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Detection of mycotoxins in opaque beer production

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Abstract

In Zimbabwe opaque beer is one of the most common alcoholic beverages. This study was aimed at detecting aflatoxins and Ochratoxin A (OTA) in commercial opaque beer brewing. Aflatoxins and OTA are mycotoxins produced by fungi. Maize, sorghum malt and opaque beer samples were used for microbiological plating and multi mycotoxin extraction. Aflatoxins, OTA, *Aspergillus spp*, total viable count, coliforms, *Lactobacillus spp*, *Salmonella*, *Shigella*, *Staphylococcus aureus* were assessed. Mycotoxins were separated using an High Performance Liquid Chromatography (HPLC). Aflatoxins were not detected in all the samples. OTA concentration for malt was 18 µg/kg, 5.2 µg/kg in beer and absent in maize. Fungi identified in maize were *Aspergillus flavus, Aspergillus niger, Aspergillus carbonarius,* and *Aspergillus fumigatus.* Malt contained *Aspergillus flavus* and *Aspergillus carbonarius.* Fungi observed in beer were *Aspergillus flavus* and *Aspergillus niger.* The beer may need heat treatment methods in order to remove the microorganisms identified and prolong shelf life of the product.

Keywords: *Mycotoxins, Aflatoxins, Ochratoxin A (OTA), Opaque beer, Food safety*

1. Introduction

Consumption of home or commercial traditional brewed beer in Africa is one main characteristic that one is deeply rooted in the African culture, Haggblade and Holzapfel (1989); Shale *et al*. (2014). Opaque beer is an alcoholic beverage which is made from grain derived by the fermentation of cereal mash with no additives added and has a characteristic pale red brown-dark brown color.

Opaque beers are very rich in calories, B-group vitamins including thiamine, folic acid, riboflavin and nicotinic acid, and essential amino acids such as lysine indicated by François *et al.* (2012). Moreover, different agricultural produce are used as opaque beer brewing material for instance sorghum malt, barley malt, corn, millet and *rapoko* which are susceptible to fungal infestation most likely at the storage site and or malting stages stated by Shale *et al.* (2014). Fungal infestation produces toxicants called aflatoxins which are harmful to human health.

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Toxigenic fungi are responsible for production of aflatoxins Dors *et al.* (2011) such as *Aspergillus flavus* and *Aspergillus parasiticus*. Dors *et al.* (2011) suggests that humans can be exposed to aflatoxins by the periodic consumption of contaminated food this leads to proliferation in nutritional deficiencies, immunosuppression and hepatocellular carcinoma. The occurrence of aflatoxins (AFs) has been found to be dominant among different types of food such as spices, cereals, oils, fruits, vegetables, milk and meat asserted by Dors *et al.* (2011). There are 18 different types of aflatoxins known the major members are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2) (Dors *et al.*, 2011).

Ochratoxin A (OTA) is a widely distributed toxin produced by *Aspergillus* and *Penicillium* genera. OTA displays variable toxicities, particularly having nephrotoxic, hepatotoxic, carcinogenic and immunotoxic effects. In humans, OTA has also been associated with urinary tract cancers and other renal diseases hence classified as a possible human carcinogen, Group 2B by the IARC (1993). The Codex Alimentarius (1991) set the maximum limit for OTA in grains at 5 μ / kg.

Contamination of foods with naturally occurring toxicant mycotoxins is unavoidable and unpredictable and poses a unique challenge to food safety postulated by Park (2002). Mycotoxins have been epidemiologically implicated as carcinogens in humans, hence are a threat to food safety. Aflatoxins and OTA detection in foods is critical to food safety and public health. These mycotoxins are hazardous to both human and animals health.

In Zimbabwe there is limited information about aflatoxins and OTA research therefore there was a need to research about them. Among other than infectious agents, mycotoxins are ranked as the most important chronic food safety risk factor, and of greater risk than synthetic food contaminants, plant toxins, food additives or pesticide residues. Hot and humid conditions encourage aflatoxin formation, Atanda *et al*. (2011) in case of opaque beer brewing sorghum malting occurs under these conditions. According to Lizárraga-Paulín *et al*. (2013) countries situated between 40 ºN and 40 ºS are most at risk of crops aflatoxin contamination. About 4.5 billion individuals in developing countries of the location mentioned are at jeopardy of chronic mycotoxins exposure Lizárraga-Paulín *et al*. (2013). Opaque beer is made from sorghum malt and maize which are all susceptible to fungal infestation during storage or production, hence the need to evaluate and quantify aflatoxins and OTA in opaque beer to ensure that the product is safe for human consumption.

Severe cases have been reported in parts of Africa for example in opaque beer in Malawi according to Matumba *et al.* (2011) 6.6-54.6 μgkg^{-1} in sorghum malt .Therefore there is a concern on the consumers of opaque beer with respect to aflatoxins and OTA. In this regard this research was carried out to determine the levels of aflatoxins and OTA.

2. Objective

Opaque beer is made from sorghum malt and maize which are all susceptible to fungal infestation during storage or production, hence the need to evaluate and quantify aflatoxins and OTA in opaque beer to ensure that the product is safe for human consumption.

3. Methodology

3.1. Sample collection

Samples were taken from an opaque beer production company (Table 1, Figure 1). Samples units included sample S1 (3 × 100 g) maize meal, sample S2 (3 × 100 g) sorghum malt and sample S3 (3 × 100 mL) of the sorghum opaque beer. The samples were collected in freshly produced supplies using sterile sampling bags (Whirl-pack, NASCO). The samples were immediately frozen at –20 ºC and transported to the laboratory, mixed thoroughly and centrifuged for 5 min at 1000 × g.

Table 1: Sampling points

Sampling point	Sample
S1	Maize
S2	Sorghum malt
S3	Opaque beer

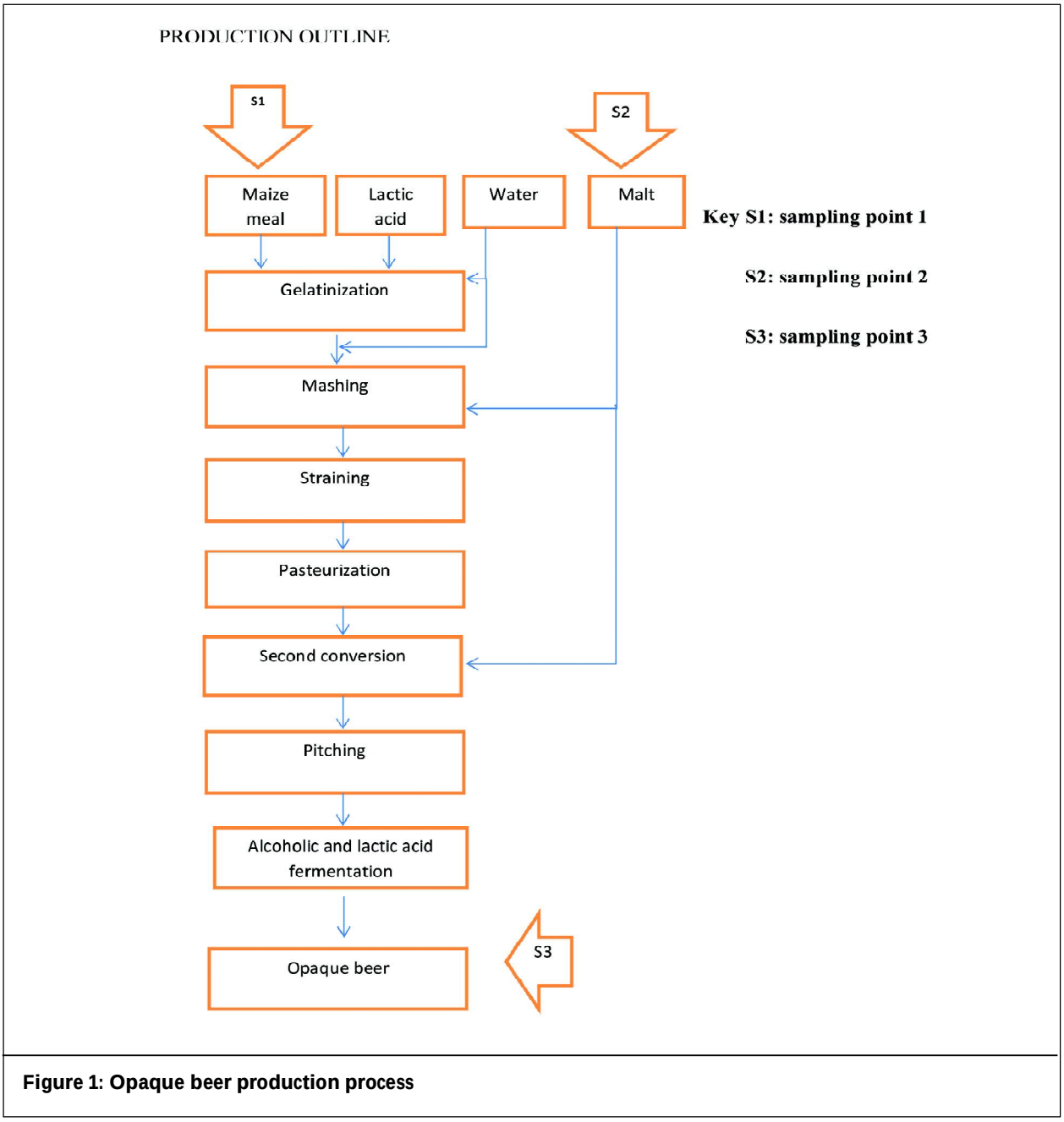

Figure 1: Opaque beer production process

3.2. Sample preparation

Maize grains were ground into small particles. Maize meal and sorghum malt were weighed 9 g for microbiological plating and 25 g for multi-mycotoxin extraction. Opaque beer was measured 9 mL for microbiological plating and 25 mL for multi-mycotoxin extraction.

4. Laboratory Analysis

4.1. Microbial analysis

Total viable count was determined and enumerated using Plate Count Agar (PCA) at an incubation temperature of 37 °C for 24 h. MacConkey Agar a selective media was used for coliforms at 37 °C for 24 h. Potato Dextrose Agar (PDA) was used for yeast and molds determination and plates were incubated at 25 °C for seven days.

The pour plate method was used to determine the presence of *Lactobacillus* species using MRS agar at 37 °C for 24 h. *Salmonella* and *Shigella*, samples were plated on XLD and were incubated for 24 h at 37 °C.TSI test was used to confirm the presence of *Salmonella* and *Shigella*. Mannitol salt agar was used for the isolation of *Staphylococcus aureus*, incubation was done at 37 °C for 24 h. A further confirmatory test using H_2O_2 was used to check for the presence of *S. aureus.*

4.2. Mycology analysis

Mycological analyses were conducted for randomly selected samples. *Aspergillus species* were cultured onto *Aspergillus flavus* and *A. parasiticus* media (AFPA). Samples were plated onto AFPA plates. The spread plate method was used for all samples. This was followed by incubation at 30 °C for five days. The isolated fungi were enumerated from plates showing well separated colonies and identified using colony color, colony texture and soluble pigmentation (Pitt and Hocking, 2009). The number of fungal colonies per gram of food was calculated and expressed as colony forming units per gram of food (c.f.u / g).

4.3. Multi-mycotoxin extraction

Multi-mycotoxin clean-up and extraction methods were implemented. This procedure is done to separate the part constituted the Acid Fraction (A.F) of the extract from the Neutral Fraction (N.F). The Figure 2 below shows a flow diagram explaining the procedure of multi-mycotoxin extraction for the A.F and N.F. Both fractions of extracts (acid and neutral) were stored at 4 °C in refrigerator until analysis by High Performance Liquid Chromatography (HPLC).

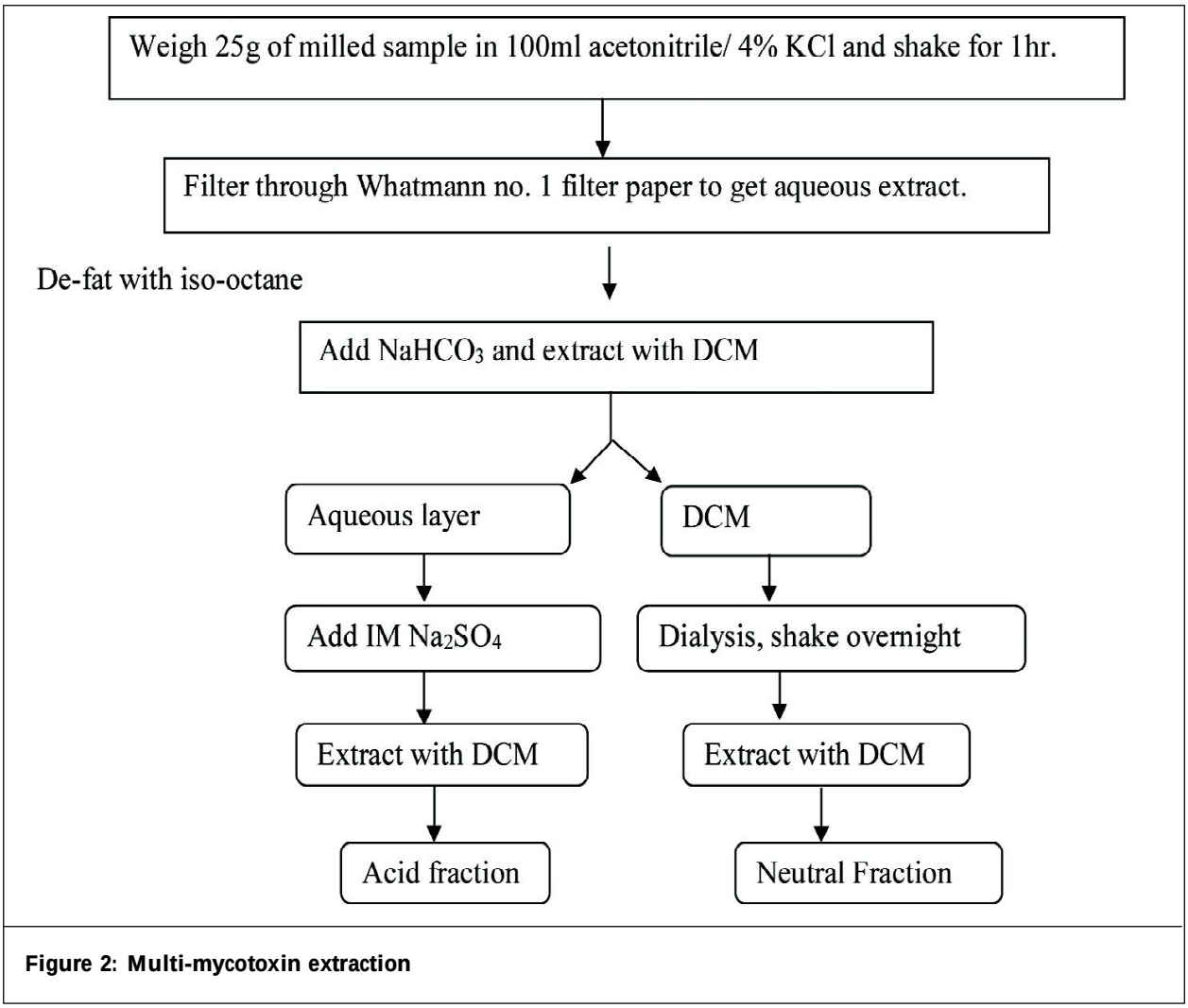

Figure 2: Multi-mycotoxin extraction

4.4. Quantitation of aflatoxins and Ochratoxin A using HPLC

Mycotoxins were isocratically separated using an HPLC Shimadzu Model LC20A System, Pump (A & B) - 2 LC-10AT, Detector - SPD-20A, Column Oven - CTO-20A, Column - C18 column, Controller - CBM-20Alite, Software – LCSOLUTION. For aflatoxins mobile phase used was H_2O:MeOH:ACN (50:40:10 v / v), i.e., water, methanol, and acetonitrile at 50 ºC and for OTA: ACN:H_2O:AA (50:48:2 v / v), i.e., acetonitrile, water, and acetic acid mobile phase was used at 40 ºC. The fluorescence detector was set at the excitation wavelength of 330 nm and emission wavelength of 450 nm. Quantification of each toxin was performed by measuring their peak areas and comparing them with their relevant standard calibration curve.

5. Results

Malt had the highest colony count of $\log_{10}$4.7 cfu / mL followed by maize $\log_{10}$3.3 cfu / mL then beer with the lowest count of $\log_{10}$1.9 cfu / mL as illustrated by Figure 3. Microorganisms were identified by their shape and color of colonies on maize, malt and beer. Total bacterial counts are used as indicators of hygiene. Bokulich and Bamforth (2013) stated that all grains (maize and sorghum) contain microflora due to microbial colonization that occurs in the field and during storage. The population of some microorganisms increases substantially during steeping and germination. Conversely the high temperatures used for pasteurization in opaque beer lower the microbial count for beer.

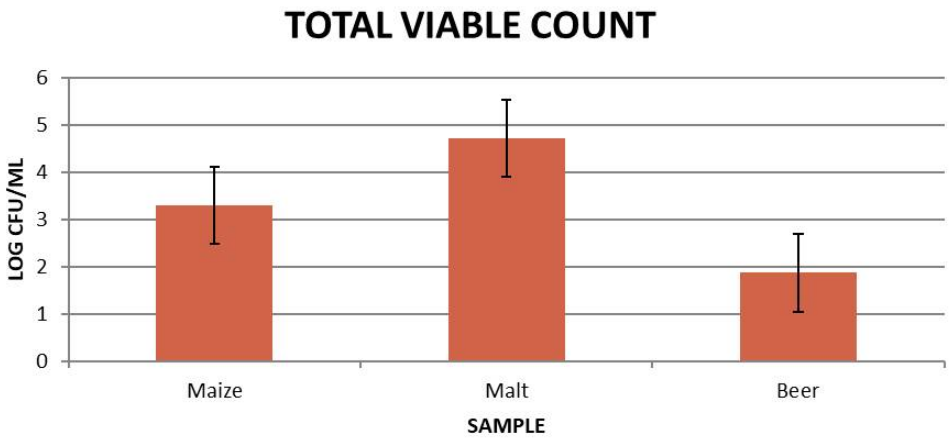

Figure 3: Total bacterial count in maize, malt and beer

In Figure 4, it is evident that maize (Log_{10}2.9 cfu / mL) and malt (Log_{10}5.0 cfu / mL) have coliforms whilst beer had no coliforms. As a measure of hygiene, effectiveness of quality systems in place coliforms were analyzed. This could indicate the effectiveness of the processes employed during opaque beer processing suggests Kutyauripo m *et al.* (2009). The failure to detect the organism does not rule out the presence of coliform in beer. Presence of coliform in maize and malt is quiet normal because coliform bacteria are found in the soil, water and animal wastes. Contamination could have occurred in the grain field.

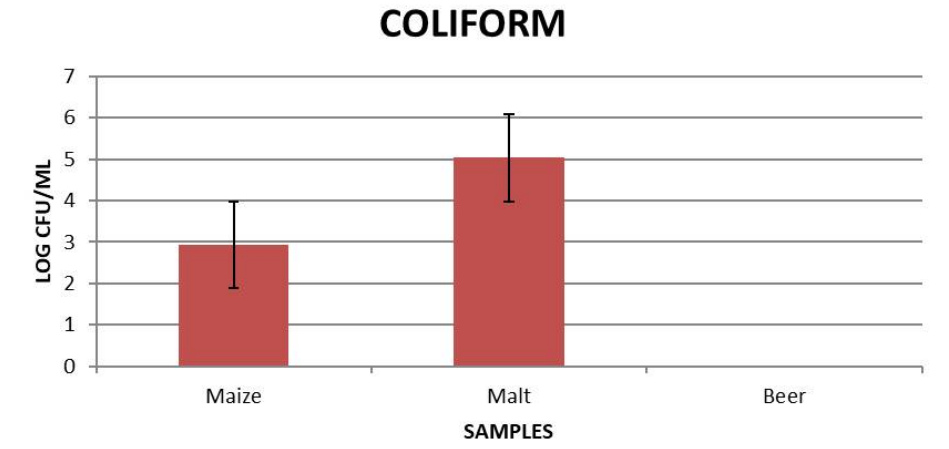

Figure 4: Coliform count in maize, malt and beer

Salmonella and Shigella were detected in maize and beer this is a signal of poor handling of raw materials and final product (beer) (Table 2). They are transmitted to food through human faecal wastes and contaminated water Bokulich and Bamforth (2013). *Salmonella and Shigella* are pathogenic microorganisms of concern their presence in beer renders the product unsafe for human consumption.

All the three samples had *S. aureus* positive which was confirmed by the formation of bubble in H_2O_2. *S.aureus* contamination is through skin contact it is prevalent in improperly cleaned hands or skin. It is important that (food handlers) are educated about personal hygiene and how to wash hands in the best effective way recommended by World Health Organization (WHO). Pathogenic and opportunistic microorganisms like *S. aureus* are very crucial to public health. It is necessary for food production companies to set up quality and food management system to reduce or eliminate these microorganisms from their production line and most importantly the final product which has a potential of endangering the consumer.

All samples were contaminated with *Lactobacillus spp.* beer having the highest count, followed by maize and malt being the lowest. The presence of LAB in the brewing raw materials (malt and maize) is through natural contamination either in the field or during storage. Opaque beer has the highest count of LAB due to the absence of antibacterial activity of hop-derived compounds that prevent LAB growth in clear beer. The bacteria beneficial lowers the pH inhibits growth of other bacterial that cannot survive in low pH however if beer is sold while microbiologically active action of *Lactobacillus spp* may end up spoiling the product (sour beer). LAB bacteria are responsible for souring the beer by the production of lactic acid, haze formation, and or diacetyl production, which gives the beer an intense aroma of artificial butter states Bokulich and Bamforth (2013).

Table 2: Determination of *Salmonella*, *Shigella* and *S. aureus*

Sample	*Salmonella and Shigella*		*S. aureus*	
	Characteristics in XLD	**Characteristics in TSI**	**Characteristics in MSA**	**Characteristics in H_2O_2**
Maize	Colorless colonies with black centres (*Salmonella*) and colorless colonies (*Shigella*)	Positive	Yellow colonies	Positive
Beer	Colorless colonies with black centers (*Salmonella*) and colorless colonies (*Shigella*)	Positive	Yellow colonies	Positive
Malt	No colonies	Negative	Yellow colonies	Positive

Table 3: Identified fungi in maize, malt and beer

Sample	Isolated fungal species
Maize	***Aspergillus flavus, Aspergillus niger, Aspergillus carbonarius, Aspergillus fumigatus***
Malt	***Aspergillus flavus, Aspergillus carbonarius***
Beer	***Aspergillus flavus, Aspergillus niger***

Although *Aspergillus flavus* was isolated from all samples, aflatoxins (Table 3) (B1, B2, G1, and G2) were not detected in any of the samples. In this research OTA was quantified in malt and beer. Fungi detected is more resistant to heat and pH. *Aspergillus carbonarius* could be responsible for production of ochratoxin since *A. ochraceus* was not identified. Taniwaki *et al*. (2003) claim that *A. carbonarius* and *A. Niger* produce OTA. It is possible that the whole *Aspergillus* species can produce OTA. The presence of *A. ochraceus* cannot be ruled out since strain identification is based primarily on morphological traits. It is also possible that the *A. ochraceus* cannot survive the pH of beer that lowers as the beer ages but the toxin produced is persistent to both heat and pH therefore it survives. OTA can be produced in the raw materials and eventually ends up in the beer.

5.1. Aflatoxins

This study investigated whether aflatoxins B1, B2, G1, G2 are present in maize, malt and beer. Aflatoxins where not detected in all the samples however this does not mean that aflatoxins are not present but could have been too low for detection using HPLC.

Figure 5 shows that malt had the highest average of 18 µg/kg, beer had an average of 5.2 µg/kg and maize had none (0). Figure 5 shows that malt and beer samples exceeded Codex Alimentarius regulated limit for OTA which is 5 µg/kg. High-levels of OTA in malt samples usually indicate that storage and/or processing conditions favored fungal growth as stated by Gumus *et al*. (2004). In the production of malt, steeping and germination stages favor fungal contamination and production of mycotoxins. The population of some microorganisms including many of the fungi increases substantially during steeping and germination. Taniwaki *et al*. (2003) claim that *A. carbonarius* and *A. niger* produce OTA. It is possible that *Aspergillus species* can produce OTA.

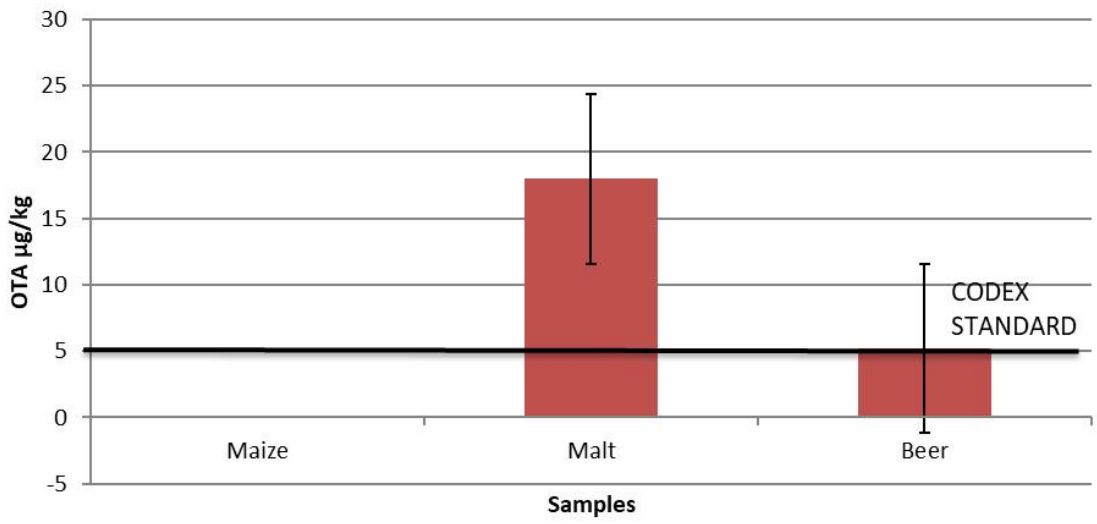

Figure 5: Levels of OTA in maize, malt and beer

6. Conclusion

In the present study, no aflatoxins were detected in the opaque beer. However the study revealed the presence of OTA in both the ingredients and the product of the opaque beer production process. Therefore, there is need to improve in the storage of the raw materials and implementation of Food Safety Management Systems in order to ensure product safety.

References

Atanda, S. A., Pessu, P. O., Agoda, S., Isong, I. U., Adekalu, O. A., Echendu, M. A. and Falade, T. C. (2011). Fungi and mycotoxins in stored foods: Nigerian stored products. *African Journal of Microbiology,* 5(25), 4373-4382.

Bokulich, N. A. and Bamforth, C. W. (2013). The microbiology of malting and brewing. *Microbiology and Molecular Biology,* 77(2), 157-172.

Codex Alimentarius Commission (1991). Rome, 1- 10 July. CL 1990/17-FAC June 1990.

Dors, G. C., Caldas, S. S., Bemvenuti, F. V., Hackbart, H. S. C., Moraes de Souza, M., Oliveira, M., Garda-Buffon, J., Primel, E. G. and Badiale-Furlong, E. (2011). Aflatoxins: Contamination, analysis and control, aflatoxins. Dr. Ramon G. Guevara-Gonzalez (Ed.) Biochemistry and Molecular Biology. InTech. Crotia 415-438.

François, L., Jacques, G., John, N., Emmanuel, B. and Philippe, T. (2012). Characteristics of African traditional beers brewed with sorghum malt. *Biotechnology Agronomy Society Environment,* 16(4), 509-530.

Gumus, T., Arici, M. and Demirci, M. (2004). A Survey of barley, malt and beer contamination with Ochratoxin A in Turkey. *Institute of Brewing,* 110(2), 146-149.

Haggblade, S. and Holzapfel, W. H. (1989). Industrialization of Africa's indigenous beer brewing. In Steinraus, K. H. (Ed.) Industrialization of indigenous fermented foods. Marcel Dekker Inc: New York: 191-283.

International Agency for Research on Cancer (IARC) (1993). Toxins derived from *Fusarium moniliforme*: Fumonisins B1, B2 and Fusarin C. *Monograph on the Evaluation of Carcinogenic Risk to Humans*. 56, 445-466.

Kutyauripo, J., Parawira, W., Tinofa, S., Kudita, I. and Ndengu C. (2009). Investigation of shelf-life extension of sorghum beer (Chibuku) by removing the second conversion of malt. *International Journal of Food Microbiology*, 129(3), 271-276.

Lizárraga-Paulín, E. G., Miranda-Castro, S. P., Moreno-Martínez, E., Torres-Pacheco, I. and Lara-Sagahón, A. V. (2013). Novel methods for preventing and controlling aflatoxins in food: A worldwide daily challenge. Mehdi Razzaghi-Abyaneh (Ed.) *Aflatoxins-Recent Advances and Future Prospects*, Intech, Crotia: 93-128.

Matumba, L., Monjerezi, M., Khonga, E. B. and Lakudzala, D. D. (2011). Aflatoxins in sorghum, sorghum malt and traditional opaque beer in Southern Malawi. *Food Control*, 22, 266-268.

Park, D. L. (2002). Effect of processing on aflatoxin. Advances in Experimental Medicine Biology. Plenum Publishers, New York: 173-179.

Pitt, J. J. and Hocking, A. D. (2009). Fungi and Food Spoilage. (3rd ed). Aspen Publishers, New York: 19-52.

Shale, K., Mukamugema, J., Lues, R. J. and Venter, P. (2014). Possible microbial and biochemical contaminants of an indeginous banana beer Urwagwa. *African Journal of Food Science*. 8(7), 379-389.

Taniwaki, M. H., Pitt, J. I., Teixeira, A. A. and Iamanaka, B. T. (2003). The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *International Journal of Food Microbiology*, 82(2), 173-179.

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