Food Control 37 (2014) 27–32

Contents lists available at ScienceDirect

Food Control

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

Common African cooking processes do not affect the aflatoxin binding efficacy of refined calcium montmorillonite clay

Sarah E. Elmore [1], Nicole Mitchell [2], Travis Mays [3], Kristal Brown [2], Alicia Marroquin-Cardona [2], Amelia Romoser [4], Timothy D. Phillips*

Veterinary Integrative Biosciences Department, College of Veterinary Medicine and Biomedical Sciences, TAMU 4458, Texas A&M University, College Station, TX 77843-4458, USA

ARTICLE INFO

Article history:
Received 4 June 2013
Received in revised form
16 August 2013
Accepted 25 August 2013

Keywords:
AFB_1
UPSN
Enterosorption therapy
Food safety
Cornmeal

ABSTRACT

Aflatoxins are common contaminants of staple crops, such as corn and groundnuts, and a significant cause of concern for food safety and public health in developing countries. Aflatoxin B_1 (AFB_1) has been implicated in the etiology of acute and chronic disease in humans and animals, including growth stunting, liver cancer and death. Cost effective and culturally acceptable intervention strategies for the reduction of dietary AFB_1 exposure are of critical need in populations at high risk for aflatoxicosis. Fermented gruels consisting of cornmeal are a common source for such exposure and are consumed by both children and adults in many countries with a history of frequent, high-level aflatoxin exposure. One proposed method to reduce aflatoxins in the diet is to include a selective enterosorbent, Uniform Particle Size NovaSil (UPSN), as a food additive in contaminated foods. For UPSN to be effective in this capacity, it must be stable in complex, acidic mixtures that are often exposed to heat during the process of fermented gruel preparation. Therefore, the objective of the present study was to test the ability of UPSN to sorb aflatoxin while common cooking conditions were applied. The influence of fermentation, heat treatment, acidity, and processing time were investigated with and without UPSN. Analyses were performed using the field-practical Vicam assay with HPLC verification of trends. Our findings demonstrated that UPSN significantly reduced aflatoxin levels (47–100%) in cornmeal, regardless of processing conditions. Upon comparison of each element tested, time appeared to be the primary factor influencing UPSN efficacy. The greatest decreases in AFB_1 were reported in samples allowed to incubate (with or without fermentation) for 72 h. This data suggests that addition of UPSN to staple corn ingredients likely to contain aflatoxins would be a sustainable approach to reduce exposure.

1. Introduction

Aflatoxins (AF) are common contaminants of staple crops, such as corn and groundnuts, and are a significant cause of concern for food safety and public health in developing countries. Aflatoxin B_1 (AFB_1) is one of four secondary metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and is the most prevalent and toxic of the AF congeners (Wild & Turner, 2002). Frequent humidity, drought, and insect damage in West Africa and South East Asia encourage pre-harvest fungal contamination, leading to chronic AFB_1 exposure in these populations (CAST, 1989). Furthermore, poor grain storage practices that lead to higher moisture levels can cause increased AF levels in harvested grains previously infected in the field (CAST, 2003). Chronic exposure is greatest in communities that produce and consume their own food (Wild & Gong, 2010) and is associated with an increased risk of hepatocellular carcinoma (HCC) (IARC, 1993, 2002; Wild & Turner, 2002). Additionally, AFB_1 is known to be hepatotoxic, genotoxic, immunosuppressive, and anti-nutritional (IARC, 2002).

In many parts of West Africa, populations are chronically exposed to AFs beginning *in utero* (Partanen et al., 2010; Turner et al., 2007; Turner, Flannery, Isitt, Ali, & Pestka, 2012). Exposure typically continues through the first years of life, with the presence of a toxic secondary metabolite of AFB_1, Aflatoxin M_1 (AFM_1), in breast milk (Gong et al., 2003; Shepard, 2008; Zarba et al., 1992),

* Corresponding author. Tel.: +1 979 845 6414.
E-mail addresses: selmore@cvm.tamu.edu (S.E. Elmore), nmitchell@cvm.tamu.edu (N. Mitchell), tmays@tvmdl.tamu.edu (T. Mays), kabrown@cvm.tamu.edu (K. Brown), aliciamarroquin@hotmail.com (A. Marroquin-Cardona), aromoser@cvm.tamu.edu (A. Romoser), tphillips@cvm.tamu.edu (T.D. Phillips).
[1] Tel.: +1 979 845 4977.
[2] Tel.: +1 979 845 4976.
[3] Tel.: +1 979 845 1454.
[4] Tel.: +1 979 540 6500.

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.08.037

and well into childhood and adulthood where exposure to AFB_1 can be present in fermented cornmeals and porridges (Andah, 1972; Lartey, Manu, Brown, Peerson, & Dewey, 1999). Recently, a sampling of corn-based weaning foods intended for children between the ages of 6 months and 2 years in the Ashanti region of Ghana was found to contain high levels of AFs (Kumi, 2011). All of the 36 samples tested were contaminated with AFs, with 83% containing concentrations above the U.S. FDA action level of 20 ppb AFB_1 and some samples ranging as high as 500 ppb. Despite the fact that fermenting and heating these weaning foods and breakfast gruels may prevent spoilage and enhance food safety, AFs are resistant to degradation by thermal inactivation and fermentation (Christensen, Mirocha, & Meronuck, 1977) and therefore remain a constant source of concern.

Methods that focus on reducing dietary exposure to AFs in contaminated foods are highly desirable as a practical strategy to mitigate the harmful effects of this toxin (Williams et al., 2004). Preferential sorption of AFs in the gastrointestinal tract with the inclusion of certain clays in the diet is one example of this type of approach. NovaSil™ (NS) is a calcium montmorillonite clay with high binding affinity and capacity for AFB_1. NS has been shown to be safe and effective in preventing aflatoxicosis in animals and reducing biomarkers of AF exposure in humans and animals (Harvey et al., 1991; Kubena et al., 1991; Lindemann, Blodgett, Kornegay, & Schurig, 1993; Mayura et al., 1998; Phillips, 1999, 2006; Phillips, Kubena, Harvey, Taylor, & Heidelbaugh, 1988; Phillips, Lemke, & Grant, 2002; Pimpukdee et al., 2004). These studies have shown that NS is effective as an enterosorbent for AFs when included in the diet at levels ranging from 0.25 to 2% (w/w) in animals (Phillips et al., 2002). Additionally, a minimal effective dose of NS 0.25% w/w delivered in capsules for three months in a high risk Ghanaian population was successful in decreasing biomarkers of AF exposure and did not interfere with the levels of serum vitamins A and E, iron, or zinc (Afriyie-Gyawu et al., 2008). Parent NS clay was refined to form UPSN (Uniform Particle Size NovaSil) through a process that served to improve the palatability and consistency of the clay for food delivery. The refining process resulted in a higher percentage of NS particle sizes between 45 and 100 μm and lower levels of quartz; however, NS and UPSN were compared and shown to have similar AF sorption properties (Marroquin-Cardona et al., 2011). Rats fed UPSN at levels as high as 2% (w/w) for 13 wk displayed no detectable toxicity (Marroquin-Cardona et al., 2011). Recently, UPSN inclusion in foods has been investigated in populations at high risk of AF exposure. In a crossover study in Ghana, UPSN was shown to be palatable and well-tolerated when added to fermented foods. Moreover, those participants consuming 0.25% UPSN exhibited significantly decreased levels of urinary AFM_1 compared to the placebo group. Also, no adverse reactions from the treatment or placebo were reported. This study indicated that UPSN (when delivered in common fermented foods) was acceptable and could safely and effectively reduce AF exposure when included in contaminated diets (Mitchell et al., 2013).

Fermentation of corn-based foods in West Africa is common and the effects of acidity and ethanol production during this process are important parameters that could interfere with toxin sorption by clay (UPSN) and thus need to be investigated. Also, knowledge regarding the effects of different cooking conditions (temperature and fermentation time) on the AF–clay complex is needed to determine AF adsorption ability of the clay in a cornmeal matrix. Hence, the objective of the present study was to determine UPSN stability and AFB_1 sorption during fermentation and heating protocols that typify the production of common corn-based foods intended for consumption in this region.

2. Materials and methods

2.1. Materials

Acetonitrile (ACN) and methanol (MeOH) utilized were HPLC analytical grade (Fisher Scientific, Fair Lawn, NJ). Ultrapure deionized water (18.2 MΩ) was generated using an Ultrapure automated filtration system (Elga™ Woodridge, IL). Aflatoxin B_1 was purchased from Sigma–Aldrich Corporation (St. Louis, MO). Cornmeal was purchased from a local grocery store in College Station, TX. Extraction equipment, including AflaTest® immunoaffinity columns, was purchased from Vicam® (Watertown, MA) and utilized according to the manufacturer's instructions. Uniform Particle Size NovaSil (UPSN) was obtained from Texas Enterosorbents (Bastrop, TX). Quantitative analysis of AFB_1 was performed using a Vicam Series 4 Fluorometer and verified on a Waters HPLC with fluorescence detection (Watertown, MA).

2.2. Cornmeal preparation

Purchased cornmeal contained an average of 1 ppb AFB_1 as measured by Vicam analysis (see Section 2.3). AFB_1 standard was diluted in water to obtain a 25 ppm stock solution for spiking cornmeal samples. The concentration was verified daily using UV spectrophotometry (Shimadzu UV-1800). Cornmeal samples (50 g) were prepared in triplicate, containing 5, 50, 100, 300, 500, and 1000 ppb AFB_1, for both the control and UPSN-treated groups. UPSN (1.5 g) was added to AFB_1-spiked cornmeal samples to comprise the clay test group. The amount of UPSN was calculated based on the high dose of UPSN that was delivered per person in clinical intervention trials in Ghana (1.5 g in each meal) (Mitchell et al., 2013). AFB_1-spiked samples and clay were mixed together for 15 s to disburse the clay throughout the cornmeal. This procedure was repeated for all samples and served as a base mixture before additional processing steps were applied as described in the following Sections 2.2.1–2.2.3.

2.2.1. Base product

AFB_1 was extracted from samples immediately following the base (non-processed) cornmeal product to assess binding capacity of UPSN for AFB_1 within the cornmeal matrix without any fermentation or cooking. The same procedure was also repeated at pH 3.5 to simulate the average pH observed during the fermentation of corn dough (Plahar & Leung, 1983). This was achieved by adjusting the mixture with HCl until stabilized at pH 3.5.

2.2.2. Fermented product

Fermentation of the base product was allowed to occur naturally in covered flasks simulating the process used in Africa. Water (50 mL) was added and mixed by agitating the flask until uniform in appearance and thoroughly moist. Mixtures were allowed to ferment for 24 or 72 h in a NUAIRE™ TS Autoflow Incubator (Plymouth, MN) maintained at 30 °C. This procedure represents both the average temperature and typical fermentation environment that would occur in West Africa. Additionally, samples with the same AFB_1 concentrations and controls were subjected to heat treatment by adding 50 mL boiling water following fermentation. Then, the dough and water were mixed together without further heating, resulting in a matrix with the consistency of a thick soup or gruel. Mixtures were allowed to sit at room temperature for 10 min prior to processing.

2.2.3. Sterilized product

Sterilized samples were produced by autoclaving the base products and water at 220 °C for 30 min prior to the 72 h