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Short communication

Aflatoxin contamination in unrecorded beers from Kenya – A health risk beyond ethanol

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ABSTRACT

Samples of unrecorded opaque beers (n = 58; 40 based on maize, 5 on sorghum and 13 on other plants) and recorded wines (n = 8) in Kenya were screened for aflatoxins using a rapid ELISA technique followed by confirmation using liquid chromatography-tandem mass spectrometry. Six of the maize beers were obtained from Kibera slums in Nairobi County. Aflatoxin contamination was detected in six unrecorded beers (10%), but in none of the recorded wines. Remarkably, three of the aflatoxin positive samples were from the Kibera slums.

The mean concentration of aflatoxins in the positive samples was 3.5 μg/L (range 1.8–6.8 μg/L), corresponding for an average consumption of 500 mL (1 standard drink) to a margin of exposure (MOE) of 36 (range: 15–58), which is considered as 'risk'. On the other hand, the alcoholic strength of the aflatoxin positive samples had a mean of 4.3% vol (range 3.5–4.8%) corresponding to a MOE of 2.5 (range of 2.2–3.0) for the equivalent consumption volume. While aflatoxins pose a risk to the consumer, this risk is about 10 times lower than the risk of ethanol.

The Joint FAO/WHO Expert Committee on Food Additives sets no acceptable daily intake for aflatoxins since they are genotoxic carcinogens and instead recommends for the reduction of aflatoxin dietary exposure as an important public health goal, particularly in populations who consume high levels of any potentially aflatoxins-contaminated food. Nevertheless, ethanol still posed a considerably higher risk in the unrecorded beers examined. However, consumers should be informed about aflatoxins, as these are an involuntary and unknown risk to them. In addition, producers should be educated about measures to reduce aflatoxins in alcoholic beverages.

1. Introduction

The consumption of unrecorded alcohol products such as artisanal beers is high in Kenya with the WHO estimate being 58% of total adult per capita alcohol consumption (WHO, 2016). Although the unrecorded production of artisanal beers is prohibited in the country, the beverages are produced at household levels and consumed during social and religious functions (Willis, 2002). A majority of these producers including women are economically dependent on the trade (Bodewes, 2010). Artisanal beers in Kenya are mainly produced from grains, and saps of plants such as *Kigelia africana*, coconut palm, cashew nuts (*Ancardium occidentale*), sugarcane and the East African doum palm (*Hyphaene compressa*) plant. The beers from fermented grains are locally known as *busaa*, with maize or sorghum, malted millet and water being the main ingredients. Maize is the preferred ingredient for preparation of these beers owing to its wide use as a staple food. To prepare *busaa*, maize flour is mixed with water to form a thick paste which is allowed to ferment for 5–7 days followed by roasting over the fire to yield brown granules which are air dried. Final fermentation is initiated by adding malted millet and sufficient water to the granules in earthen pots or other suitable plastic containers. The

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mixture is permitted to ferment for 2–3 days before consumption.

Mycotoxins are secondary fungi metabolites that attack cereal grains and fruits such as grapes during growth in the field or post harvesting, and the extent of contamination is estimated to be 25% in tropical regions (Turner, Subrahmanyam, & Piletsky, 2009). Aflatoxins and ochratoxins are the two major mycotoxins of public health significance (IARC, 2012; Miller, 1995). Aflatoxins are mainly produced by *Aspergillus flavus* and *A. parasiticus* while ochratoxin A is from *Penicillium verrucosum* and *A. alataceus* (Chu, Chang, Ashoor, & Prentice, 1975; Mably et al., 2005). There are four major types of aflatoxins namely B_1, B_2, G_1, and G_2, classified based on their fluorescence properties under UV light (blue or green) and mobility on thin-layer chromatographic plates (Bennett & Klich, 2003; Turner et al., 2009). Aflatoxins are carcinogenic and hepatotoxic secondary metabolites known to potentially contaminate numerous agricultural and animal products. Aflatoxin B_1 poses the greatest toxicological concern since it is a known potent natural carcinogen (Squire, 1981). The International Agency for Research on Cancer (IARC) classifies aflatoxins in its highest category as carcinogenic to humans (group 1) (IARC, 2012) with a proposed mechanism of hepatocellular injury via adducts that induce mutations in the gene responsible for tumour suppression (IARC, 2012; Wild & Turner, 2002). The effects upon exposure are dose-dependent with notable inter- and intra-species differences in susceptibility (Turner et al., 2015, 2009). Aflatoxins B_2, G_1 and G_2 are relatively not as potent carcinogens as B_1 (Wild & Turner, 2002).

Kenya has experienced several aflatoxin-poisonings with the first occurrence reported in 1982 attributed to the consumption of contaminated maize. Subsequent sporadic poisonings occurred in 2004, 2005 and 2006. The 2004 incident was the most severe, resulting in 317 aflatoxicosis case-patients and 125 deaths (39% mortality) (Centers for Disease Control and Prevention (CDC), 2004; Wagacha & Muthomi, 2008; Yard et al., 2013). Acute human poisoning is characterized by vomiting, abdominal pain, pulmonary or cerebral oedema, necrosis, and fatty liver. Other symptoms include anorexia, depression, jaundice, diarrhoea, and photosensitivity (Marin, Ramos, Cano-Sancho, & Sanchis, 2013). Most studies in Kenya, therefore, have focussed on grains with a few studies on unrecorded grain-based beers marked by limited sampling. To the best of our knowledge, no country-wide screening of unrecorded maize and sorghum beers and recorded wines for aflatoxins has been reported in Kenya. The purpose of this study was to screen and determine the amount of aflatoxins in unrecorded beers and wines, and conduct quantitative risk assessment using the margin of exposure approach for aflatoxin exposure in comparison to ethanol if positive samples were to be detected.

2. Methods

2.1. Sample collection

Unrecorded beers and recorded wines for this study were collected from 16 different counties in Kenya between May and July 2016 in the context of an international project designed to characterize the quality of unrecorded alcohol (Lachenmeier, Kanteres, & Rehm, 2011; Lachenmeier, Leitz, et al., 2011). The samples of unrecorded beers consisted of 45 *busaa* (40 maize and 5 sorghum based products) and 13 other plant based sap beers. Sampling of *busaa* was concentrated in areas where maize and sorghum are grown and consequently the unrecorded artisanal alcohol products are widely produced and consumed. *Busaa* samples were obtained from Nairobi (n = 6), Kisii (n = 5), Narok (n = 2), Bomet (n = 1), Kericho (n = 1), Kisumu (n = 2), Busia (n = 2), Bungoma (n = 2), Migori (n = 2), Nakuru (n = 2), Elgeyo Marakwet (n = 12), Uasin Gishu (n = 1), Taita Taveta (n = 3), Kakamega (n = 2) and Samburu (n = 2). The plant based beers from other sources were collected from the counties of Embu (n = 4), Kiambu (n = 1), Nyeri (n = 1) and Kirinyaga (n = 7). For recorded wines, 8 samples were purchased from retail outlets from Nairobi, Kenya. Most of the sampling was concentrated in the maize growing regions in Kenya where the production and consumption of the artisanal beers is most prevalent. Directly following the sampling, the samples were stabilized by adding a spatula tip of NaN_3 and stored at −18 °C until analysis.

2.2. ELISA screening

The samples were screened for aflatoxins at 2 μg/kg levels and for ochratoxin A at 3 μg/kg levels using enzyme-linked immunoassay (ELISA) kits from R-Biopharm Rhone Ltd, Darmstadt, Germany (Aflacard Total, product code: P38 for aflatoxins and Ochracard, product code: P48 for ochratoxin A).

Sample preparation and test procedures were conducted as described by the kit manufacturer. In brief, for aflatoxin determination, 5 mL of sample was cleaned by passing through a solid phase column before introducing 500 μL of the eluent onto the port of the ELISA card. This was followed by successive addition of 100 μL of conjugate, wash buffer, substrate and stop solution according to manufacturer's instructions. The methodology is based on a competitive ELISA technique (Onnelly, Allagher, & Ordan, 2010). For the ochratoxin A test, 9 mL of the filtered sample was diluted with 18 mL of phosphate buffered saline (BPS) before passing 25 mL of the solution through an immunoaffinity column and eluting the toxins from the column with methanol. The eluate, 500 μL, was introduced onto the port of the ELISA card followed by successive addition of 100 μL of conjugate, wash buffer, substrate and stop solution according to manufacturer's instructions (Gilbert & Michelangelo, 2014).

2.3. Liquid chromatography-tandem mass spectrometry

The samples that were positive for aflatoxins by ELISA were subjected to confirmation analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis using a previously validated in-house method. Certified aflatoxin standard mixture (AFB1, AFB2, AFG1 and AFG2) was from Trilogy Analytical Laboratory, Washington, MO, USA. LC grade acetonitrile, ammonium formate and methanol were from Merck, Darmstadt, Germany.

About 50 mL of sample were centrifuged for 20 min at 4000 rpm and 20 °C. Following that, 0.5 mL of the supernatant were transferred into a 2 mL vial, 50 μL of internal standard solution (certified reference standards of $^{13}C_{17}$-marked aflatoxin B_1, B_2, G_1, and G_2 obtained from Biopure, Getzersdorf, Austria), 350 μL of acetonitrile and 100 μL of water. The resulting solution was homogenized by stirring and then centrifuged for 10 min at 18,000 G. Finally, 0.5 mL of the clear supernatant was filled into a vial for analysis of which 5 μL were injected into the LC/MS/MS system (5500 QTRAP, ABI Sciex, Darmstadt, Germany) equipped with a turbo spray ion source operated in the positive ionization mode at 480 °C. Chromatographic separation was achieved on an Agilent 1260 HPLC system using a XBridge Shield column (150 mm × 3 mm; 3.5 μm) (Waters, Eschborn, Germany) maintained at 40 °C with a mobile phase consisting of methanol (A) and water (B) as eluents containing 1 mM ammonium formate, both delivered at a flow rate of 0.5 mL/min with a linear gradient elution: 0–5 min: 90–10 (A- B); 5–17 min: 5–95 (A-B); 17–24 min: 90–10 (A-B). An amount of 5 μL of each sample was injected into a flow rate of 0.5 mL/min. The proprietary software, Analyst version 1.6.2 (ABI Sciex, Darmstadt, Germany), was used for instrument control and data evaluation. The following fragments (m/z Q1/Q3) were observed in the