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Article

Determination of Levels of Ochratoxin A in Selected Cereal Grains Flour, Baked Wheat Bread and Finger Millet Brew Retailed in Market Outlets in Nairobi County, Kenya

Peter K. Karoki[1], Cecilia W. Kathurima[2], Ezekiel K. Njoroge[2], Sauda Swaleh[1], Wilson M. Njue[1,*]

[1] *Chemistry Department, Kenyatta University, Kenya*

[2] *Coffee Research Foundation, Ruiru – Kenya*

* Author to whom correspondence should be addressed; E-Mail: wilsonnjue@yahoo.com

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Abstract: Mycotoxins are of grave concern in food safety due to their health risks on both humans and animals. Ochratoxin A (OTA), a naturally occurring carcinogenic mycotoxin, was assessed in forty four (44) samples of wheat *(Triticum aestivum)* flour, sorghum (*Sorghum bicolor*) flour, finger millet (Eleusine *coracana*) flour, wheat bread and traditional finger millet malted brew (Busaa). The samples were extracted for OTA, followed by clean up using SPE cartridges before determination by High Performance Liquid Chromatography (HPLC). Results showed that most of the samples contained OTA. The levels in finger millet flour and sorghum flour were 0.6302±0.0203 ng/g and 0.7439±0.0254 ng/g respectively. White wheat flour recorded lower levels (0.1174±0.0385 ng/g) than whole wheat flour (0.6176±0.0445 ng/g). The levels in whole wheat and white wheat bread were 0.9842±0.0904 ng/g and 0.3475±0.0158 ng/g respectively. Open-exposed whole wheat bread showed significantly higher levels of OTA (2.4938±0.1172 ng/g) than in white bread (1.7633±0.0243 ng/g). The overall mean OTA content in finger millet brewers malt and traditional finger millet malted brew was 0.7658±0.0192 ng/g and 3.0189±0.9452 ng/g respectively. There was significant variation in OTA levels in samples from different markets which was attributed to difference in storage conditions and quality

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of raw materials. Although the levels of OTA in flour samples were lower than the set limits in some countries, there is potential health risk associated with chronic exposure to OTA. The outcome of this study provides baseline data on the levels of OTA in cereals flour and their processed products retailed in Nairobi, Kenya.

Keywords: Ochratoxin A, Flour, Wheat, Sorghum, Finger millet, Malted brew, HPLC.

1. Introduction

Mycotoxins, secondary metabolites of *Aspergillus* and *Penicillium* species, are considered serious threat to the quality of agricultural commodities and a variety of food substances, which results to a great risk to animals and human health worldwide (Bennett and Klich, 2003). Mycotoxin contamination of commonly consumed commodities has been associated with diseases like cancer, teratogenicity, neurotoxicity, hepatotoxicity and nephrotoxicity in consumer population in Africa and other parts of the world (Bennett and Klich, 2003). Their presence also lowers the quality of agricultural produce and other food commodities.

Ochratoxin A (OTA), whose chemical structure is shown in Figure 1, is a mycotoxin of toxicological significance found in agricultural commodities and foodstuff. *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium verrucossum* are the main mold species that produce OTA in tropical and temperate climate regions (Héctor *et al.*, 2003).

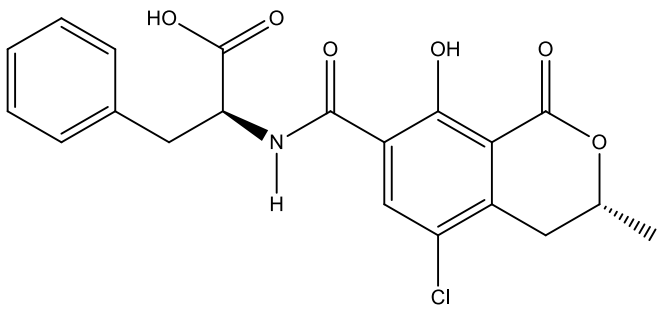

Figure 1: Structure of ochratoxin A

Many of the agricultural areas in Kenya are characterized by hot and humid climatic conditions, accompanied by regular rainfall. These conditions provide a conducive environment for proliferation of ochratoxigenic fungi (Enyiukwu *et al.*, 2014). Inadequate drying of agricultural crops following early harvesting and poor storage facilities may necessitate the growth of the fungi (Fernandez-cruz *et al.*, 2010), which are consequently carried over to food products.

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Ochratoxins contamination in cereals and their products is of economic importance in many countries of the world. However, few studies have been relatively conducted on OTA contamination in Kenyan cereals based flour and their processed products. This has partly contributed to less emphasis on legislation of maximum allowable limits of OTA in foodstuffs in Kenya. This study, therefore, aimed at documenting OTA levels in finger millet flour, sorghum flour and wheat flour and their products which include raw finger millet brewers malt, traditional finger millet malted brew (busaa) and wheat bread retailed in market outlets in Nairobi County, Kenya.

2. Materials and Methods

2.1. Sampling

A total of forty four (44) samples were collected. Finger millet flour, sorghum flour samples were obtained from Githurai, Gikomba and Nyamakima main cereal market outlets. Raw finger millet brewer malt and traditional finger millet malted brew (locally known as busaa) were purchased from local traditional brewers. Branded wheat flour and wheat bread came from grocery stores in Central Business District, Nairobi County. Sampling was done according to method by Krejcie and Morgan, 1970. Samples were procured and transported to the laboratory in sterile sample collection bags. They were thoroughly homogenized prior to extraction of ochratoxin A.

2.2. Chemicals and Reagents

Ochratoxin A standard solution (10µg/L), HPLC grade acetonitrile, HPLC grade water and acetic acid were obtained from Sigma Aldrich (UK). Chloroform, toluene, ethyl acetate-90%, formic acid-85%, anhydrous sodium sulphate, sodium bicarbonate, methyl alcohol, acetone, diethyl ether and phosphoric acid were of analytical grade.

2.3. Preparation of OTA Standards Solutions

A 10 µg/L stock solution of OTA standard was diluted to produce working standards of 2, 4, 6, 8 and 10 µg/L, and were stored at refrigerated temperature of 4 ºC before use.

2.4. Extraction and Clean-up of the Samples

2.4.1. Extraction of OTA from solid samples

Samples were extracted according to the method described by Braicu *et al.*, 2008 with slight modification. 50 mL of chloroform was added to 10 g of sample in a 250 mL conical flask and sonicated for 10 minutes at room temperature. The extract was filtered under vacuum and washed once with 50 mL of distilled water. The chloroform extract was dried over 20 g of anhydrous sodium

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sulphate, filtered, then concentrated to dryness on a rotary evaporator at 50 ºC and residue re-dissolved in 4 mL acetonitrile. The extract was stored in the refrigerator at 4 ºC awaiting analysis. The procedure was repeated for all the solid samples.

2.4.2. Extraction of OTA from malted finger millet brew (busaa)

Extraction was done according to method described by Welke *et al.*, 2010 for liquids. 10 mL of busaa was acidified to pH 2.0 using phosphoric acid and mixed intensively for 1 min. 5 mL of chloroform was added and the mixture centrifuged for 2500 rpm at 5 min. Clear organic phase at the bottom was transferred to an appropriate flask and extracted twice with 5 mL of 1.25% $NaHCO_3$ solution. Both phases were combined and pH adjusted to 2.5 with formic acid. Combined phases were then extracted with 20 mL of chloroform, evaporated to dryness using a rotary evaporator and re-dissolved in 4 mL of acetonitrile.

2.4.3. Sample clean-up

Clean-up was done using SPE columns according to the protocol highlighted by Ali *et al.*, 2010. Columns were pre-conditioned with 4 mL of deionized water before loading the sample at a flow rate of 1 mL/min and then washed thrice with 1 mL HCl (pH=1) followed by HCl (pH=1):acetonitrile (6:4) and eventually 10 mL of deionized water. Columns were then dried with 4 mL of acetonitrile-0.01% acetic acid at a flow rate of 5 mL/min before eluting OTA with 2 mL of methanol-2% acetic acid at a flow rate of 0.8 mL/min. The resulting eluents were evaporated to dryness and re-dissolved in 4 mL of pure acetonitrile before the analysis.

2.5. High Performance Liquid Chromatography (HPLC)

The specifications for HPLC used are shown in Table 1. Under the conditions in Table 1, OTA was eluted at 2.533 min as a single peak.

Table 1: Analytical specification for HPLC

Instrument	**Condition**
HPLC (KNEUER, GERMANY)	
Column	Analytical C-18 column 25 cm × 4.6 mm (5µm)
Mobile phase	acetonitrile : water : acetic acid (51:47:2)
Flow rate	1 mL/min
Injection volume	20 µL
Column temperature	Ambient temperature
Detector	fluorescence detection
Detection limit	0.03 µg/L
Excitation wavelength	332 nm
Emission wavelength	472 nm

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2.6. Calibration of HPLC Instrument

The standard calibration curve was obtained by running OTA standard solutions through the instrument. The corresponding peak areas were plotted against the standard concentrations to generate the curve as shown in Figure 2.

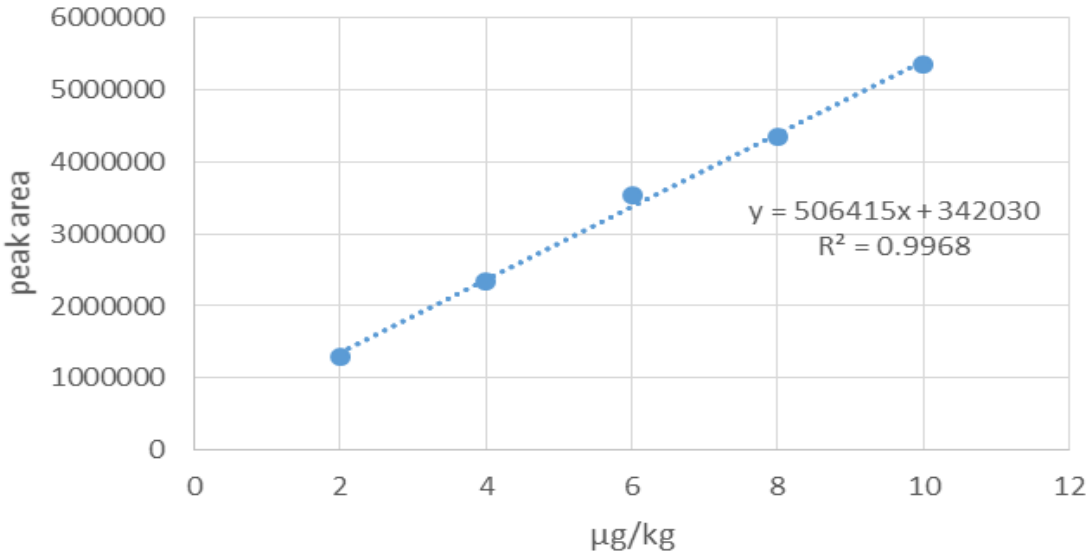

Figure 2: HPLC Calibration curve

3. Results and Discussion

The levels of OTA in finger millet flour and sorghum flour from the three market outlets are shown in Table 2. Samples from Gikomba outlet showed the highest levels of OTA at 1.4939±0.0096 ng/g and 1.9971±0.0435 ng/g in finger millet flour and sorghum flour respectively. The levels were lowest for the samples from Nyamakima outlet (0.0546±0.0104 ng/g in finger millet flour and 0.0519±0.0020 ng/g in sorghum flour). The OTA levels in samples from Githurai outlet (0.3420±0.0321 ng/g in finger millet flour and 0.1826±0.0068 ng/g in sorghum flour) were significantly higher than those obtained from Nyamakima outlet.

Table 2: OTA content (ng/g) in finger millet flour and sorghum flour

	Gikomba	**Githurai**	**Nyamakima**	**Overall Mean**
Finger millet flour	1.4939±0.0096	0.3420±0.0321	0.0546±0.0104	**0.6302±0.0203**
Sorghum flour	1.9971±0.0435	0.1826±0.0068	0.0519±0.0020	**0.7439±0.0254**

In overall, the mean OTA contamination of finger millet flour (0.6302±0.0203 ng/g) and sorghum flour (0.7439±0.0254 ng/g) were lower than the maximum allowable limit set by various countries on levels of OTA level in foodstuff. For instance, maximum allowable limit for all cereal

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products in European Union is 3 ng/g (Hans & Marco, 2005; Barber, 2007) while Indonesia has set its limits at 5 ng/g (Anukul *et al.*, 2013)

The observed significant variation in OTA levels in samples between markets was attributed to difference in storage conditions by retailers in these markets and quality of grains from main suppliers (Duarte *et al.*, 2010; Volkova, 2013). Poor storage structures with poor moisture and temperature control in Gikomba provides a favourable environment for proliferation of ochratoxigenic molds leading to higher production of OTA unlike in Nyamakima and Githurai markets. The conditions on proliferation of ochratoxigenic molds were observed by the studies undertaken (Atanda *et al.*, 2011; Milani, 2013).

Wheat flour millers pack their flour mostly in 2 and 1kg bags under different brand names. In this study anonymous brand names were used for the samples of flour from different miller companies. The concentration of ochratoxin A in whole wheat and sifted wheat flour are presented in Table 3. OTA was detected in all the samples. The overall mean concentration of OTA was significantly higher in whole wheat flour (0.6176±0.0445 ng/g) than in sifted wheat flour (0.1174±0.0385 ng/g). This difference can be attributed to the fact that some ochratoxigenic molds/OTA could have been removed during the milling process of sifted flour where outer covering of wheat corn is removed.

Table 3: Concentration of OTA(ng/g) in branded wheat flour

	Brand 1	**Brand 2**	**Brand 3**	**Overall mean**
Whole wheat flour	0.8758±0.0749	0.4674±0.0141	0.5096±0.0113	**0.6176±0.0445**
Sifted wheat flour	0.1276±0.0453	0.1098±0.0486	0.1148±0.0052	**0.1174±0.0385**

OTA concentration in whole wheat flour varied significantly from one brand to another. Brand 1 had had the highest level of 0.8758±0.0749 ng/g, followed by Brand 3 (0.5096±0.0113 ng/g), while Brand 2 recorded the lowest level of 0.4674±0.0141ng/g. This significant difference can be attributed to difference in quality control measures by various millers, which ranged from the quality of wheat grain used for processing, storage of grains prior to processing, handling of processing operations and packing and storage of processed flour prior to distribution. High quality grains and proper storage minimizes the chances for the growth of molds, hence reducing the chances of ochratoxin A production (Alberti, 2011; Zain, 2011). There was no significant variation in OTA levels in sifted wheat flour in the three brands (1, 2 & 3) analysed.

Levels of OTA was also determined in three brands of wheat bread and the results are given in Table 4. The results showed that the overall mean concentration was significantly higher in samples of whole wheat bread (0.9842±0.0904 ng/g) than in white wheat bread (0.3475±0.0158 ng/g). This significant variation could be attributed to the quality of wheat flour used in the baking process.

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Table 4: Levels of OTA(ng/g) in branded baked wheat bread retailed in Nairobi County, Kenya

	Brand D	**Brand E**	**Brand F**	**Overall mean**
Whole wheat bread	1.2594±0.0016	1.3182±0.1566	0.3751±0.0013	**0.9842±0.0904**
White wheat bread	0.1974±0.0011	0.7158±0.0273	0.1293±0.0015	**0.3475±0.0158**

The levels of OTA significantly differed from one brand to another, with Brand E recording the highest values in both whole wheat bread (1.3182±0.1566 ng/g) and white bread (0.7158±0.0273 ng/g). Brand F had the least OTA contamination in both whole wheat bread (0.3751±0.0013 ng/g) and white bread (0.1293±0.0015 ng/g). This variation can be due to quality of wheat flour used by the bakers.

The levels of OTA in samples of wheat bread under this study were lower than maximum recommended limits set by some countries on wheat bread and other wheat products. For instance, the maximum tolerable concentration of OTA in Canada and United States is 3 ng/g on wheat products (Grafenhan, 2013; Kolakowski *et al.*, 2016) while Taiwan has set a maximum allowable limit of 5 ng/g (Anukul *et al.*, 2013). Though levels of OTA in the samples studied are lower, prolonged exposure could be harmful to human health.

The effect of open-exposure of fresh bread for seventy two (72) hours on levels of OTA was examined. The results in Table 5 showed that OTA levels increased upon exposure from an overall mean of 0.9842±0.0904 ng/g to 2.1962±0.0555 ng/g in whole wheat bread samples, and from 0.3475±0.0158 ng/g to 1.3882±0.0474 ng/g in white bread.

Table 5: Levels of OTA in exposed wheat bread

	Brand D	**Brand E**	**Brand F**	**Overall mean**
Exposed whole wheat bread	2.1923±0.0084	2.5577±0.0550	1.8387±0.0783	**2.1962±0.0555**
Exposed white wheat bread	1.1634±0.0482	1.9364±0.0404	1.0648±0.0527	**1.3882±0.0474**

Similarly, levels varied significantly from brand to brand, with Brand F recording the least values of 1.8387±0.0783 ng/g and 1.0648±0.0527 ng/g in whole wheat bread and white bread respectively. Brand E recorded the highest levels of 2.5577±0.0550 ng/g and 1.9364±0.0404 ng/g for whole wheat bread and white bread respectively. This shows that baked bread is susceptible to attack by OTA producing molds on exposure. Therefore, prolonged exposed bread could be harmful for human consumption.

Finger millet flour in addition to making of hot meal, it is used in fermented form in brewing an alcoholic drink by some communities in Kenya, referred to as Busaa. In this study, OTA was assessed in finger millet brewer's malt and in Busaa, and the results are represented in Table 6. The levels of

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OTA in brewer's malt samples from Gikomba and Githurai outlets were 0.7979±0.0175 ng/g and 0.7337±0.0207 ng/g respectively. The levels in the traditional brew (busaa) from Gikomba and Githurai outlets were 3.3415±0.1634 µg/L and 2.6963±1.3267 µg/L

Table 6: OTA content in finger millet brewer's malt (ng/g) and finger millet malted brew (µg/L)

	Gikomba	**Githurai**	**Overall Mean**
Raw brewer's finger millet malt	0.7979±0.0175	0.7337±0.0207	**0.7658±0.0192**
Local finger millet brew (Busaa)	3.3415±0.1634	2.6963±1.3267	**3.0189±0.9452**

The overall OTA mean level in busaa (3.0189±0.9452 µg/L) was significantly higher than that of its starting raw malted material (0.7658±0.0192 ng/g) at 95% confidence level. This shows that OTA producing molds continue to grow during the brewing process hence higher OTA concentration in the final brew. Poor quality of brewer's raw malt, lack of quality control in brewing process and poor brewing facilities could be the reason for the continued mold growth. According to European Commission regulations on OTA, the maximum allowable limit on all cereal derived liquid products for human consumption is 3µg/L and hence there can be serious health impact on the continued consumption of busaa.

4. Conclusion

The study has provided a documentation of ochratoxin A contamination in some Kenyan cereals products retailed in Nairobi County. OTA contamination in wheat flour, wheat bread, sorghum and finger millet flour intended for direct human consumption, were lower than maximum limits set by countries with regulations on OTA levels in foodstuff. Busaa, an informally brewed traditional drink had the highest levels of OTA (3.0189±0.9452 µg/L). The public should be sensitized on health risks associated with consumption of the alcoholic brew.

There is need for further studies on OTA contamination in other cereal products like maize flour, which is a staple food product for majority of Kenyan population. Results obtained from this study could form the basis of formulating legislations of maximum allowable limits for OTA in Kenyan cereals and cereal derived products, as well as creating awareness on the importance of proper storage practice of the agricultural produce so as to minimize OTA contamination.

Potential Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgment

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