

Full Length Research Paper

## Total aflatoxin, fumonisin and deoxynivalenol contamination of busaa in Bomet county, Kenya

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Mycotoxin contamination is a common problem in developing countries, particularly in cereals, and this poses a serious health risk to its consumers. Busaa is a Kenyan traditional brew whose cereal ingredients are prone to mycotoxin contamination. This study aimed at detecting the presence and subsequently quantifying aflatoxin, fumonisin and deoxynivalenol (DON), in busaa in Bomet county, Kenya. Busaa samples were collected from homesteads involved in brewing in the north eastern part of Bomet East constituency. Mycotoxins were detected in the samples using the Enviroligix QuickTox kits and quantified using the QuickScan machine according to the manufacturer's instructions. Among the 61 samples tested, 93, 9.8 and 23% were contaminated with aflatoxin, fumonisin and DON, respectively, (mean:  $5.2 \pm 0.2$   $\mu\text{g}/\text{kg}$ , range: 2.8 to 11  $\mu\text{g}/\text{kg}$ ; mean  $1460 \pm 188$   $\mu\text{g}/\text{kg}$ , range 280 to 4000  $\mu\text{g}/\text{kg}$ , mean  $259 \pm 5.2$   $\mu\text{g}/\text{kg}$ , range 200 to 360  $\mu\text{g}/\text{kg}$ , respectively). Although traditional brews are not directly included in the European law on mycotoxins, it is important to consider their mycotoxin levels. In this study, busaa is a mainly a maize product and also the European Union (EU) guidelines on mycotoxins in maize were used as reference. It was found out that 65.6% of busaa had aflatoxin levels above the limit set in the EU guideline (4  $\mu\text{g}/\text{kg}$ ). Although, the average levels of fumonisin and DON were within the set limits (fumonisins: 4000  $\mu\text{g}/\text{kg}$ ; DON: 1750  $\mu\text{g}/\text{kg}$ ), studies have shown that chronic exposure to multiple mycotoxins has detrimental health effects. Therefore, there is need for mycotoxicological quality control of traditionally produced brews for public mycotoxicological safety.

**Key words:** Mycotoxin, traditional brew.

### INTRODUCTION

Busaa (maize beer) is a Kenyan traditional brew that has a socio-cultural significance and is mostly consumed during events such as male circumcisions, weddings and funerals. It is also commonly consumed by members of low income earning groups as a leisure activity especially

when there is a bumper harvest (Daško et al., 2005). The main ingredients of busaa are raw maize flour and semi-ground finger millet malt. Natural fermentation of the raw maize flour followed by subsequent addition of the semi-ground finger millet malt and farther fermentation results

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in busaa as the final product. The production and consumption of cereals such as maize, sorghum and millet in Africa is highly constrained by rot fungi including *Aspergillus*, *Fusarium* and *Penicillium* (Milićević et al., 2010; Shephard, 2008; Kedera et al., 1998). These fungi lower the quality of the produce through discoloration and reduction of nutritional value besides production of mycotoxins such as fumonisins, aflatoxins, ochratoxins, deoxynivalenol and zearalenone (Jacobsen, 2008). In animals, aflatoxins have been shown to be carcinogenic, mutagenic, teratogenic and immunosuppressive cause (Ajeyuyo et al., 2011) while fumonisins are generally considered to be the cause of equine leukoencephalomalacia in horses, mules, and donkeys and are strongly associated, based on epidemiology with esophageal cancer and neural tube defects in humans (Jacobsen, 2008; Wakhisi et al., 2005; Paul et al., 2001). On the other hand, the presence of DON in feeds is manifested by rejection of feeds, vomiting, diarrhea and eventual weight loss in livestock (Kuiper-Goodman, 2002). It is expected that cereals contaminated with fungi and mycotoxins are discarded but this is not always the case. Studies have shown that these cereals are sometimes consumed by humans, fed to animals or diverted to alcohol production, resulting in transfer of mycotoxins along the food chain and hence the occurrence of mycotoxicoses (Jacobsen, 2008; Moturi, 2008; Bennett and Klich, 2003; Sudakin, 2003; Binder et al., 2007).

Typically, there are five major stages in the manufacturing of beers from grains: malting, mashing, fermentation, maturation and finishing (Wood, 1998). The potential for mycotoxins or their residues to occur in busaa depends upon the initial cereal and the malt (Nkwe et al., 2005; Mbugua and Gathumbi, 2004; Mbugua and Mwaura, 1996), and the fate of the mycotoxin during malting, brewing and fermentation (Jacobsen, 2008; Wood, 1998). Previous studies have reported high incidences and levels of mycotoxins in lager beers and traditional opaque beers (Nkwe et al., 2005; Mbugua and Gathumbi, 2004; Kenji et al., 2000). This paper reports the incidences and levels of aflatoxin, fumonisin and deoxynivalenol in the Kenyan traditional brew, busaa.

## MATERIALS AND METHODS

### Study site

The study was carried out in the north-eastern part of Bomet East Constituency in Bomet county, Kenya. Agriculture is highly relied upon as the main source of income in the region but some families depend on brewing as an additional source of income.

### Sampling design

Busaa samples were collected from consenting brewers in the north-eastern part of Bomet county. Upon obtaining an informed consent from brewers, approximately 100 ml of busaa samples

were bought and aliquoted into two 50 ml sterile falcon tubes and transported to the Kenya Medical Research Institute at 2 to 8°C for mycotoxin analysis. A total of 61 samples of busaa were collected and investigated. The busaa samples were stored at -40°C prior to analysis.

### Extraction and quantification of mycotoxins

The samples were first allowed to thaw to room temperature (16 to 26°C) prior to analysis. Aflatoxin, fumonisin and deoxynivalenol were detected and quantified using the EnviroLogix QuickTox Kit for QuickScan according to the manufacturer's instructions. The EnviroLogix QuickTox kits for QuickScan fumonisin and deoxynivalenol can detect the respective residues ranging from 0.20 to 6.0 ppm, while the EnviroLogix QuickTox kits for QuickScan aflatoxin can detect aflatoxin residues ranging from 2.5 to 30 ppb. Each sample was first vortexed for 15 s prior to mycotoxin extraction. To extract aflatoxin, 5 ml of busaa was measured into a disposable sample cup. 10 ml of ethanol was added and placed on a shaker for 2 min to extract aflatoxin. The mixture was then allowed to separate into two layers. 100 µl of the top yellowish layer was diluted by addition of 100 µl of DB2 buffer in reaction vials. The buffer and the sample extracts were mixed thoroughly by drawing up and down in the pipette tip until the mixtures were uniformly yellow. Aflatoxin QuickTox Strips were placed into the reaction vials and allowed to develop for 5 min. The bottom section of each strip covered by the arrow tape was immediately cut off and discarded while the strips were inserted into the QuickScan reader for quantification.

To extract fumonisin, 5 ml of busaa were measured into a disposable sample cup and 10 ml of ethanol was added and placed on a shaker 2 min to extract fumonisin. 100 µl of the top yellowish layer of the extract was diluted with 100 µl of 50% ethanol in a dilution vial. Mixing was done by drawing up and down in the pipette tip until the mixture was uniformly yellow. 100 µl of the diluted extract was mixed with 100 µl of DB2 Buffer in a second reaction vial. Fumonisin QuickTox strips were placed in the mixtures and allowed to develop for 5 min. The bottom section of each strip covered by the arrow tape was immediately cut off and discarded then the strip was inserted into the QuickScan reader for quantification. To extract DON, 5 ml of busaa was measured into a disposable sample cup and 25 ml of room temperature tap water was added then placed on a shaker for 30 s. The extract was allowed to separate into two layers and the top tan layer was used in the test. 100 µl of the top tan layer was mixed with 100 µl of DB1 buffer in a reaction vial. DON QuickTox strips were placed in the reaction vials and allowed to develop for 10 min. The bottom section of each strip covered by the arrow tape was immediately cut off and discarded and the strip was inserted into the QuickScan reader for quantification.

## RESULTS AND DISCUSSION

This study shows that the incidence of the mycotoxins tested were as follows: aflatoxin 93% (mean: 5.2±0.2 µg/kg; range 2.8 to 11 µg/kg), fumonisin 9.8% (mean: 1460±188 µg/kg; range 280 to 4000 µg/kg) and DON 23% (mean: 259±5.2 µg/kg; range: 200 to 360 µg/kg). 65.6% of the samples had aflatoxin levels above the limit set by the European Union (4 µg/kg) (Table 1). Co-occurrence of the mycotoxins was also observed in the samples. Aflatoxin and fumonisin co-occurred in 9.8% of the samples, aflatoxin and DON co-occurred in 23% while all the mycotoxins co-occurred in 3.3% of the

**Table 1.** The incidence of aflatoxin, fumonisin and DON in busaa from Bomet County, Kenya in 2011.

Mycotoxin	Number of sample assayed	Number of samples above LOD	Incidence (%)	Mean ( $\mu\text{g}/\text{kg}$ )	Range ( $\mu\text{g}/\text{kg}$ )
Aflatoxin	61	57 <sup>1</sup>	93	5.2 $\pm$ 0.2 <sup>1</sup>	2.8 - 11
Fumonisin	61	6 <sup>2</sup>	9.8	1460 $\pm$ 188 <sup>2</sup>	280 - 4000
DON	61	14 <sup>3</sup>	23	259 $\pm$ 5.2 <sup>3</sup>	200 - 360

<sup>1</sup> LOD = 2.5  $\mu\text{g}/\text{kg}$ , <sup>2</sup> LOD = 200  $\mu\text{g}/\text{kg}$ , <sup>3</sup> LOD = 200  $\mu\text{g}/\text{kg}$ .

samples.

This study was carried out in a rural setup where agriculture is the main economic activity and brewing is practiced by some women as an additional source of income. The ingredients of the brew are usually obtained locally or from one's own produce. However, the study was carried out at a time when the region was facing maize shortage due to attack by maize lethal necrotic disease hence most brewers had temporarily stopped brewing. Additionally, most maize available for sale had been brought into the county from other regions mostly from the North Rift region of Kenya. Busaa is a product of a two-stage fermentation process. In the first stage, water is added to raw maize flour to form a stiff mixture then covered and allowed to ferment at ambient temperatures 22 to 30°C for 2 to 3 days. The acidified mixture is then roasted, usually on a large metal sheet, resulting in a desirable roasted flavor. The roasted product is mixed with water and finely ground finger millet malt is added and allowed to ferment for another 2 to 3 days. This second fermentation phase results in production of lactic acid and alcohol. Good quality busaa is opaque and creamy brown at the time of consumption due to consistent dispersion of starch and other cereal residues. Busaa should be consumed as soon as it is ready for consumption because prolonged storage results in fermentative acidification which leads to loss of consistent dispersion of starch and separation of the sediment. Due to the visual deterioration and increased sourness, the product becomes unacceptable and is usually rejected by its consumers. Therefore, there is usually a mutual agreement among brewers and consumers of busaa to first exhaust a drink from one brewer before proceeding to the next. Brewers also brew in turns so as to minimize spoilage.

This whole process of busaa production involves ingredients, moist conditions and ambient temperatures that favor fungal growth and hence mycotoxin production. Maize, the main ingredient of busaa, has been found to be an excellent substrate for mycotoxin production given a favorable environment for mycotoxigenic fungal growth (Alakonya et al., 2009). Kenji et al. (2000) reported as high as 1120  $\mu\text{g}/\text{kg}$  of total aflatoxins in malted maize and 86% incidence of aflatoxin B1. Besides, the malting process of finger millet and the fermentation of the maize flour, the dough allow the contaminating fungi to grow and produce mycotoxins especially due to the poor

hygienic handling conditions involved. Kenji (2003) studied aflatoxins in busaa in the slums of Nairobi and found out that 68% of the samples analyzed had concentrations of aflatoxin higher than 5 ppb and 17% were above 50 ppb. Nkwe et al. (2005) however detected neither aflatoxin nor fumonisin in sorghum-based traditional malt in Botswana. Deoxynivalenol has been reported to be a heat stable toxin that can be found in cereals such as wheat and maize as well as their products (Jacobsen, 2008). Although there is a roasting step during busaa preparation, the mycotoxins still persist due to their stability. Mbugua and Gathumbi (2004) also reported the presence of fumonisins and DON in Kenyan Pilsner and Tusker beers. While the incidence of fumonisin and DON were low in this study, as well as their levels being within the limits set by the European Union (400 and 1750  $\mu\text{g}/\text{kg}$ , respectively), the effects of the toxins are still a cause of concern if the volumes of busaa consumed daily are to be considered. It has been reported that consumers of busaa can drink as much as 2 L per day per person almost on a daily basis (Kenji, 2003).

The co-occurrence of multiple mycotoxins even in low quantities may have higher toxicity with serious health consequences for regular busaa consumers. Although, exposure to low levels of mycotoxins may not cause immediate effects, exposure to these toxins over a long period of time may result in long term effects. This may be attributed to the high incidence of esophageal cancer in Bomet County as reported by the findings of Wakhisi et al. (2005). Additionally, since it is a common practice in the Kenyan rural areas for busaa consumers to exhaust a drink from one brewer before proceeding to the next, there is obvious exposure to different mycotoxins occurring at different levels daily. The possibility of passage of mycotoxins from the raw materials to the brew should not be ignored as spoilt maize grains are often diverted to traditional brewing or animal feeds. The purity of the ingredients of traditional brews determines the purity of the brew as suggested by the findings of Nkwe et al. (2005)

## CONCLUSION AND RECOMMENDATION

This study confirms the presence of multiple mycotoxins in busaa from Bomet county which has the highest rate of

esophageal cancer in the world. Regular consumption of the traditional brew therefore poses a health risk to its consumers. The study was carried out at a time that the region was facing maize shortage and for more satisfactory findings of mycotoxin contamination in busaa, we recommend further studies when there is a bumper harvest. We also recommend mycotoxicological quality control of traditionally produced brew so as to ensure public mycotoxicological safety.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

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