East African Journal of Health and Science, Volume 7, Issue 1, 2024
Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

East African Journal of Health and Science
eajhs.eanso.org
Volume 7 Issue 1, 2024
Print ISSN: 2707-3912 | Online ISSN: 2707-3920
Title DOI: https://doi.org/10.37284/2707-3920

Original Article

High Levels of Fungal and Aflatoxin Contamination of the Production Stages of the Local Brew (Ajono) consumed in Uganda

Sarah Nantumbwe[1], Joseph Hokello[1], Rapheal Wangalwa[2], Magyezi Godfrey Begumisa[2], Rosemary Nalwanga[3] & Saphan Muzoora[3]*

[1] Busitema University, P. O. Box 236, Tororo, Uganda.
[2] Mbarara University of Science and Technology, P. O. Box 1410, Mbarara, Uganda.
[3] Makerere University, P. O. Box 7062, Kampala, Uganda.
*Author ORCID ID: https://orcid.org/0000-0002-6721-5933; Email: josfrah4@gmail.com

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Date Published:
13 February 2024

Keywords:
Fungi, Moulds, Yeasts, Aflatoxins, Local Brews, Fermentation.

ABSTRACT

Local brew commonly known as Ajono is a widely consumed alcoholic beverage in Uganda. Handling practices along the production stages of the local brews may create a conducive environment for fungal colonization. This study aimed to assess fungal and aflatoxin contamination along the different production stages of Ajono from Soroti District in Eastern Uganda. A total of 180 samples were collected from the different stages along Ajono production. An interview guide was used to assess the processing practices while fungal contamination was assessed using standard microbiological methods. The study found that there are three main stages during Ajono production, namely; millet grain, fermented paste, and the liquid (Ajono) stages. During the paste fermentation stage, brewers used plastic drums (50%), pits (47%) or pots (3%) as fermentation vessels. Samples from plastic drum fermentation vessels had higher levels of fungal contamination than those from the pits and pots. Furthermore, several moulds genera including Aspergillus spp., Fusarium spp., Alternaria spp., Rhizopus spp., Penicillium spp., Cladosporium spp., and Acremonium spp. were identified along the production stages, with Aspergillus spp. as the most prevalent moulds at all stages of Ajono production. Two yeast genera; Saccharomyces spp. and Candida spp. were also isolated. Total Aflatoxin B and G group was detected along the production stages of Ajono with the highest prevalence (78.6%) seen in the liquid Ajono from pit fermenters and 68.8% in drum fermenters. Overall, all three stages of Ajono production; millet grain, fermented paste, and the liquid Ajono stages were highly contaminated with aflatoxigenic fungi which may cause adverse health effects under continued and sustained consumption of the brew.

APA CITATION

Nantumbwe, S., Hokello, J., Wangalwa, R., Begumisa, M. G., Nalwanga, R., & Muzoora, S. (2024). High Levels of Fungal and Aflatoxin Contamination of the Production Stages of the Local Brew (Ajono) consumed in Uganda *East African Journal of Health and Science*, *7*(1), 124-141. https://doi.org/10.37284/eajhs.7.1.1369.

CHICAGO CITATION

Nantumbwe, Sarah, Joseph Hokello, Rapheal Wangalwa, Magyezi Godfrey Begumisa, Rosemary Nalwanga and Saphan Muzoora. 2024. "High Levels of Fungal and Aflatoxin Contamination of the Production Stages of the Local Brew (Ajono) consumed in Uganda". *East African Journal of Health and Science* 7 (1), 124-141. https://doi.org/10.37284/eajhs.7.1.1369.

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

HARVARD CITATION

Nantumbwe, S., Hokello, J., Wangalwa, R., Begumisa, M. G., Nalwanga, R., & Muzoora, S. (2024) "High Levels of Fungal and Aflatoxin Contamination of the Production Stages of the Local Brew (Ajono) consumed in Uganda", *East African Journal of Health and Science*, 7(1), pp. 124-141. doi: 10.37284/eajhs.7.1.1369.

IEEE CITATION

S., Nantumbwe, J., Hokello, R., Wangalwa, M. G., Begumisa, R. Nalwanga & S., Muzoora, "High Levels of Fungal and Aflatoxin Contamination of the Production Stages of the Local Brew (Ajono) consumed in Uganda", *EAJHS*, vol. 7, no. 1, pp. 124-141, Feb. 2024.

MLA CITATION

Nantumbwe, Sarah, Joseph Hokello, Rapheal Wangalwa, Magyezi Godfrey Begumisa, Rosemary Nalwanga & Saphan Muzoora. "High Levels of Fungal and Aflatoxin Contamination of the Production Stages of the Local Brew (Ajono) consumed in Uganda". *East African Journal of Health and Science*, Vol. 7, no. 1, Feb. 2024, pp. 124-141, doi:10.37284/eajhs.7.1.1369.

INTRODUCTION

In Africa, local brews are traditionally prepared in homesteads and many of them confer some cultural values. These local brews are believed to constitute the highest proportion of alcohol consumption in Sub-Saharan Africa (Papas *et al.*, 2010). In addition, these local brews hold various names across the different ethnic groups and are usually made from carbohydrate rich agricultural produce (Sefa-Dedeh *et al.*, 1999). In Uganda, there is a diversity of locally processed traditional brews including; Kweete, Tonto, Waragi, and Ajono (Mwesigye and Okurut, 1995). Ajono is a widely consumed alcoholic beverage in Uganda known by different names in different regions of the country. For instance, in Eastern Uganda it is called "Ajono", in Central Uganda, it is known as "Malwa" while in Northern Uganda it is known as "Kongo ting". Ajono is a traditional alcoholic beverage made principally from finger millet (*Eleusine coracana*) which is a cereal crop (Oduori and Kanyenji, 2005). It is a fermented alcoholic beverage produced mainly at the household level valued for its taste, flavour and aroma (Muyanja *et al.*, 2010). In addition to serving as a means of socio-economic empowerment of households, Ajono is used for merry making at weddings, childbirth celebrations and for consolation of bruised souls during mourning of the diseased.

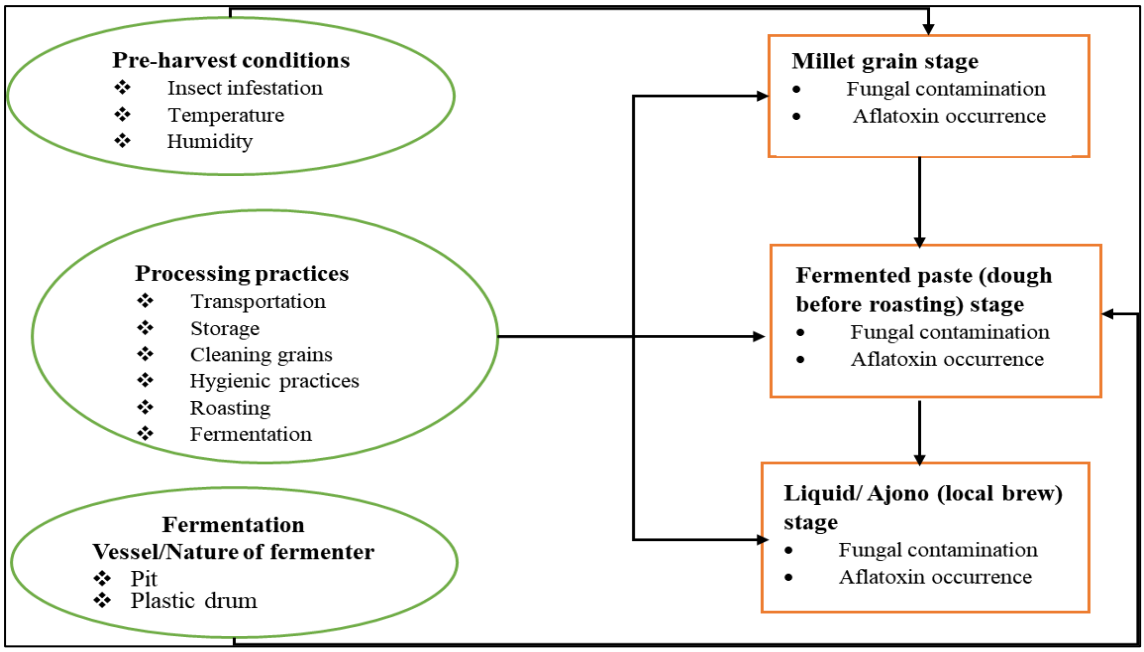
The preparation of the cereal-based local brews is carried out in environments conducive for fungal colonization and therefore, high chances of mycotoxin contamination. Handling practices involved in the preparations of the ingredients for production of the local brew can also result in mould growth. For instance, the most commonly found filamentous fungi in stored cereal grains are *Aspergillus*, *Penicillium* and *Fusarium* species (Bullermann and Bianchini, 2011). These fungi can cause food spoilage, bio-deterioration and are capable of producing different mycotoxins. *Aspergillu*s species are the most common toxigenic species in various grains, legumes, oil seeds, foods, and feeds (Bankole and Adebanjo, 2003; Bueno *et al.*, 2015; Nsabiyumva *et al.*, 2023). *Aspergillus flavus* and *Aspergillus parasiticus* are the most predominant fungi responsible for aflatoxin contamination of crops prior to and during post-harvest handling (Creppy, 2002; Payne and Yu, 2010).

Fungal invasion and contamination of cereals often begins before harvest and can continue during post-harvest handling as well as handling practices of the local brew along the production stages (Misihairabgwi *et al.*, 2018). The raw materials for local brew production may be contaminated with aflatoxins resulting from poor pre-harvest or post-harvest practices. Presence of aflatoxins in food and animal feeds is potentially hazardous to the health of both humans and animals (Samuel *et al.*, 2013). For example, aflatoxins have been associated with several health concerns including hepatocellular carcinogenesis, immunosuppression and organ toxicities (Kowalska *et al.*, 2017). In this regard, local brew production is often done following a number of stages that may involve unhygienic handling practices and processes such as fermentation and malt roasting that may predispose these brews to fungal contamination. Despite being widely consumed by the

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

population, fungal contamination due to the handling and processing practices along the different stages of Ajono production (*Figure 1*) has not been explored. Therefore, this study aimed to examine aflatoxigenic fungal contamination at the different stages of Ajono production in Soroti District in Eastern Uganda.

Figure 1: The conceptual frame-work



There are three main stages during Ajono production; millet grain stage, fermented paste stage and the liquid Ajono stage. The millet grain stage is affected by the pre-harvest conditions which may lead to fungal and aflatoxin contamination. The millet grains are processed to form the fermented paste which may lead to further contamination due to the processing practices while the fermenting vessel may cause contamination of the fermented paste. The last stage in Ajono production is the liquid Ajono stage which may be contaminated by the germinated millet flour used as malt during production.

METHODS AND MATERIALS

Study Area

The study was carried out in Soroti District in the Eastern Uganda; one of the districts where Ajono is vastly prepared and consumed (Muyanja *et al.*, 2010). Soroti District lies between latitude 1.5791°N and 1.9683°N and longitude 33.3803°E and 33.7381°E. It is bordered by Serere and Ngora Districts in the South, Kaberamaido District in the West, Amuria and Katakwi Districts in the North (*Figure 2*).

In 2014, the Uganda Bureau of Statistics (UBOS) estimated the district population to be about 296,833 (UBOS, 2017). There are predominantly two ethnicities in Soroti District namely, the Iteso and the Kumam. The main languages spoken in the district are Ateso, Kumam, and Swahili. Soroti District is made up of three counties which include; Soroti Municipality, Soroti County and Dakabela county (UBOS, 2017).

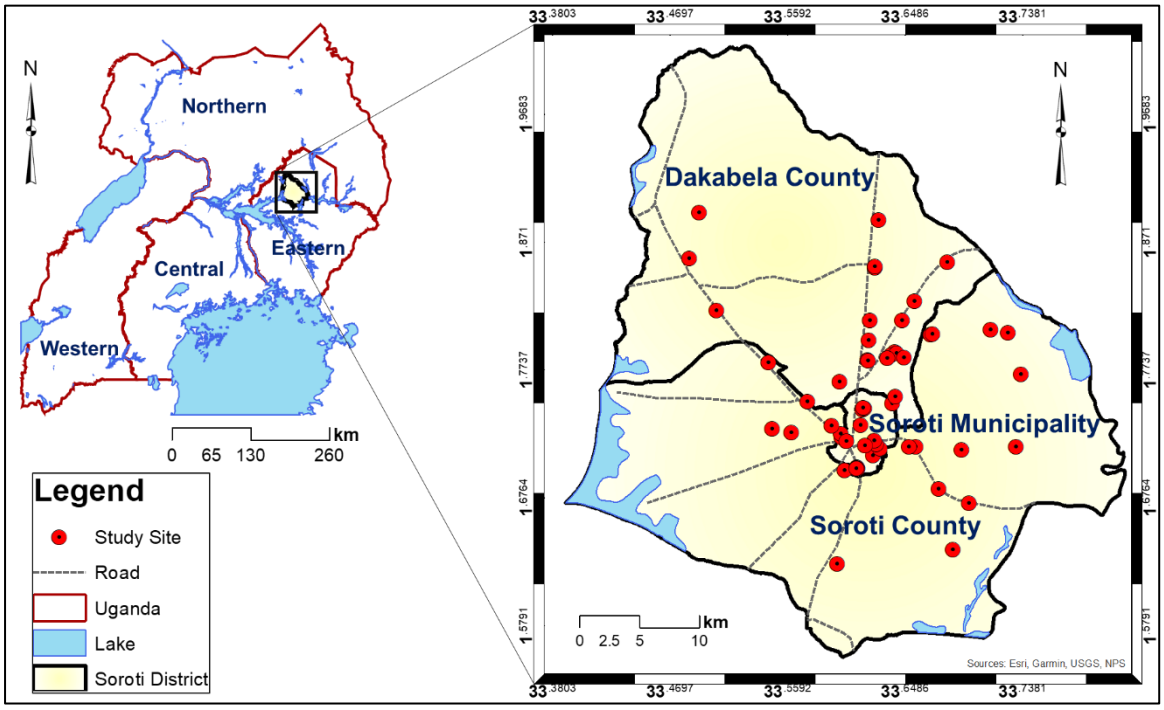
Study Design

This study followed a blended research approach involving a casual-comparative design in which comparisons of processing practices, fungal and aflatoxin contamination were done based on the stages of Ajono production in Soroti District. Documentation of the Ajono processing practices was done using face-to-face interviews. Isolation and identification of fungi, and determination of aflatoxin concentration were performed using standard microbiological methods in the Biochemistry and Microbiology research

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

laboratories at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University. This study assessed fungal and aflatoxin occurrence and concentration at three stages of Ajono production namely, millet grain, fermented paste, and liquid Ajono stages.

Figure 2: Map of Uganda showing the location of the study site



Sampling Strategy

Using Soroti District records, it was estimated that the number of Ajono production points in the study area was about 200. The district records further revealed that there were 71 large scale Ajono producers (those with more than 100 consumers daily) in the district. Therefore, this study focused on large scale Ajono producers in Soroti District largely because occurrence of mycotoxigenic fungi in the local brew would have a great impact on a number of people. The large scale Ajono producers in the district were distributed as follows; 26 producers in Soroti Municipality, 27 producers in Dakabela County and 18 producers in Soroti County. Therefore, the study was conducted on a representative sample of 60 local brewers in line with the sampling table designed by Krejcie and Morgan (Krejcie and Morgan, 1970). Using stratified random sampling, 22 local brewers were selected from Soroti Municipality, 23 local brewers were selected from Dakabela County and 15 local brewers were selected from Soroti County making a total of 60 brewers. Each local brewer was assigned a unique code and the IBM SPSS version 20 software was used to randomly select the Ajono brewers in each county of the district. The selected local brewers were interviewed on the processing practices used during Ajono production using a questionnaire designed according to Cortesa et al. (Cortese *et al.*, 2017). In addition, samples for fungal isolation from each of the three stages of Ajono production (Millet grain stage, fermented paste stage and liquid Ajono stage) were collected from each of the selected local Ajono brewer. Therefore, a total of 180 samples were collected and analyzed for fungal contamination. The 180 samples were obtained as follows; 60 samples of the raw cereals (millet grains), 60 samples of the fermented paste (dough) and 60 samples of liquid Ajono.

Sample Collection, Transportation and Storage

Millet Grains

Sixty grams (60 g) of millet grains were collected into separate sample bags. Specifically, 50 g of millet grains were placed into sterile labelled zip-

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

lock bags. Samples were then transferred into a cool box containing ice blocks and transported to the Biochemistry Research laboratory at Makerere University, stored at 4 °C and processed within 24 hrs. These samples were used for fungal culturing. Concomitantly, 10 g of the same millet grains samples were collected into labelled sterile zip-lock bags which were then transferred into a cool box without ice to serve as controls. These samples were kept at room temperature (25 °C) during transportation to the laboratory. On reaching the laboratory, the moisture content of the samples was determined immediately.

Fermented Paste

Fifty grams (50 g) of the fermented paste (dough before roasting) were collected into labelled sterile zip-lock bags, transferred into a cool box containing ice blocks and then transported to the Biochemistry Research laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) at Makerere University, stored at 4 °C and processed within 24 hrs. Each of the fermented paste samples, was collected after the first fermentation during Ajono production (7 days) from each of the local brewers. These samples were used for fungal culturing.

Liquid Ajono

Fifty millilitres (50 ml) of the freshly prepared liquid Ajono were collected into three separate 50 ml capacity labelled sterile bottles. The samples were then transferred into a cool box containing ice blocks and transported to the Biochemistry Research laboratory, COVAB at Makerere University, stored at 4 °C and processed within 24 hrs. At the final stage of production, Ajono samples were collected from each local brewer. These samples were used for lactic acid determination and fungal culturing. All samples were each sequentially collected from each local brewer at the time when they were ready.

Moisture Content Determination in Millet Grains

Briefly, 5g from each millet grain sample were weighed into a labelled pre-weighed crucible. Samples in pre-weighed crucibles were transferred into hot-air oven set at 105 °C and allowed to dry for 24 hours. After 24 hours of drying, samples were allowed to cool from desiccators, re-weighed and transferred back to the oven for 24 hours. This was repeated until a constant weight was obtained. The percentage moisture content (% MC) was determined using the equation derived by Reeb and colleagues (Reeb *et al.*, 1999);

$$\%\text{Moisture content} = \frac{\text{a(g)} - \text{b(g)} \times 100}{\text{a (g)}}$$

Whereby; a= weight of the sample before drying; b=weight of the sample after drying

Fungal Occurrence Along Production Stages of Ajono

This was divided into two phases: phase one involved isolation and enumeration of moulds and yeasts, while phase two involved identification of the moulds and yeasts genera. Total plate count method was used to enumerate mounds and yeasts whereas microscopy, biochemical tests, and germ tube were used during the identification of the fungal genera as described previously (Pepper and Gerba, 2009).

Isolation and Enumeration of Moulds and Yeasts

Isolation and enumeration of moulds and yeasts were done following the culture method described previously (Pepper and Gerba, 2009) (*Plate 1*). For each of the Ajono samples, 1 ml was mixed with 9 ml of peptone water (10^{-1}) and transferred into a sterile falcon tube. The mixture was then subjected to serial dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Using a pipette, 0.1 ml of the neat and each of the dilutions was inoculated into the Potato Dextrose Agar (PDA) and then spread using a sterile glass spreader. The inoculated petri dishes were each wrapped with parafilm and incubated at 25 °C for 24 hrs and after incubation, the petri dishes were observed for mould and yeast growth. Petri dishes that did not exhibit growth after 24 hrs of incubation were re-incubated for 6 days after

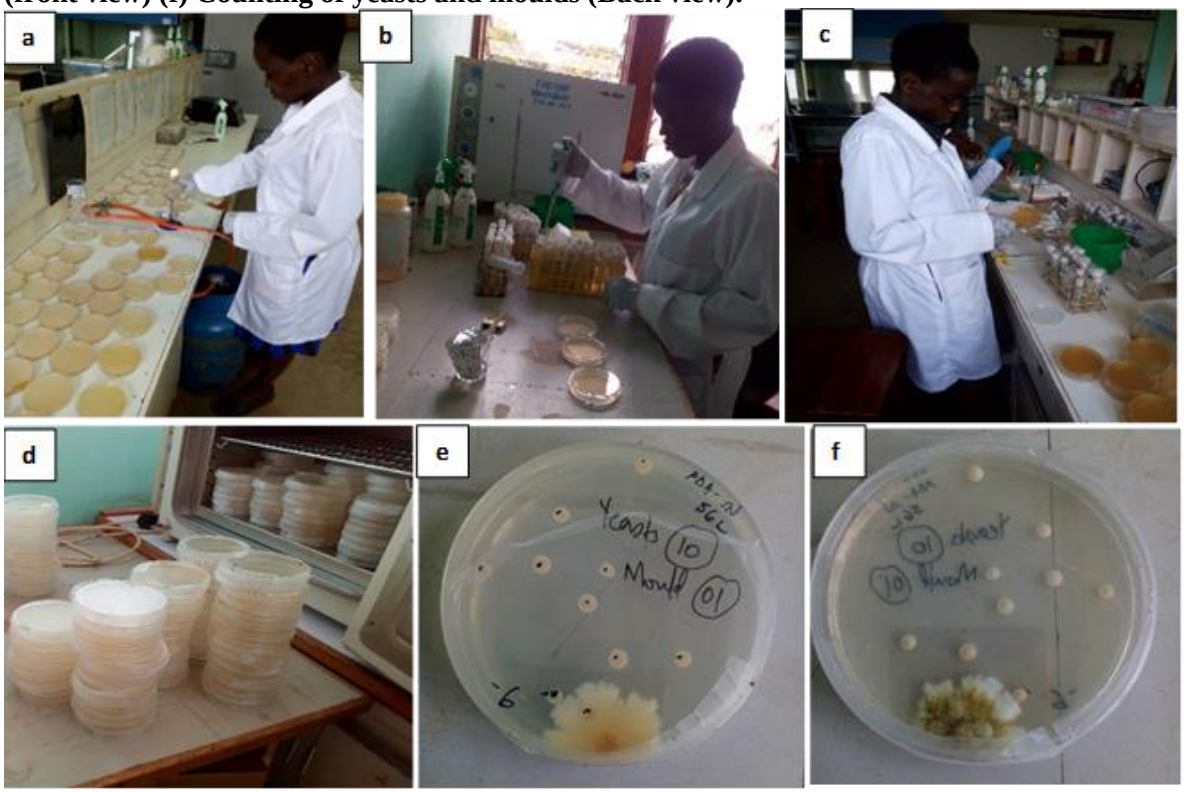
Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

which those exhibiting no fungal growth were discarded. All the used apparatus were autoclaved immediately after use at a temperature of 121 °C and a pressure of 15 psi for 30 minutes before washing thoroughly and the petri dishes were immediately placed in an incinerator (Sakai and Hiraoka, 2000). The total counts (cfu/ml) of the moulds and yeasts were computed from the following formula as adopted from Da Silva et al. ((Da Silva *et al.*, 2018);

$$\text{Total count of microorganisms} = \frac{\text{Number of colonies counted}}{\text{Dilution factor} \times \text{Volume of the inoculated sample}}$$

The total counts of moulds and yeasts were transformed into log cfu/ml as recommended by Zaragoza et al. (Zaragoza *et al.*, 2017).

Plate 1: Isolation and enumeration of moulds and yeasts. (a) Casting media (b) Serial diluting (c) Surface spreading (d) Placing petri dishes in the incubator (e) Counting of yeasts and moulds (front view) (f) Counting of yeasts and moulds (Back view).



Identification of Mould Genera

Sub-culturing of the colonies was done after 6 days of growth to obtain pure cultures. From the pure cultures, significant macroscopic characteristics were observed, read, and recorded after every two days for a duration of two weeks. Thereafter, slide cultures were prepared as follows; in an empty glass Petri dish, a sterilized set of a 7 cm diameter filter paper, a V-shaped glass rod, a glass slide, and two cover slips were placed. A PDA plate was cut into small squares of approximately 1 cm^2 each. One block of the cut PDA medium was placed in the middle of the slide. A sterile wire loop was used to inoculate the four sides of the PDA block with the fungal colony under investigation. Using forceps, a cover slip was placed on top of the inoculated PDA. To prevent drying of the agar block, the filter was wetted with sterile distilled water and the set was incubated at 25 °C for 48 hrs after which inspection for growth and sporulation was performed. The cover slip of the agar was gently lifted and placed on a slide with lactophenol cotton blue (LPCB) followed by examination under a microscope at x10 and x40

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

magnifications. Microscopic characteristics of the moulds were observed, read, and recorded. Single spore isolation method was used to culture the spores as previously described (Zhang *et al.*, 2013). All the used apparatus were autoclaved immediately after use at a temperature of 121 °C and a pressure of 15 psi for 30 minutes before washing thoroughly after which the petri dishes were immediately placed in an incinerator (Sakai and Hiraoka, 2000). A fungal guide was used to identify the moulds using their hyphae, conidia, shape, colour and size (Larone and Larone, 1987). Slide culturing was used because; it is a rapid method of preparing fungal colonies for examination and identification, permits fungi to be studied virtually in-situ with as little disturbance as possible, and fungus is identified mostly by close examination of its morphology and characteristics.

Identification of Yeast Genera

Yeast cells were obtained from the cultured media and gram staining was conducted. The cells were observed and the nature of their gram reaction was recorded that is; Gram positive, Gram negative or Gram variable. The shape, size and colour of cells were equally recorded. Biochemical tests; maltose test, glucose test, urease test and sucrose test were performed and a fungal guide was used to identify the yeasts (Larone and Larone, 1987). In addition, germ tube test was also performed to identify the presence of *Candida* spp. All the used apparatus were autoclaved immediately after use at a temperature of 121 °C and a pressure of 15 psi for 30 minutes before washing thoroughly and the petri dishes were immediately placed in an incinerator (Sakai and Hiraoka, 2000).

Detection of Aflatoxins in the Production Stages of Ajono

Aflatoxins detection involved solvent extraction of aflatoxins using a procedure described previously (Turner *et al.*, 2009) and Thin Layer Chromatography (TLC) screening was used to identify aflatoxin groups.

Quantification of Aflatoxins in Millet Grains, Fermented Paste and Liquid Ajono

Positive samples from TLC screening were further analyzed to quantify aflatoxin levels by Max Signal ®Total Aflatoxin ELISA Test Kit according to manufacturer's instruction. Briefly, five grams (5 g) each of the ground millet grain and fermented paste or five millilitres (5 ml) of liquid Ajono samples were weighed into a 50 ml conical flask and 25 ml of 70% methanol was added and the mixture vortexed for 10 minutes. The sample was centrifuged at 4,000×g for 10 minutes at room temperature (25 °C). Then, 300 µl of the supernatant was transferred to a new 2 ml eppendorf tube containing 900 µl of solution C (Mixture of 7 volumes of 100% methanol with 23 volumes of 1× Oil extraction buffer) and the sample was vortexed for 1 minute. Fifty microliters (50 µl) of each sample were added to the wells in duplicates. Then, 50 µl of each standard was also added to different wells in duplicate using a new pipette tip. Standards were added from low to high concentrations. Thereafter, 100 µl of Aflatoxin-HRP conjugate was added to each well. The solution in the wells was mixed for 1 minute by gently tapping the plate. The plate was covered and then incubated for 30 minutes at room temperature (25 °C). After incubation the plate was washed three times with washing buffer. The plate was then inverted and tapped against paper towel to remove residual washing buffer. Immediately, to avoid drying of the plate, 100 µl of TMB substrate was added to each well. The solution in the wells was mixed for 1 minute by tapping the plate gently. The plate was covered with aluminium foil and then incubated for 15 minutes at room temperature (25 °C). After incubation, 100 µl of stop solution was added to each well to stop the substrate reaction. A lint-free wipe was used to clean the bottom of the wells before inserting the plate to the ELISA plate reader. The absorbance values were obtained at OD 450 nm. A standard curve was constructed using the mean relative absorbance obtained from the reference standard against its concentration in parts per billion (ppb). The concentrations (ppb)

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

of the samples were computed accordingly using the plotted standard curve.

RESULTS

Demographic Characteristics of the Ajono Brewers in Soroti District

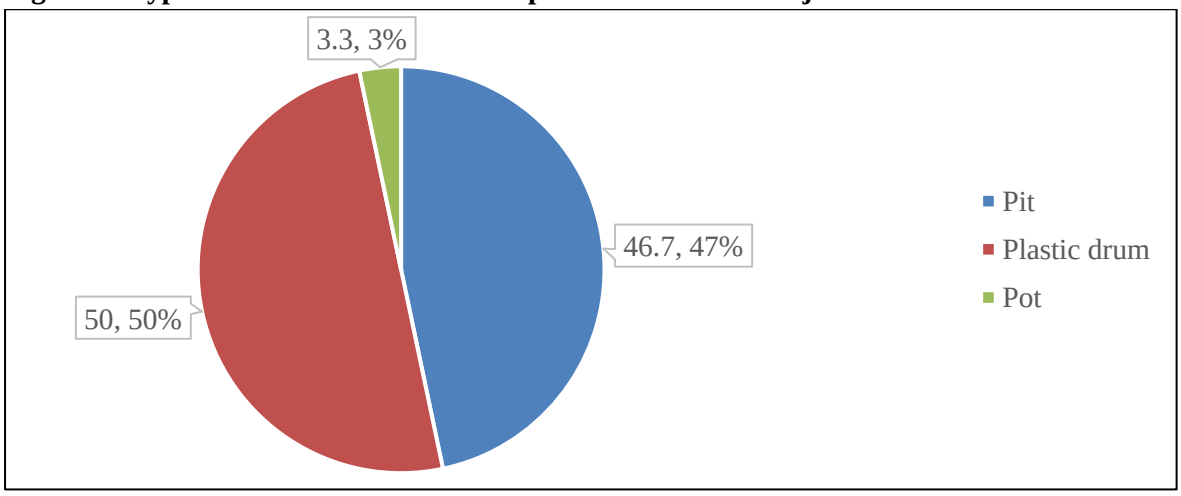
The demographic characteristics of Ajono brewers in Soroti District were analyzed (*Table 1*) and results showed that the majority (58%) of the brewers had no formal education while the vast majority (96%) were females. Ajono production in Soroti District involved three major stages; millet grain, fermented paste and liquid Ajono stages.

Processing practices along the production stages of Ajono were established to find out the source of fungal and aflatoxin contamination of the local brew. The results of millet grain processing and handling are indicated in *Table 2*. It was established that the vast majority (75%) of the local brewers used small sized millet grains that are red in colour for Ajono production. A large percentage (96%) of the brewers obtained the millet grains from lock-up shops. The study also found that, 95% of the brewers preferred stored millet grains. In addition, 84% of the brewers preferred grains that were stored for three months in polypropylene bags. Furthermore, only 13% of Ajono brewers sorted and de-hulled the millet grains themselves.

On the other hand, all brewers germinated and milled the grains during the production of Ajono. Processing and handling practices of the fermented paste were assessed for documentation.

The proportion of Ajono brewers who used different fermenting vessels were determined and found that plastic drums (50%) and pits (47%) were the dominant while pots (3%) were the least used vessels (*Figure 3*). It was noted that Pots were used only when the brewer had small orders from the consumers while the fermented paste was stored in plastic sheets. Results indicated that all Ajono brewers cleaned the containers (buckets) used to process Ajono. However, the brewers did not boil water that was added to the sundried roasted paste and all brewers stored Ajono in uncovered buckets and did not wash their hands during Ajono production.

Figure 3: Types of fermenters used in the production chain of Ajono



Three different types of fermenters are used during the production of Ajono; pit, plastic drum and pot with the plastic drum being the most commonly used fermenter.

Majority of the brewers used plastic drums (50%) and pits (47%) for fermentation and results indicated varying levels of moisture in different containers. The mean percentage moisture content of grains used with pit fermenters was 9.21% ± 0.20, while plastic drum fermentation had 10.76% ± 0.76 moisture content.

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Table 1: Demographic characteristics of Ajono brewers in Soroti District.

Characteristics	Category	Location				
		Soroti Municipality (n = 22)	Dakabela County (n = 23)	Soroti County (n =15)	p-value	Over all (n = 60)
Education level	No formal Education	13 (59.1%)	13(56.5%)	9 (60.0%)	0.590	35 (58.3%)
	Primary	3 (13.6%)	6 (26.1%)	4 (26.7%)		13 (21.7%)
	Secondary	5 (22.7%)	3 (13.0%)	1 (6.7%)		9 (15%)
	Tertiary	1 (4.5%)	1 (4.3%)	1 (6.7%)		3 (5%)
Brewer's gender	Male	0 (0.0%)	1 (4.3%)	1 (6.7%)	0.509	2 (3.3%)
	Female	22 (100%)	22(95.7%)	14 (93.3%)		58 (96.7%)

No significant association was recorded between education level and brewer's gender at p = 0.05 tested using Chi-square test statistic.

Table 2: Processing practices along production stages of Ajono local brew in Soroti District.

Processing Practices	Categories		Brewer's Location				
			Soroti Municipality (n = 22)	Dakabela County (n = 23)	Soroti County (n = 15)	p-value	Overall (n = 60)
Nature of grains	Size	Large	6 (27.3%)	3 (13.0%)	6 (40%)	0.141	15 (25.0%)
		Small	16 (72.7%)	20 (87.0%)	9 (60%)		45 (75.0%)
	Colour	Red	16 (72.7%)	20 (87.0%)	9 (60%)	0.243	45 (75.0%)
		Others	6 (27.3%)	3 (13%)	6 (40%)		15 (25.0%)
	Source	Lockup	22 (100%)	23 (100%)	13(86.7%)	0.509	58 (96.7%)
		Garden	0 (0%)	0 (0%)	2 (13.3%)		2 (3.3%)
Storage of grains	Grains stored		20 (90.9%)	22 (95.7%)	15 (100%)	0.355	57 (95%)
	Grains used immediately		2 (9.1%)	1 (4.3%)	0 (0%)		3 (5.0%)
	Length of storage	1 month	3 (15%)	2 (9.1%)	4 (26.7%)	0.434	9 (15.8%)
		3 months	17 (85%)	20 (90.9%)	11(73.3%)		48 (84.2%)
Sorting		Sorted	5 (22.7%)	3 (13.0%)	0 (0%)	0.136	8 (13.3%)
		Did not sort	17 (77.3%)	20 (87%)	15 (100%)		52 (86.7%)
Dehulling		Dehulled	5 (22.7%)	3 (13.0%)	0 (0%)	0.136	8 (13.3%)
		Did not dehull	17 (77.3%)	20 (87%)	15 (100%)		52 (86.7%)

Results presented as number of respondents (percentage). All (100%) Ajono brewers germinated grains and milled them using a machine. No significant association was recorded between processing practices and location of the brewers at p = 0.05 tested using Chi-square test statistic.

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Isolation of Fungal Genera along the Production Stages of Ajono

To identify the fungal genera along the production stages of Ajono, isolation of moulds and yeasts were performed and results of fungal genera in pit and plastic drum fermenters are presented (Table 3). Overall, 7 moulds and 2 yeast genera were isolated along the three stages of production of Ajono. Furthermore, a general decrease in the prevalence of moulds along the production stages of Ajono was noted.

Basing on the nature of the fermenter, plastic drum generally had the highest diversity of moulds including *Aspergillus, Fusarium*, *Alternaria*; *Rhizopus*, *Penicillium*, *Cladosporium*, and *Acremonium*. On the other hand, pit fermenters had only four genera of moulds namely; *Aspergillus, Fusarium*, *Alternaria* and *Rhizopus*. Results also revealed that *Aspergillus* spp. were the most prevalent moulds along the production stages of Ajono in both pit and plastic drum fermenters while *Acremonium* spp. were the least prevalent along production stages of Ajono (*Table 3*). In addition, pit registered a 10.8% reduction in prevalence of *Aspergillus* spp. compared to plastic drum that registered only 3.1% reduction when the millet grains were fermented into paste (*Table 3*). In regard to yeast occurrence, *Saccharomyces* spp. increased along the production stages of Ajono. In addition, all (100%) Ajono samples had *Saccharomyces* in both pit and plastic drum fermenters. Results indicated that unlike in the plastic drums, *Candida* spp. occurred at all stages of Ajono production in pit fermenters and were more prevalent (7%) at the paste stage of pit fermentation (*Table 3*).

Aflatoxin Contamination and Concentration along the Production Stages of Ajono

High prevalence (78.6%) of total Aflatoxin B and G were observed in the liquid stage when pit fermenters were used while the liquid stage had 68.8% of total Aflatoxin A and G for plastic drum fermenters (*Figure 4a*). The highest prevalence of Aflatoxin B (31.3%) was recorded in samples of the liquid stage from plastic drum fermenters. In addition, all liquid Ajono samples from both pit and plastic drum fermenters did not have Aflatoxin G. Unlike the liquid and paste stages, the grain stage had only Aflatoxin G with its highest prevalence (6.3%) recorded in samples for plastic drum fermenters (*Figure 4a*).

The mean aflatoxin concentration along the production stages of Ajono were compared with maximum acceptable levels for the European Union (EU) and East Africa Community (UNBS) (*Figure 4b*). It was found that in the plastic drum fermenter, the paste stage and liquid stage had aflatoxin levels significantly ($P<0.05$) above the maximum acceptable EU levels (4 ppb) for total aflatoxins in cereals and cereals products. More so, only the paste stage of plastic drum fermenter had aflatoxin levels (14 ppb) above the maximum acceptable EAC levels (10 ppb) although the difference was not significant.

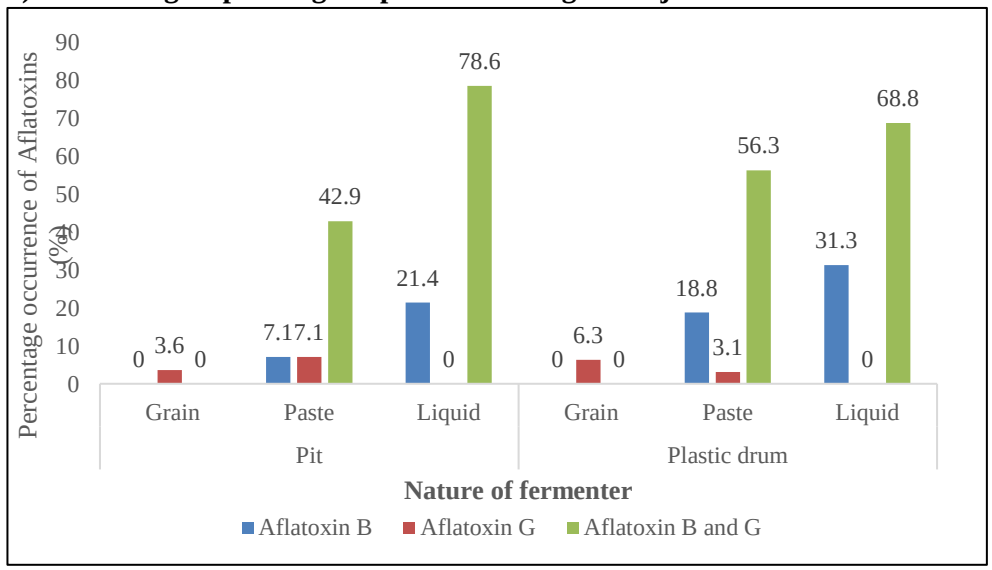
Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Table 3: Mould and yeast genera identified along the production stages of Ajono

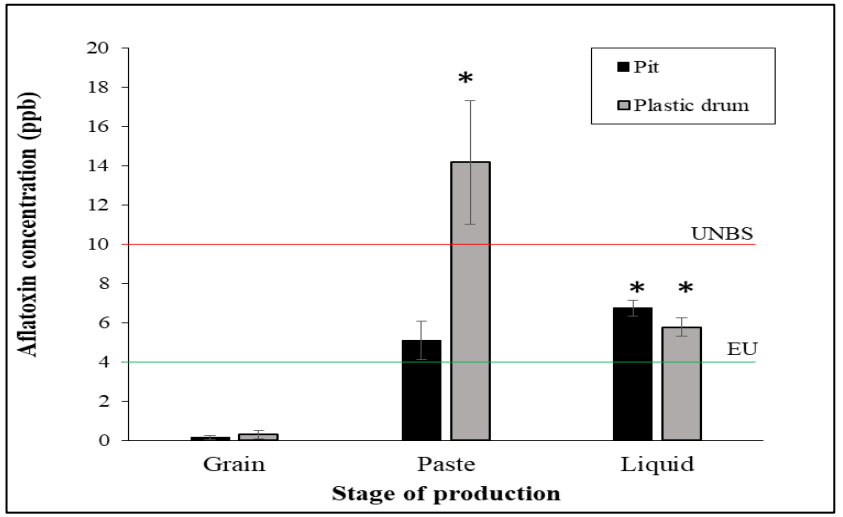
Fungi	Genera	Nature of fermenter					
		Pit			Plastic drum		
		Millet grain (n=28)	Fermented Paste (n=28)	Ajono (n=28)	Millet grain (n=32)	Fermented Paste (n=32)	Ajono (n=32)
Mould	*Aspergillus spp.*	12 (42.9%)	9 (32.1%)	4 (14.3%)	20 (62.5%)	19 (59.4%)	16 (50.0%)
	Fusarium spp.	4 (14.3%)	3 (10.7%)	2 (7.1%)	9 (28.1%)	7 (21.9%)	9 (28.1%)
	Alternaria spp.	2 (7.1%)	0 (0%)	0 (0%)	3 (9.4%)	2 (6.3%)	5 (15.6%)
	Rhizopus spp.	1 (3.6%)	0 (0%)	0 (0%)	2 (6.3%)	1 (3.1%)	2 (6.3%)
	Cladosporium spp.	0 (0%)	0 (0%)	0 (0%)	2 (6.3%)	1 (3.1%)	3 (9.4%)
	Penicillium spp.	0 (0%)	0 (0%)	0 (0%)	1 (3.1%)	1 (3.1%)	2 (6.3%)
	Acremonium spp.	0 (0%)	0 (0%)	0 (0%)	1 (3.1%)	1 (3.1%)	1 (3.1%)
Yeast	*Saccharomyces spp.*	13 (46.4%)	21 (75%)	28 (100%)	17 (53.1%)	28 (87.5%)	32 (100%)
	Candida spp.	1 (3.5%)	2 (7.1%)	1(3.5%)	0 (0%)	0 (0%)	0 (0%)

Figure 4: Aflatoxin contamination and concentration along the production stages of Ajono

a) Aflatoxin groups along the production stages of Ajono



b) Aflatoxin concentration along production chain of Ajono compared with Standard limits.



** Indicates mean values significantly above acceptable EU levels (4ppb).*

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

DISCUSSION

The majority of Ajono brewers (58%) in Soroti District had no formal education (*Table 1*). This may be attributed to the fact that Ajono production is a tradition in Eastern Uganda that can be learnt at any time and does not require complex equipment that would need expertise and technical skills from school. These findings are in line with the findings of Musa and Akande who reported that 56% of food handlers in Nigeria had no formal education (Musa and Akande, 2003). The lack of formal education among the Ajono brewers may imply that they have little or no understanding of the risks of hygiene and fungal contamination of the local brew or how to avoid them. This is indicative of the fact that most of the brewers lacked adequate knowledge on the best processing practices like drying of the grains, hygienic practices and proper storage conditions among others that greatly influence fungal and aflatoxin contamination in the local brew.

Majority of the brewers used small sized millet grains that are red in colour during Ajono production. This may be due to the desired quality of the local brew in terms of taste, flavour, odour, and colour. In addition, small sized red millet grains may be preferred because of their tendency to resist pest infestation (Proctor, 1994). A larger percentage of the brewers obtained millet grains from lock-up shops compared to harvest from individual's gardens. This may be attributed to the fact that lock-up shops were easily accessible with adequate supply of the millet grains to the Ajono producers. Subsequently, most brewers preferred millet grains that were stored for at least three months (*Table 2*). This may be attributed to the fact that long term storage provides grains that are ready for Ajono production. However, the fact that the storage conditions and the handling practices at the lock-up shops may not be well regulated and could have exposed the millet grains to fungal and aflatoxin contamination. In Nigeria, Shehu and Bello reported that storage of millet grains in unfitting conditions promotes the growth of aflatoxigenic fungi (Shehu and Bello, 2011). More so, mycotoxin levels were reported to be higher in stored grains than in grains that were not stored (Okeke *et al.*, 2015). This study also showed that majority of the brewers did not sort and dehull the millet grains to be used for Ajono production. This may be attributed to the laborious and time-consuming nature of these activities. Consequently, using grains that are not sorted and dehulled may lead to fungal proliferation and aflatoxin contamination. In addition, all the millet grains used in Ajono production were milled using a machine to save time and their market demands. However, Obilana highlights that milling cereals using machines reduces nutritional status and the shelf-life (Obilana, 2003).

All Ajono brewers germinated a portion of the millet grains to produce malt which was used in the production of Ajono as indicated in *Table 2.* Mastanjevic et al. (2019) explained that malting is needed to develop enzymes useful in alcohol production. However, fungi originating from field and storage are reported to thrive under malting conditions. Therefore, this process could be a potential source of fungal and aflatoxin contamination during Ajono production. Results also indicated that a very small proportion of brewers dried the millet grains for Ajono production. This may be attributed to the fact that drying millet grains requires time. Drying is very key in controlling fungal growth in grains because it reduces the moisture content to safe storage levels (Shier *et al.*, 2005; Canty *et al.*, 2014). The fact that few brewers dried the grains may have exposed the grains to fungal contamination and consequently aflatoxin occurrence. This is evidenced by the high moisture content above 8% reported in this study. Similarly, Tola and Kebede observed that growth of aflatoxigenic fungi can occur in stored cereals when moisture exceeds 8% (Tola and Kebede, 2016).

Fermented Paste Processing and Handling

Majority of Ajono brewers used either plastic drums (50%) or pits (47%) as their preferred vessels of fermentation unlike the usage of pots (*Figure 3*). This was probably because plastic drum fermenters provided a large surface area for

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Ajono production as compared to pits and pots. In addition, brewers preferred plastic drums over pit probably because the process of making pits is laborious and time consuming. Muyanja et al. (2010) also report the use of pit fermenters during local brew production. A large percentage of brewers used cold water and plant leaves to clean plastic drum and pot fermenters. Usage of plant leaves for cleaning the fermenters may be highly favoured due to the fact that they are readily available, cheaper with some plants having antimicrobial properties and bitter flavour (Oguntoyinbo, 2014). Therefore, cleaning the fermenters reduces fungal contamination and thus increasing the shelf-life of the local brew.

Ajono Handling Practices

All Ajono brewers cleaned the containers used for Ajono production. This may be attributed to the fact that failure to clean the containers is associated with bad odour, taste, and flavour of the brew. In addition, all brewers did not boil water that was added to the sundried roasted fermented paste during Ajono production. This may be attributed to the fact that boiling water requires heating materials such as charcoal or firewood which would increase the cost of production. The use of unboiled water during Ajono production is a threat to the health of the consumers since mould contamination has been reported in unboiled water (Jonathan *et al.*, 2016). Nevertheless, all brewers added germinated ground grains (malt) to the fermented paste because germinated grains harbour yeasts which are needed to break down the sugars to produce alcoholic beverage. Unfortunately, the mould load increases as germination of cereals occurs consequently exposing the brew to mould contamination (Krasauskas, 2017). Moreover, the unhygienic practices of brewers such as storage of Ajono in uncovered buckets and absence of handwashing during production increase the susceptibility of Ajono to fungal contamination and consequently aflatoxin occurrence. Findings of this study are in line with the findings of (Zaukuu *et al.*, 2016) who reported unhygienic practices in an indigenous beer made from sorghum in Ghana.

Fungal Occurrence along the Production Stages of Ajono Local Brew

Moulds Diversity along the Production Stages of Ajono

This study identified seven mould genera: *Aspergillus*, *Fusarium*, *Alternaria*, *Rhizopus*, *Penicillium*, *Cladosporium*, and *Acremonium* along the production stages of Ajono (*Table 3*). This is consistent with previous findings that food poisoning in Africa is mainly due to microbial contamination (Oladayo *et al.*, 2022). *Aspergillus* spp. was the most prevalent along production stages of Ajono for both pit and plastic drum fermenters. This may be attributed to the hot and humid climate of the study area which may have favoured *Aspergillus* proliferation and subsequent aflatoxin production along production stages of Ajono. *Aspergillus* spp survive at temperatures ranging from 12 °C to 48 °C (Payne and Yu, 2010; Nji *et al.*, 2022). In addition, improper handling and processing practices coupled with the unhygienic practices during Ajono production may have favoured fungal contamination. In particular, most brewers preferred millet grains stored for at least 3 months which may have favoured *Aspergillus* colonization of the millet grain while in storage consistent with previous findings (Thathana *et al.*, 2017). *Aspergillus* were reported to be more prevalent than *Fusarium* and *Penicillium* genera in alcoholic beverages (Adekoya *et al.*, 2018) and in maize (Nsabiyumva *et al.*, 2023). Unlike the millet grain stage, presence of lactic acid during the fermented paste stage may have reduced the *Aspergillus*. Further reduction during the liquid stage may have been due to the roasting of the fermented paste and the alcoholic fermentation during Ajono production.

Yeast Diversity along the Production Stages of Ajono

Results indicated occurrence of only two yeast genera: *Saccharomyces* and *Candida* along the production stages of Ajono (*Table 3*). *Saccharomyces* were the most prevalent yeast

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

genera along the production stages of Ajono irrespective of the nature of fermenter (*Table 3*). This was because lactic acid bacteria produce desirable acids like lactic acid that enhance development of yeasts during fermentation. Indeed, different studies on traditional fermented beverages have indicated that predominant microorganisms in beverages were mainly lactic acid bacteria and yeast (Aka *et al.*, 2014; Wafula *et al.*, 2022). In particular, it was reported that the majority of yeasts involved in alcoholic fermentation belong to the genus *Saccharomyces* (Demuyakor and Ohta, 1993). However, some strains of *Saccharomyces* spoil wines and other alcoholic beverages by producing gassiness, turbidity and off-flavours associated with hydrogen sulphide and acetic acid (Rawat, 2015).

Detection and Quantification of Aflatoxins along the Production Stages of Ajono

A higher prevalence of total aflatoxin B and G group in the liquid stage was observed in brewers who used pit fermenter. Unlike the pit fermenters, plastic drum fermenters had a higher prevalence of total aflatoxin B and G in paste stage (*Figure 3*). This is probably because plastic drums were not tightly covered which allowed penetration of oxygen that may have favoured *Aspergillus parasticus* to produce Aflatoxin B and G (Kumar *et al.*, 2019). In addition, liquid and paste stage may have had a high moisture content that is known to promote fungal growth. Results indicated that aflatoxins were prevalent in the liquid stage probably due to unhygienic handling practices of the local brew that may have exposed it to *Aspergillus flavus* contamination. Presence of aflatoxins G was observed in millet grains only probably because of the temperatures that the millet grains were subjected to during the pre-harvest, storage and post-harvest handling which may have favoured the production of Aflatoxin G by *Aspergillus parasticus* (Abbas *et al.*, 2009; Nji *et al.*, 2022).

Unlike the millet grains, the liquid Ajono samples had aflatoxin levels that were significantly above maximum acceptable EU limits (4ppb) for total aflatoxins. The fact that majority of brewers had no formal education implies that Ajono brewers may have limited knowledge on good processing and handling practices during Ajono production. The findings of this study are in line with previous findings which reported low levels of aflatoxins in sorghum grains but significantly higher aflatoxin levels in the malt prepared for beverages (Matumba *et al.*, 2011). Another study reported high aflatoxin exposures in adults and children in Kenya, Tanzania and Uganda (Kimanya, 2015). Exposures to Aflatoxins is reportedly associated with epidemics of acute toxic hepatitis in Africa (Hussein and Brasel, 2001). Another study reported that approximately 250,000 deaths are caused by hepatocellular carcinomas in China and Sub-Saharan Africa annually (Groopman and Kensler, 2005). Furthermore, aflatoxins have been reported to induce immunosuppression, weight loss, increased liver and kidney diseases and hepatitis in various species of animals (Hussein and Brasel, 2001).

CONCLUSION

Ajono production in Soroti District is marked by three major stages: millet grain stage, fermented paste stage and liquid Ajono stage. Fermentation being a key process in Ajono production, was majorly carried out in plastic drums and pits as vessels. However, the local brewers who were largely women used unhygienic handling practices that consequently predisposed the local brew to fungal and Aflatoxin contamination. Although the grain stage was highly contaminated with moulds and is thus the most susceptible stage of contamination, there was fungal contamination along all the production stages of Ajono. Mould contamination generally decreased along the production chain of Ajono, and *Aspergillus* spp. were the most prevalent moulds at all three stages of Ajono production. In regard to yeast, *Saccharomyces* spp. had the highest prevalence along the stages of Ajono production. There was a higher prevalence of fungi in plastic drum fermenters compared to pit and pot fermenters. There was a higher prevalence of fungi in plastic drum fermenters compared to pit and pot fermenters. Aflatoxin contamination occurred at

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

all stages of Ajono production. However, the liquid Ajono stage had Aflatoxin levels significantly above the maximum acceptable levels for EU for both pit and plastic drum fermenters. The plastic drum as a fermenting vessel produced fermented paste with high levels of aflatoxins compared to pit fermenters. On the other hand, millet grain stage had the least aflatoxins levels below EU regulatory limits. All samples had aflatoxin levels significantly below the maximum regulatory levels for the EAC.

ACKNOWLEDGEMENTS

The authors thank all the study participants for their valuable time given during data collection. This study was supported by funds from the Deutscher Akademischer Austauschdienst (DAAD) project and the Africa Centre of Excellence in Materials, Product Development and Nanotechnology (MAPRONANO ACE) to Sarah Nantumbwe.

Conflict of Interest Statement

The authors declare no conflict of interest.

Availability of Data Statement

The data supporting this study are included within the article.

REFERENCES

Abbas, H., Wilkinson, J., Zablotowicz, R., Accinelli, C., Abel, C., Bruns, H., & Weaver, M. (2009). Ecology of aspergillus flavus, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. *Toxin reviews, 28*(2-3), 142-153.

Adekoya, I., Obadina, A., Adaku, C. C., De Boevre, M., Okoth, S., De Saeger, S., & Njobeh, P. (2018). Mycobiota and co-occurrence of mycotoxins in south african maize-based opaque beer. *International journal of food microbiology, 270*, 22-30.

Aka, S., Konan, G., Fokou, G., Dje, K. M., & Bonfoh, B. (2014). Review on african traditional cereal beverages. *Am. J. Res. Commun, 2*(5), 103-153.

Bankole, S., & Adebanjo, A. (2003). Mycotoxins in food in west africa: Current situation and possibilities of controlling it. *African journal of Biotechnology, 2*(9), 254-263.

Bueno, D., Istamboulie, G., Muñoz, R., & Marty, J. L. (2015). Determination of mycotoxins in food: A review of bioanalytical to analytical methods. *Applied Spectroscopy Reviews, 50*(9), 728-774.

Bullermann, L., & Bianchini, A. (2011). The microbiology of cereals and cereal products. *Food Qual, 18*, 26-29.

Canty, M. J., Fogarty, U., Sheridan, M. K., Ensley, S. M., Schrunk, D. E., & More, S. J. (2014). Ergot alkaloid intoxication in perennial ryegrass (lolium perenne): An emerging animal health concern in ireland? *Irish Veterinary Journal, 67*(1), 1-7.

Cortese, D., Pairotti, M. B., Giachino, C., & Bonadonna, A. (2017). The art of craft beer in a traditional wine nation: A preliminary study in a model area. *Calitatea, 18*(161), 149-156.

Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in europe. *Toxicology letters, 127*(1-3), 19-28.

Da Silva, N., Taniwaki, M. H., Junqueira, V. C., Silveira, N., Okazaki, M. M., & Gomes, R. A. R. (2018). *Microbiological examination methods of food and water: A laboratory manual*: CRC Press.

Demuyakor, B., & Ohta, Y. (1993). Characteristics of single and mixed culture fermentation of pito beer. *Journal of the Science of Food and Agriculture, 62*(4), 401-408.

Groopman, J. D., & Kensler, T. W. (2005). Role of metabolism and viruses in aflatoxin-induced liver cancer. *Toxicology and applied pharmacology, 206*(2), 131-137.

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Hussein, H. S., & Brasel, J. M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology, 167*(2), 101-134.

Jonathan, S., Oghodero, O., & Asemoloye, M. (2016). Incidence of molds in treated and untreated drinking water of selected local governments in ibadan, south-western nigeria. *Res. Rev. Biosc, 11*(4), 108-118.

Kimanya, M. E. (2015). The health impacts of mycotoxins in the eastern africa region. *Current Opinion in Food Science, 6*, 7-11.

Kowalska, A., Walkiewicz, K., Kozieł, P., & Muc-Wierzgoń, M. (2017). Aflatoxins: Characteristics and impact on human health. *Advances in Hygiene and Experimental Medicine, 71*, 315-327.

Krasauskas, A. (2017). Fungi in malting barley grain and malt production. *Biologija, 63*(3).

Krejcie, R. V., & Morgan, D. W. (1970). Determining sample size for research activities. *Educational and psychological measurement, 30*(3), 607-610.

Kumar, M., Kumar, H., Topno, R. K., & Kumar, J. (2019). Analysis of impact of anaerobic condition on the aflatoxin production in aspergillus parasiticus speare. *Agricultural Science Digest-A Research Journal, 39*(1), 75-78.

Larone, D. H., & Larone, D. H. (1987). *Medically important fungi: A guide to identification* (Vol. 196): Citeseer.

Matumba, L., Monjerezi, M., Khonga, E. B., & Lakudzala, D. D. (2011). Aflatoxins in sorghum, sorghum malt and traditional opaque beer in southern malawi. *Food Control, 22*(2), 266-268.

Misihairabgwi, J., Ishola, A., Quaye, I., Sulyok, M., & Krska, R. (2018). Diversity and fate of fungal metabolites during the preparation of oshikundu, a namibian traditional fermented beverage. *World Mycotoxin Journal, 11*(3), 471-481.

Musa, O., & Akande, T. (2003). Food hygiene practices of food vendors in secondary schools in ilorin. *Niger Postgrad Med J, 10*(3), 192-196.

Muyanja, C., Birungi, S., Ahimbisibwe, M., Semanda, J., & Namugumya, B. (2010). Traditional processing, microbial and physicochemical changes during fermentation of malwa. *African Journal of Food, Agriculture, Nutrition and Development, 10*(10).

Mwesigye, P. K., & Okurut, T. O. (1995). A survey of the production and consumption of traditional alcoholic beverages in uganda. *Process Biochemistry, 30*(6), 497-501.

Nji, Q. N., Babalola, O. O., Ekwomadu, T. I., Nleya, N., & Mwanza, M. (2022). Six main contributing factors to high levels of mycotoxin contamination in african foods. *Toxins, 14*(5), 318.

Nsabiyumva, G., Mutegi, C. K., Wagacha, J. M., Mohamed, A. B., Njeru, N. K., Ndayihanzamaso, P., Niyuhire, M. C., Atehnkeng, J., Njukwe, E., & Callicott, K. A. (2023). Aflatoxin contamination of maize and groundnut in burundi: Distribution of contamination, identification of causal agents and potential biocontrol genotypes of aspergillus flavus. *Front Microbiol, 14*, 1106543.

Obilana, A. (2003). Overview: Importance of millets in africa. *World (all cultivated millet species), 38*, 28.

Oduori, C., & Kanyenji, B. (2005). Finger millet in kenya: Importance, advances in r&d, challenges and opportunities for improved production and profitability. *Finger Millet Blast Management in East Africa. Creating opportunities for improving production and utilization of finger millet, 10*.

Oguntoyinbo, F. A. (2014). Safety challenges associated with traditional foods of west africa. *Food Reviews International, 30*(4), 338-358.

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Okeke, C. A., Ezekiel, C. N., Nwangburuka, C. C., Sulyok, M., Ezeamagu, C. O., Adeleke, R. A., Dike, S. K., & Krska, R. (2015). Bacterial diversity and mycotoxin reduction during maize fermentation (steeping) for ogi production. *Front Microbiol, 6*, 1402.

Oladayo, T., Miteu, G., Addeh, I., Folayan, E., Olayinka, T., Adegboyega, J., Ojeokun, O., Ogah, S., & Benneth, E. (2022). Most prominent factors of food poisoning in africa: Nigeria based perspective. *IPS Journal of Nutrition and Food Science, 1*(1), 11-17.

Papas, R. K., Sidle, J. E., Wamalwa, E. S., Okumu, T. O., Bryant, K. L., Goulet, J. L., Maisto, S. A., Braithwaite, R. S., & Justice, A. C. (2010). Estimating alcohol content of traditional brew in western kenya using culturally relevant methods: The case for cost over volume. *AIDS and Behavior, 14*, 836-844.

Payne, G. A., & Yu, J. (2010). Ecology, development and gene regulation in aspergillus flavus. *Aspergillus: molecular biology and genomics*, 157-171.

Pepper, I., & Gerba, C. (2009). Cultural methods in environmental microbiology. In: London: Elsevier.

Proctor, D. (1994). *Grain storage techniques: Evolution and trends in developing countries*: Food & Agriculture Org.

Rawat, S. (2015). Food spoilage: Microorganisms and their prevention. *Asian journal of plant science and Research, 5*(4), 47-56.

Reeb, J. E., Milota, M. R., & Association, W. D. K. (1999). Moisture content by the oven-dry method for industrial testing.

Sakai, S.-i., & Hiraoka, M. (2000). Municipal solid waste incinerator residue recycling by thermal processes. *Waste management, 20*(2-3), 249-258.

Samuel, S. M., Aiko, V., Panda, P., & Mehta, A. (2013). Aflatoxin b-1 occurrence, biosynthesis and its degradation. *Journal of Pure and Applied Microbiology, 7*(2), 965-971.

Sefa-Dedeh, S., Sanni, A., Tetteh, G., & Sakyi-Dawson, E. (1999). Yeasts in the traditional brewing of pito in ghana. *World Journal of Microbiology and Biotechnology, 15*, 593-597.

Shehu, K., & Bello, M. T. (2011). Effect of environmental factors on the growth of aspergillus species associated with stored millet grains in sokoto. *Nigerian Journal of Basic and Applied Sciences, 19*(2).

Shier, W., Abbas, H., Weaver, M., & Horn, B. (2005). The case for monitoring aspergillus flavus aflatoxigenicity for food safety assessment in developing countries. *Aflatoxin and food safety*, 291-311.

Thathana, M. G., Murage, H., Abia, A. L. K., & Pillay, M. (2017). Morphological characterization and determination of aflatoxin-production potentials of aspergillus flavus isolated from maize and soil in kenya. *Agriculture, 7*(10), 80.

Tola, M., & Kebede, B. (2016). Occurrence, importance and control of mycotoxins: A review. *Cogent Food & Agriculture, 2*(1), 1191103.

Turner, N. W., Subrahmanyam, S., & Piletsky, S. A. (2009). Analytical methods for determination of mycotoxins: A review. *Analytica chimica acta, 632*(2), 168-180.

UBOS. (2017). The national population and housing census 2014–area specific profile series. *Pigm Resin Technol, 37*, 01-50.

Wafula, E. N., Muhonja, C. N., Kuja, J. O., Owaga, E. E., Makonde, H. M., Mathara, J. M., & Kimani, V. W. (2022). Lactic acid bacteria from african fermented cereal-based products: Potential biological control agents for mycotoxins in kenya. *Journal of Toxicology, 2022*.

Zaragoza, J., Bendiks, Z., Tyler, C., Kable, M. E., Williams, T. R., Luchkovska, Y., Chow, E.,

Article DOI: https://doi.org/10.37284/eajhs.7.1.1369

Boundy-Mills, K., & Marco, M. L. (2017). Effects of exogenous yeast and bacteria on the microbial population dynamics and outcomes of olive fermentations. *MSphere*, *2*(1), e00315-00316.

Zaukuu, J. L. Z., Oduro, I., & Ellis, W. O. (2016). Processing methods and microbial assessment of pito (an african indigenous beer), at selected production sites in ghana. *Journal of the Institute of Brewing*, *122*(4), 736-744.

Zhang, K., Yuan-Ying, S., & Cai, L. (2013). An optimized protocol of single spore isolation for fungi. *Cryptogamie, Mycologie*, *34*(4), 349-356.

