Food Control 56 (2015) 114–118

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Aflatoxin contamination in Haitian peanut products and maize and the safety of oil processed from contaminated peanuts

Jeremy R. Schwartzbord [a, *], Dan L. Brown [b]

[a] Institute of Comparative and Environmental Toxicology, Cornell University, Morrison Hall, Rm 343, Ithaca, NY 14853, USA
[b] Department of Animal Science, Cornell University, Morrison Hall, Room 267, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form
6 March 2015
Accepted 14 March 2015
Available online 21 March 2015

Keywords:
Aflatoxins
Contamination
Haiti
Peanuts
Maize
Edible oil

ABSTRACT

The primary objective of this study was to monitor aflatoxin contamination in Haitian samples of raw peanuts (n = 21), peanut butters (n = 32), and maize (n = 30) obtained in Port-au-Prince and Cap Haitien, Haiti, during 2012 and 2013. Our secondary objective was to explore a process that uses a locally produced Haitian spirit (*clarin*) to transform oil from contaminated peanuts into a safe, edible product. Immuno-affinity column chromatography and fluorometry (VICAM Aflatest) detected aflatoxins in 14%, 97%, and 30% of raw peanuts, peanut butters, and maize samples, respectively, and the concentration of total aflatoxins was greatest in peanut butters (median: 137 μg/kg, maximum: 2720 μg/kg). The concentration of aflatoxin in extracted oil was on average 10% of that in un-extracted oil which, in turn, had a concentration that was only 5% of the original contaminated peanuts. Therefore, aflatoxin concentration in the final product was 99.5% less than that found in the original peanuts, even without pre-filtration. Our extraction experiments testing laboratory-grade ethanol and *clarin* provide evidence that the latter can serve as a low-cost alternative to effectively reduce aflatoxin concentrations in oil pressed from high aflatoxin peanuts.

1. Introduction

Aflatoxins are toxic secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* and include a stable and highly oxygenated structure of 5 fused rings and a lactone moiety. Aflatoxin B1, the most abundant of aflatoxins, is a causative agent in hepatocellular carcinoma and is associated with immune-dysfunction and protein deficiency syndromes such as kwashiorkor (Coulter et al., 1986; Turner, Moore, Hall, Prentice, & Wild, 2003; Wogan, 1992). Though found in a range of crops, including spices (Hammami et al., 2014), tree nuts (Georgiadou, Dimou, & Yanniotis, 2012), maize and peanuts (Jager, Tedesco, Souto, & Oliveira, 2013), aflatoxin contamination is most prevalent in the latter two, occurring both before and after harvest (Williams et al., 2004). When stressed by drought and pest pressure, maize and peanuts are most prone to infection by toxigenic *Aspergillus* and contamination with aflatoxin (Pitt, Taniwaki, & Cole, 2013). In addition, warm, humid storage conditions result in post-harvest fungal growth and increased aflatoxin concentrations (Turner et al., 2005). Consequently, aflatoxins are often detected in foods from tropical countries where irrigation and pest management practices are lacking and food storage is poor (Williams et al., 2004).

In Haiti, exposure to aflatoxins, as indicated by circulating blood-albumin was detected among outpatients residing in Port-au-Prince (Schwartzbord et al., 2014), and aflatoxin contamination has been documented for Haitian maize (Castor, Mirocha, & Chang, 1987) and peanut butters (Filbert & Brown, 2012). Filbert and Brown collected peanut butter samples in Port-au-Prince during December of 2009 and October 2010 (Filbert & Brown, 2012). Using immuno-affinity column chromatography coupled with fluorometric detection, they found that aflatoxin levels ranged from 7.9 to 799.8 μg/kg aflatoxin, and 16 out of 18 samples had more than 20 μg/kg, the US Food and Drug Administration (FDA) regulatory limit. Aflatoxin contamination in Haitian maize, a staple crop, has been described in one peer-reviewed study (Castor et al., 1987). Castor's team collected maize samples from markets during

Abbreviations: FDA, US Food and Drug Administration; HPLC, high pressure liquid chromatography; CL, Clarin; HA, High Aflatoxin Oil; LA, Low Aflatoxin Oil; ND, Nord Department of Haiti.

* Corresponding author. Tel.: +1 650 534 6350.
E-mail addresses: Jrs477@cornell.edu (J.R. Schwartzbord), Dlb20@cornell.edu (D.L. Brown).

http://dx.doi.org/10.1016/j.foodcont.2015.03.014
0956-7135/

January, July, and October of 1983 and January of 1984, and total aflatoxins were measured using HPLC. Twenty-two percent of the 268 samples had greater than 20 μg/kg aflatoxin.

A primary motivation for examining aflatoxin in the food supply of a resource-limited country is to explore feasible processes that will attenuate contamination to acceptable levels. Removal of contaminated kernels by visual, tactile and density segregation are examples of effective physical separation and are feasible among Haitian food processors (Filbert & Brown, 2012). In places where poverty is pervasive, however, the subsequent challenge after separating contaminated kernels is that they will be discarded by the processor but obtained in local, unregulated markets and construed as edible among food insecure individuals (Matumba, Van Poucke, Monjerezi, Njumbe Ediage, & De Saeger, 2015). Pursuant to minimizing aflatoxin exposure among the poorest of consumers, it is essential to prevent highly contaminated kernels from reentering food chains, and decontamination of such kernels should complement sorting practices. Many chemical decontamination processes exist and are reviewed extensively by Leibetseder (2006), including treatments to lessen the potency of the aflatoxin molecule and solvent extraction techniques. Chemical treatment with ammonia (Weng, Martinez, & Park, 1994) and oxidizing agents such as ozone (Luo, Wang, Wang, Li, Bian, et al., 2014; Luo, Wang, Wang, Li, Wang, et al., 2014), for instance, have been shown to reduce aflatoxin concentration to 1–36% and 11–13% original levels, respectively, depending on treatment parameters. Also effective is solvent extraction using aqueous ethanol, which reduces aflatoxins to 2–7% original levels in cottonseed and peanut meals (Rayner, Dollear, & Codifer, 1970). Ammoniation, ozone treatment, and ethanol extraction are not equally feasible in lesser-developed countries. Anhydrous ammonia and ozone, for instance, are often not readily available in lesser-developed countries such as Haiti. Furthermore, residual ammonium following ammonia treatment is not permissible in human food, and ozone hastens lipid peroxidation in oil seeds. Imported ethanol and locally produced ethanol, however, are available in Haiti, the latter being less costly and more economically accessible to small-scale peanut processors.

Given that Filbert and Castor *et al.* reported their results in 2012 and 1987, respectively, the primary purpose of our study was to monitor more recent aflatoxin contamination of Haitian peanuts and locally produced maize during 2012 and 2013. As a corollary, we sought to identify a safe, value-added product made from formerly aflatoxin-contaminated peanuts, and we considered production of edible oil as potentially suitable to Haitian food processors. Therefore our secondary purpose was to determine aflatoxin carryover from contaminated kernels to un-refined, edible oil and the efficacy of extraction, comparing both laboratory-grade ethanol and a locally procured Haitian spirit, on the residual aflatoxin concentrations found in such oil. This comparison was made because extraction using a local ethanol would be more feasible among small-scale food processors, who produce the majority of peanut butter sold in Haiti.

2. Materials & methods

2.1. Food samples

Samples were collected during three periods. During July of 2012, 14 peanut butters and 21 peanut samples were obtained from open-air markets in Port-au-Prince and Cap Haitien. In December 10 maize samples of approximately 1.0 kg each were obtained from the Telele, Croix du Bouquets, and Croix du Bosales markets in Port-au-Prince. The third period was September through December of 2013, during which 21 peanut butters were purchased around Cap Haitien, and 20 maize samples were obtained at four farmer association depots and three mills in the Nord Department.[1] At depots, where farmers sort and grade the maize as fit for human consumption or animal feed, representative samples were taken and kept separate based on classifications described by farmers onsite. Whole, sound ears of corn were generally directed to the mill, while those with visible rot and free kernels on the ground were directed to animal feed. At mills, a sampling probe was used to obtain kernels from the bottom, middle, and upper parts of storage sacks weighing 50–100 kg. Milled maize was sampled where available and included grain for maize porridge ("mayi moulen"), fine maize flour ("mayi farin"), and bran destined for animal feed ("mayi pay"). Of the 20 maize samples collected, 11 were directed to human consumption but not yet milled, 5 were directed to human consumption and milled, and 4 were directed to livestock feed and included milled and non-milled maize. Each sample weighed 1.5–2.0 kg and was taken from a 50–150 kg storage sack. Moisture for whole grain samples was measured the day of collection. Samples were stored at −30 °C until milling with a hammer-mill.

2.2. Determination of aflatoxin with immuno-affinity column chromatography and fluorometric detection

Aflatoxin was measured using the VICAM Aflatest system (Journal AOAC, 17th edition, 2000, 972.26). Each peanut butter was emptied from its original jar and mixed thoroughly with a spatula before sub-samples (25 g) were taken for analysis. Peanut samples were ground with a small food processor prior to sub-sampling. Maize samples included whole cobs (10–15) and free kernels (1.5–2.0 kg) obtained from farmers and mill depots. Kernels from whole cobs were removed by hand, and each sample was ground in a hammer mill with a 4.0 mm screen. Sub-samples (50 g) were taken for analysis. Aflatoxin was extracted from samples (60% or 80% methanol for peanut or maize samples, respectively) using a blender at high speed for 1 min. Extract was filtered with fluted filter paper (VICAM) into a glass beaker and diluted with de-ionized water (1:2 and 1:5 dilutions for peanut-products and maize, respectively), followed by filtration with a glass microfiber filter. Dilute filtrates for peanut (10 ml or 1.0 g sample equivalent) and maize (2 ml or 0.2 g equivalent) were passed through an immune-affinity column. For samples outside the range of detection (0–100 μg/kg and 0–300 μg/kg for peanut and maize products, respectively) filtrate was further diluted 1:5 (10 ml dilute filtrate and 40 ml de-ionized water) or 1:10 (5 ml dilute filtrate and 45 ml de-ionized water). The column was washed with de-ionized water twice and eluted into a borosilicate culture tube with 1.0 ml of HPLC-grade methanol. To the eluate was added 1.0 ml of brominated water, and the sample was vortexed. After 1.0 min, fluorescence of the sample was read (Excitation: 360 nm, Emission: 440 nm). Maize reference samples with no detectable aflatoxins, 50.8, 9.6, 5.9, and 1.7 μg/kg were obtained (Trilogy Labs, Washington, Missouri), and peanuts butters with no detectable aflatoxins were spiked with 1, 2, 3, 4, 10, 25, and 400 μg/kg. The spiking standard (Trilogy Labs) contained 5 μg/ml total aflatoxins (2 μg AFB1, 2 μg AFG1, 0.5 μg AFB2, and 0.5 μg AFG2 per ml acetonitrile). All reference samples were assayed in triplicate, and the limit of detection (LOD), limit of quantitation (LOQ), recovery range %, and relative standard deviation (RSD %) were determined. We set our LOD to the lowest reference sample whose mean result was significantly different (Student's t-test, $p < 0.05$) from that of the reference without additional aflatoxins and our LOQ to the level

[1] Mill operators granted access for sampling on the condition that the locations remain anonymous.