoclonal antibodies was experimented in triplicate, using commercial kits (AFLACARD® Total) manufactured by R-Biopharm Rhone Limited. Analysis methodologies were performed strictly as indicated by the manufacturer (extraction, purification, concentration and detection). This screening procedure is intended to serve as an indicator of the presence of aflatoxin at levels of 4 ppb.

Quantitative mycotoxins analysis

The LC-MS/MS method used in this study for quantitative mycotoxins analysis was similar to those optimized and validated recently by Ediage et al. (2011).

Reagents and Preparation of standard solutions

All reagents (potassium chloride, phosphoric acid, hydrochloric acid) were of PA grade. All solvents (methanol, acetonitrile, propanol-2-ol, n-hexane and chloroform) were of HPLC grade. Double demineralized water was used for the preparation of all aqueous solutions. Standard toxins, AFB1, AFB2, AFG1 and AFG2 and ochratoxin A (OTA) were supplied by Sigma Chemicals. Standard solutions AFB1, AFB2, AFG1, AFG2 and OTA were prepared by dissolving 10 mg of AFB1, AFB2, AFG1 and AFG2 in 1 ml of methanol.

Samples preparation

All samples were solvent extracted and prepared, using previously published method for aflatoxins B1, B2, G1, G2 and ochratoxin A (Ediage et al., 2011).

Chromatographic conditions

The column used was a 150 × 2.1 mm i.d., 5 µm symmetry RP-18, with a 10 × 2.1 mm i.d. sentry guard column of the same material (Waters, Zellik, Belgium). Mobile phases were methanol/5 mmol/l ammonium formate solution in water (20:80, v/v) (mobile phase A) and methanol/5 mmol/l ammonium formate solution in water (90:10, v/v) (mobile phase B). The flow rate was 0, 3 ml/min and the injection volume was 20 µl.

Mass spectrometry conditions

Detection and quantification were performed with a Waters Acquity UPLC apparatus coupled to a Micromass Quattro Micro triple quadrupole spectrometer (Waters, Milford, MA, USA). The instrumental control and data processing utilities included the use of the Masslynx 4.1 software. The mass spectrometer analysis were carried out, using selected reaction monitoring (SRM) channels in positive electrospray ionization (ESI+) mode. The following were the instrumental settings: source and desolvation temperatures of 150 and 350°C, respectively; capillary voltage of 3.2 kV; and cone nitrogen and desolvation gas flows of 200 and 500 Lh^{-1}, respectively. The MS parameters for each mycotoxin were similar to those optimized and reported by Monbaliu et al. (2008).

Statistical analysis

Experiments were performed in triplicate, and data analyzed were mean subjected to one-way ANOVA. Means were separated by the Tukey's multiple range test when ANOVA was significant ($P < 0.05$) (SPSS 10.0; Chicago, IL, USA).

RESULTS AND DISCUSSION

Results of the microbiological analysis of the peanut cake samples from markets are given in Table 1. The mean total bacterial count was higher than 10^4 CFU g^{-1}, for all analyzed samples. They ranged between 5.4×10^4 and 7.2×10^6 CFU g^{-1}. Total coliform count ranged between 1.6×10^1 and 14.0×10^2 CFU g^{-1}, while the fungal count was between 1.0 to 8.1×10^2 CFU g^{-1}. Bacteria isolated from peanut cake were *Eschericha coli, Klebsiella spp.* and *Clostridium spp.* The fungal isolates included *Aspergillus flavus, Aspergillus parasiticus Aspergillus spp., Fusarium spp.,* and *Penicillium spp.* being the dominant microflora in decreasing sequential order (Table 2). Table 3 shows the result of aflatoxins screening test, obtained using commercial kits (AFLACARD® Total) and the results after LC-MS/MS analysis. All samples showed aflatoxin B_1 concentration between 25.54 and 455.22 µg/kg. The OTA and different types of aflatoxins (AFB_1, AFB_2, AFG_1, and AFG2) content in peanut cake were presented respectively in Tables 4 and 5.

Peanut cake products are good sources of proteins (Akano and Atanda, 1990; Bankole et al., 2005). However, the results obtained from microbial analysis of peanut cakes product show that they were contaminated with microorganisms of public health concern as for other commodities marketed in the same conditions (Aissi et

Table 1. Microbiological quality control in peanut cake (UFC/g) (N = 45).

Locality investigated	Total bacterial count	Total coliforms count	Faecal coliforms count	*E. coli* count	A.S.R spores count	Mould and yeast count	*S. aureus* count
Adjarra (n = 3)	1.4×10^{6a}	1.15×10^{2a}	1.0×10^{2a}	08^{a}	00	4.0×10^{2a}	00
Ouando (n = 3)	8.8×10^{5a}	2.0×10^{2a}	1.5×10^{2a}	12^{a}	00	1.4×10^{2a}	00
Dantokpa (n = 3)	6.4×10^{4b}	1.6×10^{2a}	1.3×10^{2a}	14^{a}	02^{a}	8.1×10^{2a}	00
Fidjrossè (n = 3)	8.2×10^{4b}	2.5×10^{2a}	1.5×10^{2a}	08^{a}	02^{a}	1.2×10^{2a}	00
Pahou (n = 3)	7.7×10^{4b}	1.65×10^{2a}	1.0×10^{2a}	10^{a}	00	3.5×10^{2a}	00
Ouidah (n = 3)	9.5×10^{4b}	7.5×10^{2a}	4.5×10^{2a}	07^{a}	04^{a}	1.9×10^{2a}	00
Comè (n = 3)	7.2×10^{6a}	4.5×10^{2a}	2.3×10^{2a}	07^{a}	04^{a}	1.0×10^{2a}	00
Bohicon (n = 4)	6.9×10^{5a}	1.6×10^{1b}	1.1×10^{1b}	09^{a}	00	7.1×10^{2a}	00
Abomey (n = 4)	8.7×10^{5a}	4.5×10^{2a}	2.2×10^{2a}	10^{a}	00	2.9×10^{2a}	00
Covè (n = 4)	6.7×10^{6a}	2.0×10^{2a}	1.0×10^{2a}	14^{a}	00	7.3×10^{2a}	00
Perma (n = 3)	7.3×10^{4b}	3.0×10^{1b}	1.0×10^{1b}	06^{a}	00	5.2×10^{2a}	00
Tigninti (n = 3)	5.4×10^{4b}	7.5×10^{1b}	4.2×10^{1b}	08^{a}	00	6.3×10^{2a}	00
Santa (n = 3)	9.7×10^{4b}	11.0×10^{2a}	5.0×10^{2}	10^{a}	00	6.9×10^{2a}	00
Ourbouga (n = 3)	6.1×10^{4b}	14.0×10^{2a}	11×10^{2}	07^{a}	00	5.2×10^{2a}	00
European criteria (2005)	*	> 10	1	*	0	*	> 10

*, Absence of criterion. Values are means. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 2. Moulds isolated in peanut cake product (kulikuli) marketed in Benin (N = 45).

Mould	Frequency (%)
Aspergillus flavus	27.5
Aspergillus parasiticus	12.3
Aspergillus spp.	1.7
Fusarium spp.	25
Penicillium spp.	25
Others	8.5

N, Number of samples.

Table 3. Comparative aflatoxins content using Screening and Chromatographic methods.

Locality investigated	AFLACARD® total	AFB1 (µg/kg)
Adjarra (n = 3)	Detected	367.54^{a}
Ouando (n = 3)	Detected	384.24^{a}
Dantokpa (n = 3)	Detected	455.22^{a}
Fidjrossè (n = 3)	Detected	204.25^{b}
Pahou (n = 3)	Detected	422.93^{a}
Ouidah (n = 3)	Detected	240.18^{b}
Comè (n = 3)	Detected	215.12^{b}
Bohicon (n = 4)	Detected	133.78^{b}
Abomey (n = 4)	Detected	259.40^{b}
Covè (n = 4)	Detected	84.75^{c}
Perma (n = 3)	Undetected	36.97^{c}
Tigninti (n = 3)	Undetected	12.18^{c}
Santa (n = 3)	Undetected	25.54^{c}
Ourbouga (n = 3)	Undetected	33.54^{c}

Values are mean. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

al., 2009). The high total bacterial and coliform count may have been as a result of the low level of hygiene maintained during the processing and sale of peanut cake product. This includes the handlers, quality of ingredients used for dressing and the utensils. The open-air exposure of products while they were displayed for sale can also serve as source of contamination. The detection of *Escherichia coli* and *Klebsiella* spp., which are enteric bacteria, confirmed the poor hygienic practice among handlers of products. The isolation of coliforms and other food pathogens (*Clostridium spp.*) from kulikuli, pose a serious threat to food safety, due to the fact that kulikuli are ready to eat foods, which are consumed without further processing. Similar results were found on the other street foods. The germs most identified in these foods were mainly *Staphylococci* and enterobacteria. According to Food and Agriculture Organization (FAO)/ World Health Organization (WHO) (1998), epidemological data in hospital showed a prevalence of 19% of diarrheal disease worldwide and bacterial diarrhea was estimated between 20 and 70% of the cases. The causes were related to poor hygiene found in the assessment of hazards and identification of critical points in the food processing chain (Leclerc et al., 2002).

Table 4. Ochratoxin A contents in peanut cakes (µg/kg).

Locality investigated	OTA content
Adjarra (n = 3)	0.8[a]
Ouando (n = 3)	< 0.3
Dantokpa (n = 3)	0.3[a]
Fidjrossè (n = 3)	< 0.3
Pahou (n = 3)	< 0.3
Ouidah (n = 3)	2.0[b]
Comè (n = 3)	< 0.3
Bohicon (n = 4)	< 0.3
Abomey (n = 4)	< 0.3
Covè (n = 4)	< 0.3
Perma (n = 3)	0.4[a]
Tigninti (n = 3)	< 0.3
Santa (n = 3)	0.7[a]
Ourbouga (n = 3)	< 0.3

Values are means. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

The results of aflatoxins analysis using kits AFLACARD® show the presence of aflatoxins in peanut cake product, with a limit of detection of 4 ppb. For accurate quantification, the use of more efficient analytical methods is required and LC-MS/MS technique revealed very high levels of aflatoxins. The levels were 25.54 to 455.22 µg/kg for AFB1, 33.94 to 491.20 µg/kg for AFB2, 0.41 to 100.33 µg/kg for AFG1 and 22.04 to 87.73 µg/kg for AFG2, indicating that the preservation methods used by traders promote the growth of fungi and aflatoxin production in ''kuliklui'' or peanut from which the cake were made was infected by toxinogenic fungi, especially *Aspergillus flavus* and *Aspergillus parasiticus.* However, Ogunsanwo et al. (2004) has reported that positive correlations were between loss of aflatoxins in the peanut seeds and the roasting conditions. Indeed, seeds dry-roasted at 140°C for 40 min resulted in 58.8 and 64.5% reductions in AFB1 and AFG1. Those roasted at 150°C for 25 min resulted in 68.5 and 73.3% reductions in AFB1 and AFG1, respectively. Roasting at 150°C for 30 min led to 70.0 and 79.8% reductions in AFB1 and AFG1, respectively. Siwela et al. (2011) also underlined the role of processing on potential reduction of toxin in peanut butter.

This high contamination level is also similar to those obtained by other workers in Nigeria (Akano and Atanda, 1990; Adebesin et al., 2001). Other works have revealed high levels of aflatoxins in Nigerian dry-roasted groundnuts. Aflatoxin B1 was found in 64.2% of samples, aflatoxins B2, G1 and G2 were detected in 26.4, 11.3 and 2.8% of the samples, respectively (Bankole et al., 2005). These results are also in agreement with those of Fandohan et al. (2005) on the study of mycotoxin contamination in cereals during storage and those of Ediage et al. (2011). Higher levels of aflatoxins have also been reported in other areas of the world (Nigeria: 20 to 455 µg/kg; Mozambique: 3 to 5500 µg/kg; Pakistan: 24 to 800 µg/kg; and Brazil: 5 to 22500 µg/kg) (Hüni et al., 1990).

According to Pittet (1998), the mycotoxins produced by *Aspergillus* spp. are of greatest significance in peanuts; and peanut products include aflatoxins and ochratoxin A (OTA). In this study, the maximum amount of OTA detected in peanut cake products is lower than European Community limits (5 ng/g in raw cereal and 3 ng/g in processed food) (European Community, 2006).

The coexistence of aflatoxins and ochratoxin A in peanut cake product should be taken into consideration as claimed by the European community (CEC, 1998). This is particularly important in regard to possible synergism and additive effects of these mycotoxins. Such co-contamination has been previously observed with other food samples, such as wheat (Vrabcheva et al., 2000), breakfast cereal (Pfohl-Leszkowicz et al., 2004; Molinie et al., 2005) or olives (El-Adlouni et al., 2006).

Therefore, special attention should be paid to certain critical points in the chain of processing, including sorting. Indeed, according to Hell et al. (2010), sorting can remove a major part of aflatoxin contaminated units, but levels of mycotoxins in contaminated commodities may also be reduced through food processing procedures that may involve processes such as washing, wet and dry milling, grain cleaning, dehulling, roasting, baking, frying, and extrusion cooking. These methods and their impact on mycotoxin reduction was reviewed by Fandohan et al. (2008).

Conclusion

This survey underlined the risk of contamination of peanut cake product by aflatoxigenic moulds and aflatoxins. Microbiological standards have not been established for processed peanut cake product in Benin. Due to the significance of the faecal-oral route transmission for many bacterial food-borne diseases, basic hygiene measures could assume a decisive importance in food safety management. It is also advisable that more attention (in the storage and selling methods) should be paid to its microbial quality and relevant quality control unit should be reactivated to assess the quality of the peanut kernels from which peanut cake and other products are made.

ACKNOWLEDGMENTS

We are grateful to the National Institute of Agricultural Research of Benin (INRAB) and the International Agency of Atomic Energy (AIEA) for their financial support. We would also like to sincerely thank Professor Sarah De Saeger and Mr. Emmanuel Njumbe Ediage (Laboratory

Table 5. Aflatoxins contents in peanut cake marketed in Benin.

Locality investigated	Aflatoxin (ppb)			
	AFB1	AFB2	AFG1	AFG2
Adjarra (n = 3)	367.54[a]	208.32[a]	100.33[a]	87.73[a]
Ouando (n = 3)	384.24[a]	405.21[a]	14.98[b]	81.41[a]
Dantokpa (n = 3)	455.22[a]	491.20[a]	94.17[a]	14.76[b]
Fidjrossè (n = 3)	204.25[b]	238.05[a]	14.76[b]	48.91[b]
Pahou (n = 3)	422.93[a]	412.34[a]	92.16[a]	11.43[b]
Ouidah (n = 3)	240.18[b]	100.34[b]	12.67[b]	10.31[b]
Comè (n = 3)	215.12[b]	321.56[a]	42.78[b]	40.24[b]
Bohicon (n = 4)	133.78[b]	150.43[b]	16.29[b]	20.98[b]
Abomey (n = 4)	259.40[b]	265.45[a]	31.56[b]	24.27[b]
Covè (n = 4)	84.75[c]	92.27[b]	43.56[b]	26.18[b]
Perma (n = 3)	36.97[c]	100.45[b]	16.32[b]	21.36[b]
Tigninti (n = 3)	12.18[c]	96.32[b]	11.34[b]	24.57[b]
Santa (n = 3)	25.54[c]	52.26[b]	10.25[b]	22.04[b]
Ourbouga (n = 3)	33.54[c]	33.94[b]	0.41[b]	22.47[b]

Values are means. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

of Food Analysis, Ghent University, Belgium) for technical assistance.

REFERENCES

Adebesin AA, Saromi OT, Amusa NA, Fagade SO (2001). Microbiological quality of some groundnut products hawked in Bauchi, a Nigerian City. J. Food Technol. Afr. 6(2):53-55.

Akano DA, Atanda O (1990). The present level of aflatoxin in peanut. Lett. Appl. Microbiol. 10:187-189.

Aissi VM, Soumanou MM, Bankolè H, Toukourou F, de Souza CA (2009). Evaluation of Hygienic and Mycological Quality of Local Cheese Marketed in Benin. Austr. J. Basic Appl. Sci. 3(3): 2397-2404.

Bankole SA, Ogunsanwo BM, Eseigbe DA (2005). Aflatoxins in Nigerian dry-roasted groundnuts. Food Chem. 89:503–506.

Betran FJ, Isakeit T (2003). Aflatoxin accumulation in Maize hybrids of different maturities. Agron. J. 96:565-570.

Cardwell K, Cotty PJ (2002). Distribution of Aspergillus section flavi among field soils from the four agroecological zones of the Republic of Benin,West Africa. J. Plant Dis. 86:434-439.

CEC (1998). Commission Regulation (EC) No. 1525/98. Off. J. Eur. Commun., L20/143, 17th July.

Cotty PJ, Bhatnagar D (1994). Variability among atoxigenic Aspergillus flavus strains to prevent aflatoxin contamination and production of aflatoxin biosynthetic pathway enzymes. Appl. Environ. Microbiol. 60:2248-2251.

Craufurd PQ, Prasad PVV, Waliyar F, Taheri A (2006). Drought, pod yield, pre-harvest Aspergillus infection and aflatoxin contamination on peanut in Niger. Field Crops Res. 98(1):20-29.

El-Adlouni C, Tozlovanu M, Natman F, Faid M, Pfohl-Leszkowicz A (2006). Preliminary data on the presence of mycotoxins (ochratoxin A, citrinin and aflatoxin B1) in black table olives ‘‘greek style” of Moroccan origin. Mol. Nutr. Food Res. 50:507–512.

European Community (2006). Final report of a mission carried out in China, in order to assess the facilities and measures in place for the control of aflatoxin contamination in peanuts intended for export into the EU. Report DG (SANCO)/8126/2006. Off. J. Eur. Union L199 p. 32.

Ediage EN, Di Mavungu JD, Monbaliu S, Van Peteghem C, De Saeger S (2011). A validated multianalyte LC-MS/MS method for quantification of 25 mycotoxins in cassava flour, peanut cake and maize samples. J. Agric. Food Chem. 59:5173–5180.

Egal S, Hounsa A, Gong YY, Turner PC, Wild CP, Hall AJ, Hell K, Cardwell KF (2005). Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. Int. J. Food Microbiol. 104(2):215-224.

Elisha B (2004). Influence of maternal nutritional status during pregnancy on birth weight in northern Benin: case of district of Natitingou. Master degree. Faculty of Agronomy. Univ. Abomey-calavi. Bénin p. 150.

Fandohan P, Zoumenou D, Hounhouigan DJ, Marasas WFQ, Wingfield MJ, Hell K (2005). Fate of aflatoxins and fuminosins during the processing of into food products in Benin. Int. J. Food Microbiol. 98:249-259.

Fandohan P, Hell K, Marasas WF (2008). Food processing to reduce mycotoxins in Africa. Pre- and postharvest management of aflatoxin in maize. In Leslie, J.F., Bandyopadhyay, R., Visconti, A., (Eds) Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI Publishing, Wallingford, UK, pp. 302-309.

FAO/WHO (1998). Forty-ninth meeting of the Joint Expert Committee on Food Additives. Food Agric. Organ. United Nation. Rome, p. 140.

Gong YY, Cardwell KK, Hounsa A, Eggal S, Turner PC, Hall A J, Wild CP (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: A cross-sectional study. Br. Med. J. 325:20–21.

Hell K, Cardwell KF, Setamou M, Poehling HM (2000). The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. J. Stored Prod. Res. 36:365–382.

Hell K, Cardwell K, Poehling H (2003). Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin. J. Phytopathol. 151:690-698.

Hell K, Fandohan P, Bandyopadhyay R, Cardwell K, Kiewnick S, Sikora R, Cotty P (2008). Pre- and postharvest management of aflatoxin in maize. In Leslie, J.F., Bandyopadhyay, R., Visconti, A., (Eds) Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI Publishing, Wallingford, UK, pp. 210-219.

Hell K, Mutegi C, Fandohan P (2010). Aflatoxin control and prevention strategies in maize for Sub-Saharan Africa. 10th International Working Conference on Stored Product. Protection. Julius-Kühn-Archiv, 425:534-541.

Honfo FG, Hell K, Akissoe N, Dossa RAM, Hounhouigan JD (2010). Diversity and nutritional value of foods consumed by children in two agro-ecological zones of Benin. Afr. J. Food Sci. 4:184-191.
Hüni K, Schneider J, Zanetti G, Rihs T (1990). Survey of aflatoxin content in Swiss concentrate for dairy cattle. J. Environ. Pathol. Toxicol. Oncol. 10:160-161.
IARC (1993). Some naturally occurring substances: Food items and constituents, heterocyclic amines and mycotoxins. IARC monographs on evaluation of carcinogenic risk to humans. International Agency for Research on Cancer 56, Lyon, France.
JECFA (1998). Safety Evaluation of Certain Food Additives and Contaminants: Aflatoxins (WHO Food Additives Series 40), 49th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Geneva, International Programme on Chemical Safety, World Health Organization.
Jolly CM, Bayard B, Vodouhe S (2009). Risks of Ingestion of Aflatoxin-Contaminated Groundnuts in Benin: Scale Measurements, Beliefs, and Socioeconomic Factors. Risk Anal. 29:1395-1409.
Leclerc H, Schwarrtzbrod L, Dei-Cas E (2002). Microbial agents associated with waterborne diseases. Crit. Rev. Microbiol. 28:371-409.
Molinie A, Faucet V, Castegnaro M, Pfohl-Leszkowicz A (2005). Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B1: development of a method for simultaneous extraction of ochratoxin A and citrinin. Food Chem. 92:391-400.
Monbaliu S, Van Poucke C, Van Peteghem C, Van Poucke K, Heungens K, De Saeger S (2008). Development of multi-mycotoxin liquid chromatography/tandem mass spectrometry method for sweet pepper analysis. Rapid Commun. Mass Spectrom. 23:3-11.
Morenoa EC, Garciab GT, Onoc MA, Vizonid E, Kawamurae O, Hirookaf EY, Onoa SYE (2009). Co-occurrence of mycotoxins in corn samples from the Northern Region of Parana State, Brazil. Food Chem. 10:21-37.
Murphy PA, Hendrich S, Bryant CM (2006). Food Mycotoxins – An Update. J. Food Sci. 71(5):51-65.
Mutegi CK, Ngugi HK, Hendriks SL, Jones RB (2009). Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. / Int. J. Food Microbiol. 130:27–34.
Ogunsanwo BM, Faboya OOP, Idowu OR, Lawal OS, Bankole SA (2004). Effect of roasting on the aflatoxin contents of Nigerian peanut seeds. Afr. J. Biotechnol. 3(9):451-455.
Pildain MB, Frisvad JC, Vaamonde G, Cabral D, Varga J, Samson RA (2008). Two novel aflatoxin-producing Aspergillus species from Argentinian peanuts. Int. J. Syst. Evol. Microbiol. 58:725-735.
Pfohl-Leszkowicz A, Molinie´A, Castegnaro M (2004). Presence of ochratoxin A, citrinin and fumonisin B1 in breakfast cereals collected in French market. Comparison of OTA analysis using or not immunoaffinity clean-up before HPLC. The Revista Mexicana de Mycologia 19:7-15.
Pittet A (1998). Natural occurrence of mycotoxins in foods and feeds: an updated review. Rev. Med. Vet. 149:479-492.
Singh K, Frisvad JC, Thrane U, Mathu SB (1991). An illustrated manual on identification of some seed borne Aspergilli, Fusaria, Penicillia and their mycotoxins. Heller up, Denmark: Danish Government, Institute of seed pathology for developing countries. P. 133.
Siwela AH, Mukaro KJ, Nziramasanga N (2011). Aflatoxin carryover during large scale peanut butter production. Food Nutr. Sci. 2:105-108.
Sultan Y, Magan N (2010). Mycotoxigenic fungi in peanuts from different geographic regions of Egypt. Mycotox. Res. 26:133–140.
Vrabcheva T, Usleber E, Dietric R, Martlbauer E (2000). Co-occurrence of ochratoxin A and citrinin in cereals from Bulgaria villages with a history of Balkan endemic nephropathy. J. Agric. Food Chem. 48:2483-2488.
Wagacha JM, Muthomi JW (2008). Mycotoxin problem in Africa: Current Status, implications to food safety and health and possible management strategies. Int. J. Food Microbiol. 124:1-12.
Wild CP (1996). Summary of data on aflatoxin exposure in West Africa. In K. F. Cardwell (Ed.), Proceedings of the workshop on mycotoxins in food in Africa, November 6–10, 1995 Cotonou, Benin: International Institute of Tropical Agriculture. p. 26.
Wild CP, Hall AJ (2000). Primary prevention of hepatocellular carcinoma in developing countries. Mutat. Res. 462:381–393.
Williams J, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2003). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80:1106–1122.