Discover Food

Research

Mycoflora diversity, exposure to mycotoxins, and cancer risk characterization associated with the consumption of two traditionally fermented alcoholic beverages in the Ho municipality of Ghana

Nii Korley Kortei[1,2] · Harrison Nii Odartey Lamptey[1] · Benjamin Kingsley Harley[3] · Ernest Yeboah Boateng[4] · Felix Kwashie Madilo[5] · Sylvia Baah-Tuahene[6] · Vincent Kyei-Baffour[7] · Leslie Owusu Ansah[8] · Seidu A. Richard[9] · George Tawia Odamtten[10]

Received: 26 March 2024 / Accepted: 13 November 2024
Published online: 27 November 2024

Abstract
Traditionally fermented alcoholic drinks are made with locally sourced ingredients and traditional knowledge, and they are typically drank in the immediate area of production. The ingredients are sources of microbial contamination in these drinks. This study aimed to update the mycoflora and evaluate levels of Ochratoxin A (OTA) and aflatoxins contamination of some local beverages (Raffia Sap 'Palm wine' and sorghum beer 'Pito') in the Ho municipality, Volta Region, Ghana. Standard mycological analyses were carried out on the samples and the plates were incubated for 5–7 days at 28 ± 1 °C. Mycotoxin levels in the samples were quantified using High-Performance Liquid Chromatography coupled to a fluorescence detector (HPLC-FLD) was used. Deterministic models recommended by a Joint FAO/WHO Expert Committee on Additives were used to assess cancer risk. The fungal counts of palm wine samples in the Ho municipality recorded were in a range of 3.29- 4.59 log10 CFU/ml, while *pito* samples recorded a fungal range between 2.55 and 4.08 log10 CFU/ml. A total of 13 fungal species, namely; *Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Fusarium oligosporus, Fusarium verticillioides, Trichoderma harzianum, Rhizopus stolonifer, Penicillium verrucosum, Rhodotorula mucilaginosa., Mucor racemosus* and *Yeasts* were isolated from both palm wine and *pito* samples. Notably, ochratoxin A (OTA) quantities ranged between 5.50 and 14.93 µg/kg for both samples while Total aflatoxins ranged between LOD-10.70 µg/kg for *pito*. No aflatoxins were detected in palm wine. Consumer risk assessment values computed for both moderate and heavy drinkers ranged between 4.08–40.20 ng/kg bw/day, 13.88–50.44, and 0.314–1.156 cases/10,000 person/year for Total aflatoxins while ochratoxin A contents ranged 4.08–40.20 ng/kg bw/day, 0.44–4.38, and 0.131–1.286 cases/10,000 person/year correspondingly for Estimated Daily Intake (EDI), Margin of Exposure (MOE), and Cancer Risks for the age categories considered. Generally, mean fungal counts in both palm wine and *pito* beverages were high and were found to be unsatisfactory while the fungi species in these beverages showed

✉ Nii Korley Kortei, nkkortei@uhas.edu.gh | [1]Department of Nutrition and Dietetics, School of Allied Health Sciences, University of Health and Allied Sciences, PMB 31, Ho, Ghana. [2]Department of Sports Nutrition, School of Sports and Exercise Medicine, University of Health and Allied Sciences, PMB 31, Ho, Ghana. [3]Department of Pharmacognosy and Herbal Medicine, School of Pharmacy, University of Health and Allied Sciences, PMB 31, Ho, Ghana. [4]Department of Basic Sciences, School of Basic and Biomedical Sciences, University of Health and Allied Sciences, PMB 31, Ho, Ghana. [5]Department of Food Science and Technology, Ho Technical University, P.O. Box HP 217, Ho, Ghana. [6]Department of Industry and Services, Council for Scientific and Industrial Research- Science and Technology Policy Research Institute, Accra, Ghana. [7]Food and Drugs Authority, Cantonments, P.O. Box CT 2783, Accra, Ghana. [8]Council for Scientific and Industrial Research- Food Research Institute, P. O. Box M20, Accra, Ghana. [9]Department of Biochemistry and Forensic Sciences, School of Chemical and Biochemical Sciences, CK Tedam University of Technology and Applied Sciences (CKT-UTAS), P. O. Box 24, Navrongo, Ghana. [10]Department of Plant and Environmental Biology, College of Basic and Applied Sciences, University of Ghana, P. O. Box LG 55, Legon, Ghana.

great diversity. Cancer Risk assessments for the mycotoxins suggested a potential health hazard to the consumers of these beverages in the Ho municipality.

Keywords Ochratoxin A · Aflatoxins · *Pito* · Palm wine · Fungi · Population exposure

1 Introduction

Traditionally fermented alcoholic beverages are brews that are native to a region and were created by the indigenes themselves using time-honored procedures from locally sourced and homegrown raw components [1–3]. Since ancient times, traditional drinks have played an important part in African native traditions and customs. Traditional drinks are part of our cultural legacy and are particularly employed in our homes as a sign of welcome, in traditional naming and conjugal ceremonies, in customary invocations, and in folk medicine [4–7]. These age-old favorites are present in most, if not all, of the world's nations, and Ghana is no exception.

The beer that the Babylonians produced and exported to Egypt circa 3000 B.C. was probably the result of both alcoholic and lactic fermentations in antiquity. Modern African beers made from sorghum, maize, and millet all have lactic fermentation as a key component, which makes them safe and popular in tropical climates. [8]. Evidence from science shows that moderate alcohol use is beneficial since it reduces the risk of kidney stones, diabetes, bone fractures in men, and overall mortality in middle-aged and older persons. It also has a good impact on bone mineral density (BMD) [9, 10]. The cardiovascular system is undoubtedly harmed by heavy or binge drinking, which raises the risk of total and cardiovascular mortality, coronary and peripheral artery disease, heart failure, stroke, hypertension, dyslipidemia, and diabetes mellitus [9]. Heavy drinking or binge drinking is also associated with certain health risks, such as ischemic heart disease [11, 12]. It is hypothesized that often consuming small amounts of alcohol is healthier than infrequently consuming larger amounts.

Sorghum beer (*Pito*) and raffia sap (Palm wine) are two of Ghana's most widely consumed drinks at adored traditional festivities, representing the Ghanaian culture. Originally manufactured from sorghum and/or millet, *pito* has a golden yellow to dark brown look with a taste that can range from mildly sweet to quite acidic [6, 13]. *Pito* is widely consumed in Ghana and its neighboring countries such as Burkina Faso, Togo, and Nigeria. The majority of recent studies have focused on the alcoholic *pito*. [14]. Traditionally prepared by residents of the country's northern area (Ghana), pito is now produced all over the country as a result of migration. In Ghana, *pito* comes in four different varieties: *Nandom, Kokomba, Togo,* and *Dagarti*. Their distinctive qualities are due to variations in the wort extraction and fermenting processes [15].

Palm wine ('*nsafufuo*' in Akan) is widely consumed and is produced from sugary palm saps. *Elaeis guineensis, Raphia sp.,* or *Cocos nucifera* are the most common sources of palm-based beverages, and when they are fermented into a non- or low-alcoholic beverage, they are known as palm wine [5]. The most frequently tapped palms are raffia palms and oil palms. Within 24 h, fermentation occurs rapidly to bring the alcohol content to a range of 1.5 to 2.1% [16]. More than 10 million people enjoy palm wine, another prominent alcoholic beverage in West Africa because it is a traditional, sweet, fizzy beverage that is popular among people in Black Africa, Latin America, and Asia [17]. Most traditional festivities in Africa involve its presentation and widespread consumption. The use of raffia sap is expanding as a result of increased humanitarian demands and environmental concerns [5]. It's interesting to note that the role of palm wine in the region's fight against malnutrition has been acknowledged by both the government and medical specialists. The anti-oxidant properties of palm wine are widely recognized, and rat models have demonstrated its health-promoting benefits. It also contains a lot of vitamins and trace elements, which are historically thought to be good for nursing mothers' general welfare. [18, 19].

Natural contaminants like fungi and their metabolites, which are present in an array of food commodities and significantly vary in species, percentage of occurrence, chemical composition, and symptomatology in humans and animals after exposure [6, 20], in addition to the health effects of alcohol mentioned above, also present some challenges The majority of food quality and quantity degradation is caused by the existence and proliferation of fungus. Agricultural raw materials used as ingredients such as fruits, grains, seeds, etc. have been linked to the introduction of fungi into processed foods [20, 21]. Despite numerous reports on the variety and amounts of fungi and their metabolites in raw materials used in the preparation and dispensation of traditional beverages, very few studies have looked at the transfer of fungal spores from ingredients to beverages, their concentrations in the beverages, and consumer exposure rates [22]. Contrarily, according to [23], filamentous molds are renowned for their unusual ability to produce alcoholic beverages that have a variety of nutritional benefits as well as organoleptic features like flavor, taste, and color.

Mycotoxins are poisonous byproducts of certain fungi (toxigenic fungi), primarily from the *Aspergillus, Penicillium,* and *Fusarium* genera that can have a range of negative consequences on equally people and animals, from hypersensitive reactions to fatalities [24]. In 1974, an outbreak of aflatoxin in India claimed 106 lives [25]. In 2014, a different one in Kenya caused 317 cases and 125 fatalities [26]. 20 fatalities and 68 cases were reported in Tanzania in 2016 [27]. The two most dangerous and prevalent mycotoxins are ochratoxin A (OTA) and aflatoxins (AFs). Ochratoxins are formed by a lone strain of *Penicillium* (*P. verucosum*) but by several Aspergillus species, *A. ochraceus* and *Aspergilli* of the section Nigri, especially *A. carbonarius*. Aflatoxins are primarily produced by two closely related Aspergillus species, *A. flavus* and *A. parasiticus*, and are thought to be mutagenic and carcinogenic in animals and humans [28]. The International Agency for Research on Cancer (IARC) of the WHO has categorized both aflatoxins and ochratoxin A as Group 1 and 2B (possible human carcinogens), respectively [29, 30]. Both mycotoxins are regarded as being extremely dangerous since they may contribute to the development of neurotoxicity, immune-neurotoxity [31], and above all, hepatocellular carcinoma (HCC) by causing DNA adducts which alter the genetic makeup of liver cells [32, 33]. The sixth most prevalent form of cancer overall is liver cancer [34]. According to current estimates, Ghana records more than 24,000 new cases of cancer annually. Approximately 15,802 Ghanaians passed away from cancer in 2020, inclusive. Among the top causes in both sexes was liver cancer [29, 30, 34]. and it has been hypothesized that mycotoxins, particularly aflatoxins, may be a contributing factor in 4.6%–28.2% of all HCC cases [35]. HCC is more likely to occur in people with chronic hepatitis B virus (HBV) infections who have also been exposed to mycotoxins, especially AFB1 [32, 36, 37].

Consumers and governments around the world now recognize food contamination by fungi and mycotoxins as one of the most serious threats to food safety. Unfortunately, many countries, including Ghana, do not have strict control procedures for traditional brewing, fermentation, and preservation of these traditionally fermented alcoholic beverages. As far as we are aware, no research has been done on the mycofloral profile and mycotoxin exposure to people in Ghana's native alcoholic beverages. This study therefore sought to update the fungal species diversity, mycotoxin exposure, and cancer risk assessment linked to the consumption of some traditionally fermented alcoholic beverages in Ghana.

2 Materials and methods

2.1 Study site and study population

This study was a quantitative experimental study performed in the Ho municipality (6° 31′ 43″ N 0° 28′ 13″ E), Volta Region. The traditionally fermented alcoholic beverages were purchased from 3 vendors per location from the 3 zones (upper, middle, and lower), in the Ho municipality. All other procedures were carried out in the University of Health and Allied Sciences (UHAS) food microbiology laboratory located on the Dave campus and the Food Laboratory Department, Food and Drugs Authority (FDA), Accra.

Ghana's Volta Region's administrative center is located at Ho Municipal. It belongs to one of the twenty-five (25) Districts in the area. Additionally, this municipality is the most prosperous hub of the area. From the information kept by the Ghana Statistical Service, the municipality includes 772 villages and a 2660 square kilometer land area.[38]. The study's sampling locations were divided into 3 regions or zones: upper, middle, and lower regions.

3 Sampling method

3.1 Sample collection, and processing

Fresh palm wine and sorghum beer samples were purchased from retailers in the Ho municipality. A convenient sampling method was used. Ho municipal was divided into 9 zones/location and 5–6 samples (3 points per location/zone) were collected from locations in each region (zone) for both Palm wine and *Pito*. In total 50 (n = 50) samples were collected for both Palm wine and *Pito* (500 ml or 0.5 L) from locations in the Ho municipality.

Within two hours of collection, each sample was taken and stored in sterile vials with a volume of about 100 ml. It was then transported to the UHAS laboratory for microbiological examination in an ice chest freezer (Thermos 7750, China) with cold packs at a temperature of 10 °C under aseptic conditions [39].

3.2 Fungal plating

One milliliter of each of the samples was diluted into ten milliliters (ml) of distilled water to form the stock solution. Each stock solution was serially diluted up to a 10^{-3} concentration in 9 ml of sterile distilled (0.1%) water. Each serial dilution was plated onto Dichloran Rose Bengal Chloramphenicol (DRBC) at a volume of one milliliter and incubated at 28 ± 1 °C for 5–7 days. For a second time, the same process was used, and one milliliter of each serial dilution was plated onto an Oxytetracycline Glucose Yeast Extract (OGYE) agar base according to the instructions by [40] and [41].

3.3 Fungal enumeration and identification

A colony counter carried out the enumeration. Standard form counts of fungi were made and then converted to a logarithmic scale. Using the formula, the colony-forming unit (CFU) per milliliter was calculated.

$$\text{CFU/ml} = \frac{\text{number of colonies} \times \text{reciprocal of the dilution factor}}{\text{culture plate volume}} \quad (1)$$

Using the formula, the percentage of fungal species was calculated.

$$\text{Percentage}\,(\%)\,\text{occurrence of fungal species} = \frac{\text{Number of fungal species}}{\text{Total number of fungi isolated}} \times 100 \quad (2)$$

*The mean of 6 samples were taken to represent the value of a sample Eg. Palm wine sample 1 (PWS1) = Av. 6 samples.

3.4 Identification

Molds that grew were recognized by their morphological and cultural traits (using guides for standard mold identification) [42, 43] (Table 2, Figure 7). Both macroscopically (based on the texture and color of the plate) and microscopically (using cultural and morphological characteristics under the microscope) the fungi were identified. (Figure 8).[43–45].

*The mean of 6 samples were taken to represent the value of a sample (Eg. PWS1 = Av. 6 samples)

3.5 Determination of pH

Using a tabletop pH meter (Jenway 3510, United Kingdom), the pH of the samples was assessed by calibrating with standard buffers of pH values 4.0 and 7.0. The measurements were made when the samples were at room temperature, and the average of three results was calculated.

4 Determination of aflatoxins

4.1 Abstraction of samples

According to the official European Committee for Standardization (CEN) technique, EN14123 [46] for aflatoxin extraction, AFB1, AFB2, AFG1, and AFG2 were removed from the samples. About twenty-five millilitres (25 ml) of the material was extracted using methanol in 200 ml of water (8 + 2) and 5 g of NaCl. Hexane (100 ml) was added to more than half of the samples. At 3000 and 3500 revolutions per minute, the mixture was homogenized for 3 min. The extracts were filtered, and 10 ml of the filtrate was added to 60 ml of phosphate buffer saline (PBS) for solid-phase extraction using an immune-affinity column (TC-C18 (2), 170, 5 µm, 4,6 × 250 mm; thus pore size of 170, particle size of 5.0 µ, inner diameter of 4.6 mm, length of 250 mm and carbon load of 12%) that was preconditioned and was particular for AFB1, AFB2, AFG1, and AFG2. The preconditioned column was loaded with the 70 ml filtrate-PBS combination, which was then permitted to elute naturally at a flow rate of 1 ml/min. After that, 15 ml of distilled water

was removed from the area at a flow rate of 5 ml/min. Aflatoxins were separated into 2 phases and then gravity-eluted into a 5 ml volumetric flask using 0.5 and 0.75 ml of HPLC-grade methanol, respectively. To bring the eluate volume to 5 ml, deionized water was utilized. The eluate was vortexed, and 2 ml was pipetted into HPLC vials for measurement.

4.2 HPLC parameters

Injection volume: 10 µl flow rate: 1 ml/min, column temperature: 35 ℃, excitation wavelength: 360 nm, emission wavelength: 440 nm, mobile phase composition: water/acetonitrile/MeOH (65:15:20 v/v/v), post-column derivatization: Kobra cells. HPLC Column Specification; TC-C18 (2), 170, 5 µm, 4,6 × 250 mm; thus pore size of 170, particle size of 5.0 µ, inner diameter of 4.6 mm, length of 250 mm and carbon load of 12%, 5 µm particle size, 250 A pore size, Acetonitrile (MeCN), Methanol (MeOH).

Supplier of Column R- Biopharm, Block 10 campus, West Scotland Science Park, Acre Road, Glasgow, Scotland G20 OXA.

4.3 Limit of detection/quantification (LOD/LOQ)

By creating a calibration curve around the standard used for spiking, 5 µ/kg (the lowest concentration range of the calibration curve), the limits of detection and quantification (LOD/LOQ) of the HPLC were estimated. Based on the standard deviation (SD) of responses (σ) and the slope (S) of the calibration curves for the aflatoxin STD and the spiked extracts, the parameters LoD and LoQ were determined.

The LOD and LOQ were calculated as follows because Blank might not give any signal.;
LOD for Ochratoxins—0.83 µg/kg.
LOQ—2.49 µg/kg.
LOD for AFB_1, AFB_2- 0.15 µg/kg; AFG_1 and AFG_2—0.13 µg/kg,
LOQ—0.39 and 0.45 µg/kg.

4.4 Measurement of accuracy

To ensure the measurement accuracy of the assay, a pure aflatoxin reference solution was spiked. Three levels of spiking were carried out at the lower, middle, and upper concentration ranges of the calibration curve concentrations (5 ppb, 15 ppb, and 30 ppb). The spike volumes for pure standards were calculated as follows:

$$\left[\text{Sample weight (g)} * \text{spike concentration (ppb)}\right] / \left[\text{Concentration of standard (ug/ml)}\right] \quad (3)$$

Aflatoxin-free sample (blank) was spiked with equal amounts, and the % recovery from the spiked sample was determined as;

$$[(\text{Concentration measured in spike} - \text{Concentration measured in the blank}) / (\text{spiked amount})] * 100 \quad (4)$$

4.5 Measurement of precision

To guarantee the measurement precision of the procedure, repeatability and intermediate precision studies of an internal reference material (IRM) should be used. The same analyst performed 10 parallel IRM extractions using the same HPLC at the same time for the repeatability analysis, and the relative standard deviation between the results was computed. Ten IRM extractions were performed with intermediate precision on several days by various analyzers, and the relative standard deviation of the results was premeditated. The comparative standard deviations were computed as;

$$\left[\text{Standard deviation} / \text{mean}\right] * 100 \quad (5)$$

CEN official method EN14123 described by [46].

4.6 Required performance criteria for accuracy and precision

Repeatability: Less than 15% of reproducible outcomes had a relative standard deviation.

Intermediate Precision: Under conditions of intermediate precision, the relative standard deviation of the results was less than 20%.

Recovery: The measurement procedure's percent recovery ranged from 80 to 120%.

*Linearity***:** Linearity of the regression curve was 0.99 (B1, B2, G1) and 0.98 (G2).[40]

4.7 Experimental data

Repeatability: Relative standard deviation;

B1 = 5.5%; B2 = 6.7%; G1 = 7.4%; G2 = 12.1% and Total aflatoxins = 5.2%.

Intermediate Precision (Reproducibility): Relative standard deviation;

B1 = 13.2%; B2 = 13.4%; G1 = 13.7%; G2 = 12.2% and Total aflatoxins = 11.9%.

*Recovery***:** Percent recovery of measurement procedure (CEN official method EN14123) [40, 46].

5 Determination of Ochratoxin A

5.1 Chemicals and standards

The OTA analytical standard was provided by Sigma-Aldrich (St. Louis, MO, USA). The HPLC grade solvents utilized in the creation of the mobile phase were all purchased from Merck in Darmstadt, Germany. Methanol and hexane of analytical grade were provided for the extraction by Sigma-Aldrich. Whatman no. 4 filters (Whatman Ltd, Maidstone, UK) were used to filter all homogenized mixes and eluates, respectively. De-ionized water was obtained using a Millipore Elix Essential filtration system (Bedford, MA, USA). OCHRA PREP immunoaffinity columns from RBiopharm, Rhone Limited. These columns had a recovery rate of at least 90% and a concentration capability of 100 ng/ml. PBS (phosphate-buffered saline) was made by dissolving PBS tablets in Sigma-Aldrich distilled water. 99.0% sodium chloride was supplied by Sigma-Aldrich. A six-time calibration was performed on the pure Ochratoxin A standard at concentrations of 5 g/kg, 10 g/kg, 15 g/kg, 20 g/kg, 25 g/kg, and 30 g/kg. Linearity for the regression curve was deemed acceptable at 0.99 or 99% [64].

5.2 Determination of Ochratoxin

The CEN official method EN14123 [47] was used to determine ochratoxin A. By thoroughly mixing the entire batch, 500 ml of *pito* and palm wine were sampled. The beverages were divided into four equal parts using a pipette. The remaining two parts was stored, and the process were repeated until a representative 500 ml sample was obtained. In a 4:1 ratio, 25 ml of samples were extracted with 5 g sodium chloride and 200 ml methanol in distilled water. Hexane (100 ml) was added to the *pito* and palm wine mixture, and the samples were homogenized for 3 min (i.e., 3000 rpm for 2 min and at 3500 rpm for 1 min). Two organic layers were formed by the beverage mixture (the hexane upper layer and methanol lower layer). The *pito* and palm wine mixtures' lower methanol layers were filtered through Whatman number 4 filter paper. Filtrates in the amount of ten milliliters (10 ml) were employed for a solid-phase extraction and cleanup. To 10 ml of filtrates, Phosphate Buffer Saline (PBS) was added, and the mixture was agitated. Immunoaffinity columns specific for ochratoxin A were preconditioned and the antibodies in the column were activated by eluting 10 ml of phosphate buffer saline through columns at a flow rate of 3 ml min 1. A precise 50 ml portion of the 160 ml filtrate-PBS combination was placed onto the pre-conditioned immunoaffinity columns tailored for ochratoxin A, and the columns were then left to drain naturally. Before eluting at a rate of 5 ml/min, the columns were washed three times with 5 ml of PBS. A vacuum pump was used to blast air through the columns to eliminate any wash solvent molecules. After one minute, ochratoxin A was eluted twice into a 5 ml volumetric flask: once with 1 ml of the purest methanol and once more with 1 ml. The column was blown with air to collect all of the eluates. The eluates were diluted to a level of 4 ml using aqueous acetic acid (1%) and vortexed before being pipetted into HPLC vials for measurement.[64].

5.3 HPLC parameters

OTA was measured using an Agilent HPLC 1260 infinite series high-performance liquid chromatography system equipped with a quaternary pump and fluorescence detection. Chem Station's Open Lab Edition was used for data collection and quantification. The Agilent HPLC, which had a fluorescence detector, was configured to excite at 333 nm and emit at 467 nm. The column compartment temperature was set to 30 °C. The mobile phase consisted of a 40:30:30 mixture of 5 mM sodium acetate, acetic acid (pH 2.4), methanol, and acetonitrile. The administration mode was isocratic, with a flow rate of 1 ml/min and an injection volume of 10 µl. The timer was set for ten minutes. [47] and [64] (CEN official method EN14123).

6 Consumer risk assessment of exposure to mycotoxins (ochratoxin A and aflatoxin B1) via consumption of *pito* and palm wine

6.1 Exposure estimation

The regular body weight, the quantity of samples ingested daily, and the average amount of mycotoxin (ochratoxin A and aflatoxin B1) consequent from the *pito* and palm wine samples were used to compute the estimated daily intake (EDI). The EDI for mean OTA and AFB1 was calculated beforehand using the formula shown in Eq. (5) and represented in grams per kilogram of body weight per day (g/kg b.w/day). [48, 49].

$$EDI = \frac{daily\ intake\ (food) \times mean\ level\ of\ my\ cotoxin}{Av.Body\ weight} \tag{6}$$

We categorized our individuals into heavy and moderate drinkers of *pito* and palm wine beverages and made some assumptions on the average daily intake of traditionally fermented alcoholic beverages in Africa (Ghana) as 0.045 (0.020–0.070 kg/day) for moderate drinkers and > 0.070 kg/day for heavy drinkers according to [50],

The following are the various age groups as defined by the European Food Safety Authority (EFSA) [51], along with the estimated average weights for each in Ghana that were used in this study: Adolescents: 46.25 (38.5–54) kg [54], Adults: 60.7 kg [55], Children: – 26 (24–28) kg [52, 53].

6.2 Cancer risk characterization for Ochratoxin A and aflatoxin B1

Using the Margin of Exposure (MOEs) procedure, the hazard assessments for genotoxic chemicals such as ochratoxins and aflatoxins are correctly computed by dividing the BMDL for ochratoxins, 130 $ngkg^{-1}bwweek^{-1}$, by the anticipated MOEs [56] and 120 $ngkg^{-1}bwweek^{-1}$ [56, 57] ensuing in an average of 125 $ngkg^{-1}bwweek^{-1}$ (17.86 $ngkg^{-1}bwday^{-1}$) and 400 $ngkg^{-1}bwday^{-1}$ for Ochratoxin A and aflatoxin exposures correspondingly as expressed in Eq. (6).

$$MOE = \frac{Bench\ mark\ dose\ lower\ limit}{EDI(Exposure)} \tag{7}$$

An adverse health risk is realized if MOEs are less than 10,000.

6.3 Probable risk of liver cancer from food sample consumption

Liver cancer can arise as a result of both aflatoxins and ochratoxins [58, 59]. Therefore, the risk of liver cancer in adult Ghanaian consumers was estimated using aflatoxins and ochratoxins [60]. The average hepatocellular carcinoma (HCC) potency, which is a product of the EDI value and the average HCC potency figure, was determined by summing the individual potencies of Hepatitis B surface antigen (HBsAg) (HBsAg-positive and HBsAg-negative groups).

For this computation, the JECFA [61] estimated potency values for OTA, which equated to 0.01 cancers $year^{-1}10{,}000^{-1}population/ngkg^{-1}bwday^{-1}$ (uncertainty range: 0.002–0.03) in HBsAg-negative individuals and 0.3 cancers $year^{-1}$ $10{,}000^{-1}$ $population/ngkg^{-1}bwday^{-1}$ (uncertainty range: 0.05–0.5) in HBsAg-positive individuals [59]. Furthermore, 92.26% (100—7.74%) was inferred for HBsAg-negative categories, whereas the average HBsAg + prevalence rate for

Ghana [62] was 7.74% (adult – 8.36%, 14.3%-adolescents, 0.55%-children). As a result, the following estimate of Ghana's average cancer potency was made using Eq. (7), which was suggested by [59] and [63]:

$$\text{Average potency} = \left[0.03 \times \text{HBsAg} - \text{negative individuals in Ghana}\right] + \left[0.01 \times \text{HBsAg} - \text{positive individuals} / \text{prevalence rate in Ghana}\right] \quad (8)$$

$(0.3 \times 0.077) + (0.01 \times 0.9226)$.
$= 0.0323$ cancers per year per 10,000 population per ng ochratoxins $kg^{-1}bwday^{-1}$.
While the average potency of Aflatoxins $= 0.0396$ $kg^{-1}bwday^{-1}$ per 10,000 population
Thus, the Cancer Risk was estimated using the following formula in Eq. (8) [59, 63, 64]:

$$\text{Cancer risk} = \text{Exposure (EDI)} \times \text{Average potency} \quad (9)$$

6.4 Statistical analysis

After performing the pH and fungal count tests in triplicate, the data was subjected to a one-factor analysis of variance (ANOVA). The results are shown as means plus standard deviation. The study employed mean counts that were converted to logarithmic values using standard forms. Mycotoxin analysis was done using regression analysis.

The Duncan's multiple range test (DMRT) was employed to identify differences between means using the Statistical Package for the Social Sciences (SPSS) software version 22, with a significance level of 5% ($p < 0.05$) deemed appropriate.

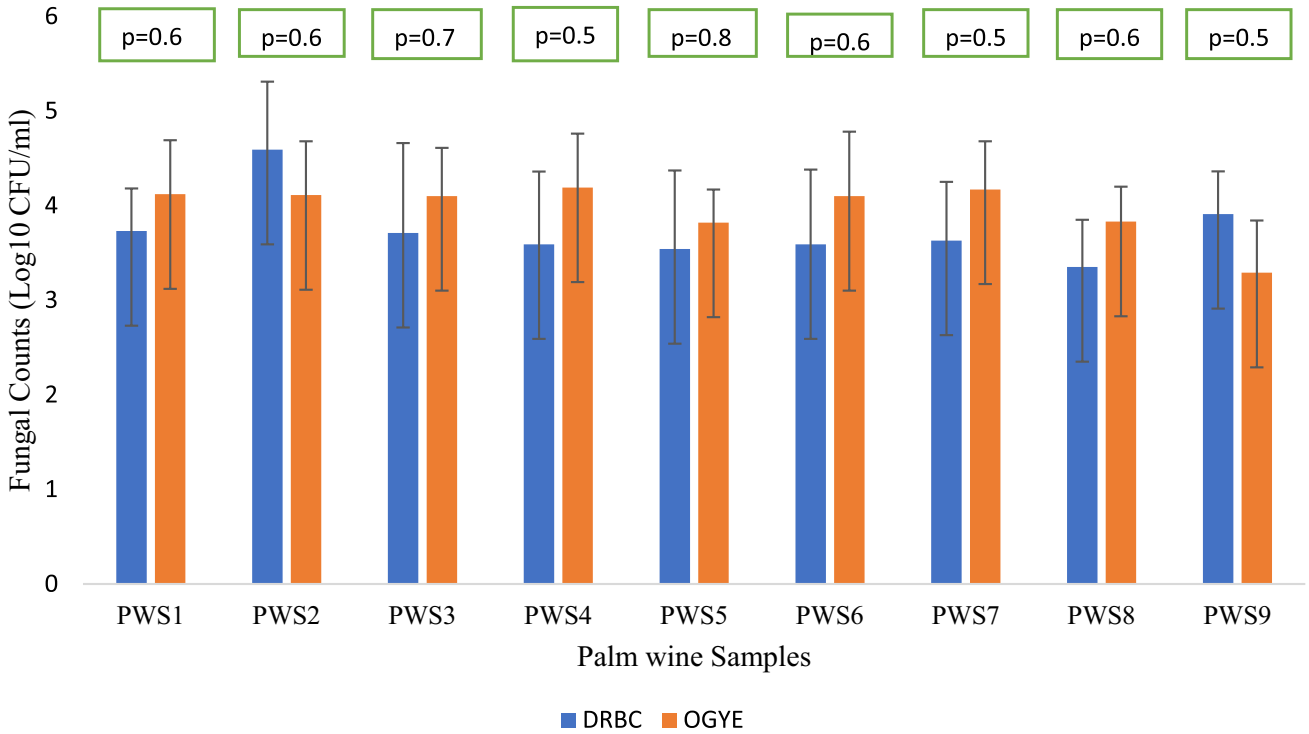

Fig. 1 Mean Fungal counts of Palm wine samples enumerated on DRBC and OGYE media incubated for 5–7 days at 28 ± 1 °C

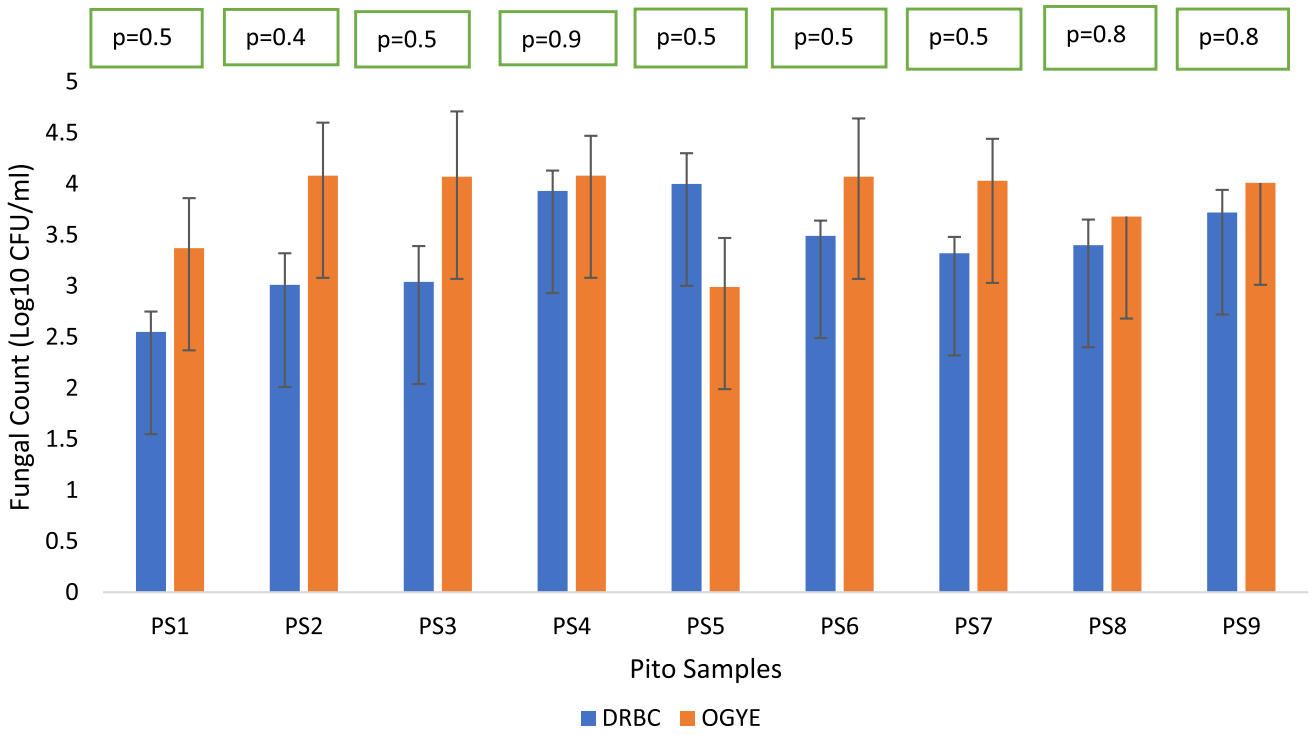

Fig. 2 Mean Fungal counts of *Pito* samples enumerated on DRBC and OGYE media incubated for 5–7 days at 28 ± 1 °C

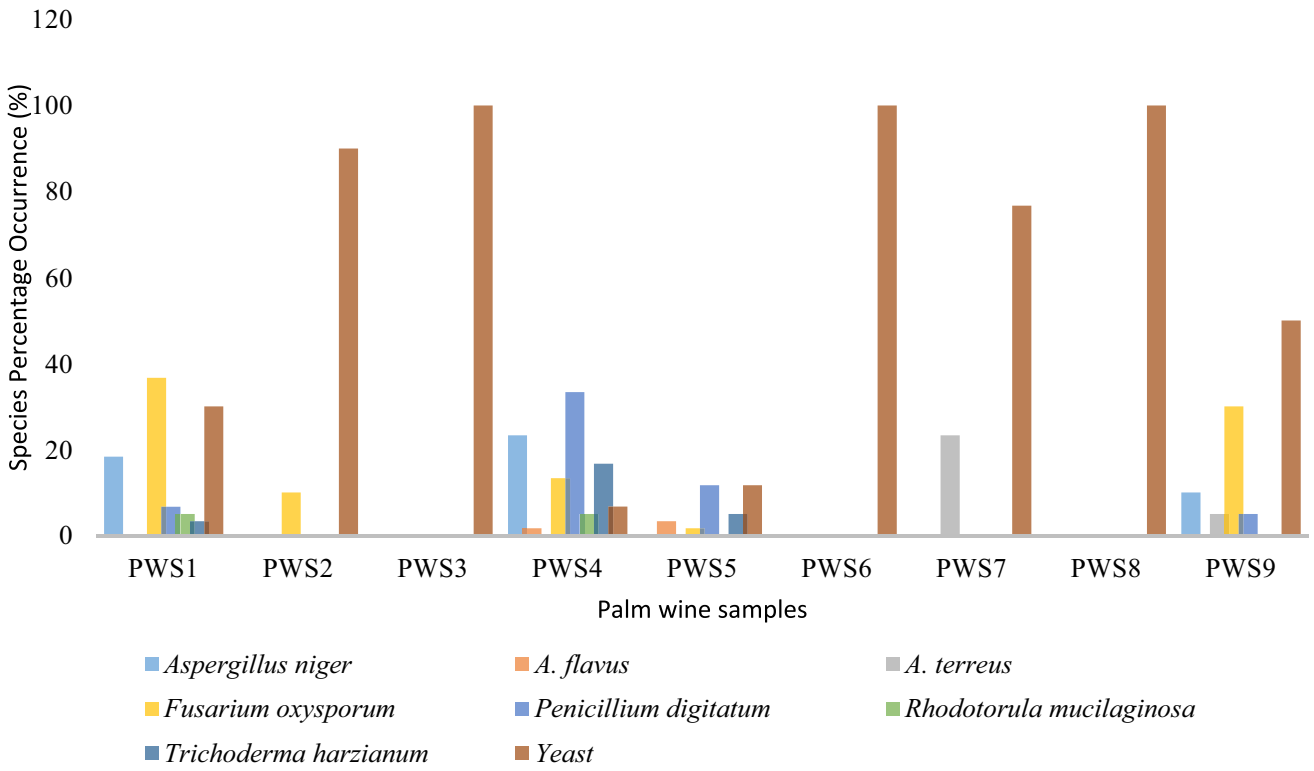

Fig. 3 Percentage occurrence (%) of fungal species cultured on DRBC medium at. 28 ± 1 °C

7 Results

7.1 Mean fungal counts of palm wine and *pito*

The results of the various fungal counts of palm wine and *Pito* from various locations are represented in Figs. 1 and 2. Palm wine samples had fungal counts ranging from 3.29 to 4.59 $\log_{10}$ CFU/ml, with the lowest value recorded by PWS9 and the highest value by sample PWS2. There were significant differences ($p < 0.05$) in the mean fungal counts of palm wine samples.

7.2 Mean fungal counts of *pito*

For DRBC and OGYEA plated *Pito* samples, fungal counts ranging from 2.55 to 4.08 $\log_{10}$ CFU/ml was recorded, with the lowest value recorded by PS1 and highest value by sample PWS2. There were no significant differences ($p > 0.05$) in the mean fungal counts of *pito* samples recorded on both media. (Fig. 3).

7.3 Percentage (%) Occurrence of *fungi*

Approximately, up to 13 fungal species, namely; *Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Fusarium oligosporus, Fusarium verticillioides, Trichoderma harzianum, Rhizopus stolonifer, Penicillium verrucosum, Rhodotorula mucilaginosa., Mucor racemosus and Yeast* fitting to eight (8) genera with names: *Aspergillus, Fusarium, Trichoderma, Rhizopus, Penicillium, Rhodotorula, Mucor* and *Yeast* were identified from both palm wine and *pito* samples (Figs. 7 and 8).

In the palm wine samples, a total of 11 fungal species were isolated (Figs. 3 and 4) with the percentage distribution as follows from both media; *Aspergillus niger* (4.85%), *Aspergillus flavus* (1.41%), *Aspergillus terreus* (1.70%), *Fusarium*

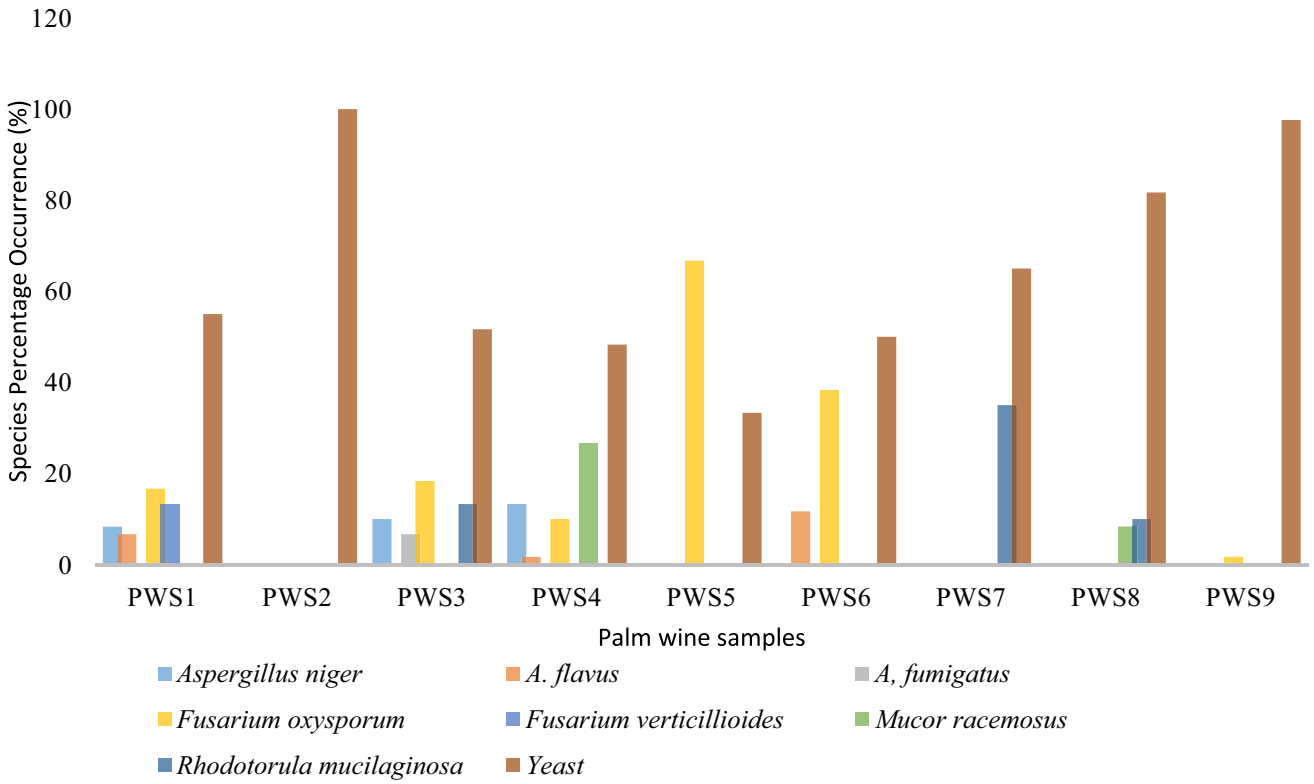

Fig. 4 Percentage occurrence (%) of fungal species cultured on OGYE medium at 28 ± 1 °C and identified in palm wine and identified in palm wine

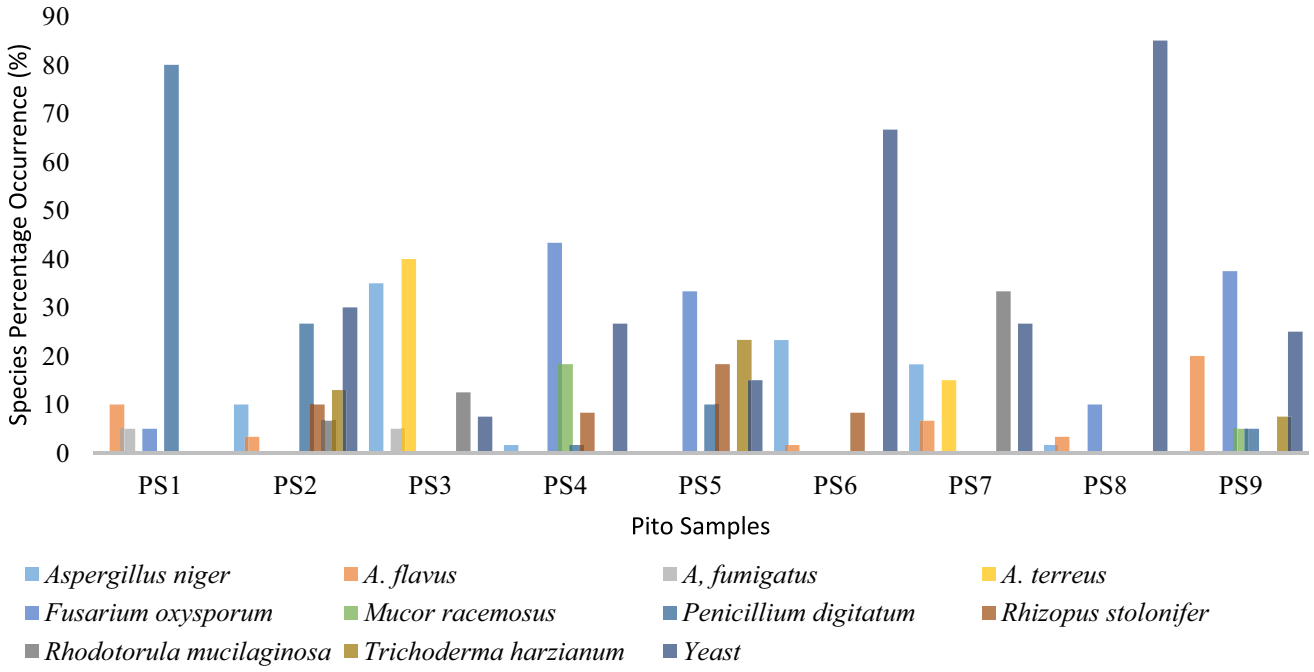

Fig. 5 Percentage occurrence (%) of fungal species cultured on DRBC medium at 28 ± 1 °C and identified in *pito*

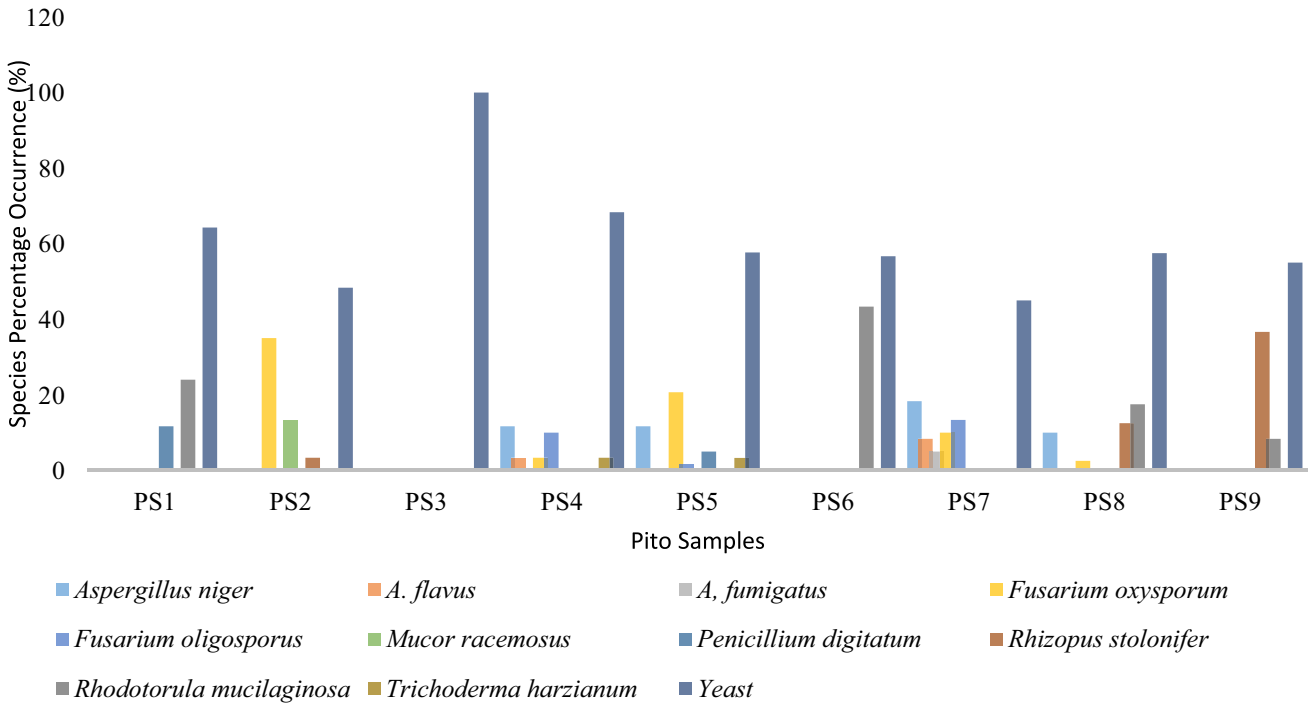

Fig. 6 Percentage occurrence (%) of fungal species cultured on OGYE medium at 28 ± 1 °C and identified in *pito*

oxysporum (13.93%), *Penicillium verrucosum* (3.40%), *Rhodotorula mucilaginosa* (3.84%), *Trichoderma harzianum* (1.50%), *and Yeast* (66.29%), *Aspergillus fumigatus* (0.37%), *Fusarium verticillioides* (0.75%), *Mucor racemosus* (1.95%) *and yeast* (66.29%) belonging to eight (8) genera namely: *Aspergillus, Fusarium, Trichoderma, Rhizopus, Penicillium, Rhodotorula, Mucor* and *Yeast.*

Pito samples recorded a total of 12 isolated (Figs. 5 and 6) fungal species, namely; *Aspergillus niger* (7.87%), *Aspergillus terreus* (7.03%), *Aspergillus flavus* (3.15%), *Aspergillus fumigatus* (0.83%), *Fusarium oxysporum* (7.18%), *Fusarium Oligosporus*

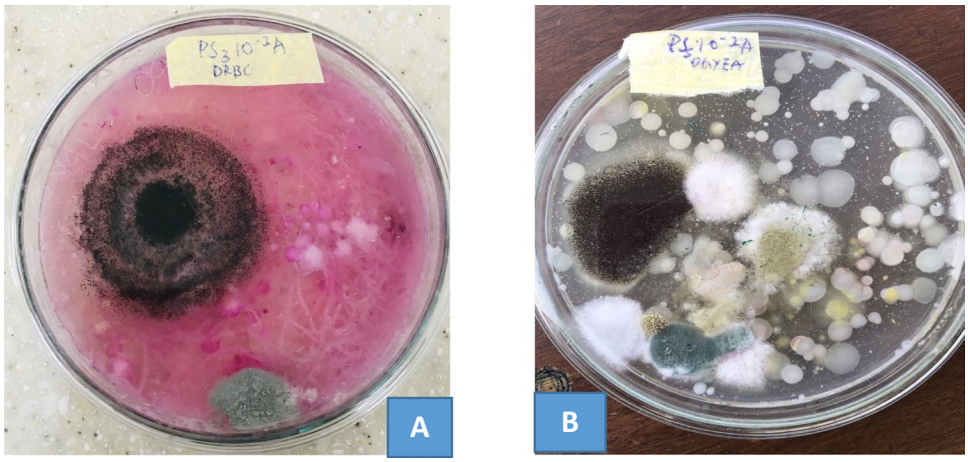

Fig. 7 A Macroscopic view of (**A**): *A. niger* (black) and *A. fumigatus* (grey) isolated from palm wine and cultured on DRBC. **B**: *F. oxysporum* (white fluffy), *P. verrucosum* (bluish green) and Yeast (creamy) isolated from palm wine and *pito* and cultured on OGYE

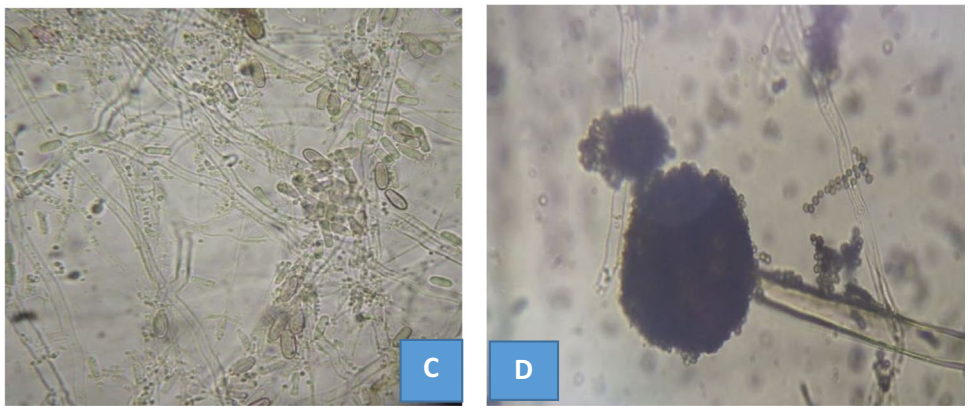

Fig. 8 Microscopic views of (**C**) *Fusarium oxysporum* (×400) and (**D**) *Aspergillus niger* (×400)

(1.39%) *Trichoderma harzianum* (2.8%), *Penicillium verrucosum* (7.78%), *Rhodotorula mucilaginosa* (8.09%), *Mucor racemosus* (2.04%), *Rhizopus stolonifera* (5.41%) *and Yeast* (46.42%) belonging to eight (8) genera namely: *Aspergillus, Fusarium, Trichoderma, Rhizopus, Penicillium, Rhodotorula, Mucor* and *Yeast*.

The microscopic and large cultural morphologies of some of the fungi that were found are displayed in Figs. 7 and 8.

Both traditionally fermented alcoholic beverages investigated had microbial counts which were generally between 2.55 and 4.59 Log_{10} CFU/ml which is interpreted as being unsatisfactory for ready-to-eat foods according to guidelines prescribed by the International Commission for Microbiological Specifications for Foods (ICMSF) [65] (Table 1).

8 pH

8.1 Palm wine and *pito* samples

Palm wine samples recorded a range between 4.35 and 4.50. Statistically, the samples differed significantly ($p < 0.05$) (Table 2). They were acidic in taste according to the pH scale. *Pito* samples recorded a range of between 4.29 and 4.39. Statistically, the samples were significantly different ($p < 0.05$) (Table 3). They fell in the acidic range of the pH scale.

9 Ochratoxin content

9.1 Palm wine

According to the findings, the mean OTA load in the Ho Municipality was at 5.5 µg/kg, which ranged between 0.00 and 34.00 µg/kg with a variance of 90.01.

Table 1 ICMSF guide on how to interpret data for particular foodborne pathogens in ready-to-eat food generally (colony-forming unit (CFU/g)

Hazard	Result (CFU/g)	Interpretation	Likely cause
Fungi	$<10^2$ or 2 $\log_{10}$	Acceptable	
	10^2–$<10^4$ 2–4 $\log_{10}$	Peripheral/borderline	Process safeguards not fully implemented or potential contamination of raw materials
	$>10^4$ 4 $\log_{10}$	Unacceptable (possibly harmful to health and/ or unfit for human consumption)	Insufficient cooling and storage time and temperature management, allowing spores to sprout and grow

Source: ICMSF [65]

Table 2 pH results for Palm wine and *Pito* Samples

Palm wine	Mean ± st.dev	*Pito*	Mean ± st.dev
PWS1	4.44 ± 0.01^{b}	PS1	4.33 ± 0.02^{a}
PWS2	4.50 ± 0.02^{c}	PS2	4.39 ± 0.01^{c}
PWS3	4.42 ± 0.02^{b}	PS3	4.29 ± 0.03^{a}
PWS4	4.37 ± 0.01^{a}	PS4	4.32 ± 0.00^{ab}
PWS5	4.45 ± 0.01^{b}	PS5	4.40 ± 0.01^{c}
PWS6	4.43 ± 0.02^{b}	PS6	4.33 ± 0.03^{a}
PWS7	4.35 ± 0.03^{a}	PS7	4.41 ± 0.01^{c}
PWS8	4.36 ± 0.01^{a}	PS8	4.35 ± 0.01^{a}
PWS9	4.35 ± 0.01^{a}	PS9	4.29 ± 0.01^{a}

Means within a column with different superscripts are significantly different ($p < 0.05$)

Table 3 Aflatoxins B1, B2, G1 and G2 levels (µg/Kg) in *pito*

	N	Range	Mean		Std. deviation	Variance	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Std. error	Statistic	Statistic	Statistic	Std. error	Statistic	Std. error
B1	50	30.87	7.2062	1.2903	9.123	83.246	0.900	0.337	− 0.466	0.662
B2	50	12.18	2.471	0.535	3.7850	14.326	1.337	0.337	0.505	0.662
G1	50	5.80	0.84	0.2314	1.6368	2.679	1.846	0.337	2.243	0.662
G2	50	1.98	0.195	0.0717	0.5076	0.258	2.438	0.337	4.673	0.662
Total	50	42.00	10.7082	1.9323	13.664	186.696	0.836	0.337	− 0.811	0.662
Valid N (listwise)	50									

Table 4 Ochratoxin amounts (µg/Kg) in *pito* and palm wine samples

	N	Range	Mean	Std. Deviation	Variance	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
OTA *Pito*	50	68.57	14.933	22.95	526.96	1.175	0.337	− 116	0.662
OTA Palm wine	50	34.00	5.5	9.48	90.01	1.633	0.337	1.834	0.662
Valid N (listwise)	50								

9.2 Pito

The mean OTA load in *pito* was at 14.933 µg/kg, and was in the range of 0.00–68.57 µg/kg with a variance of 526.96.

9.3 Aflatoxins B1, B2, G1, and G2 levels of *pito*

About 48% (24/50) of *pito* samples from the Ho municipality tested positive and recorded a mean of 10.708 µg/Kg. Values ranged between LOD-42.00 µg/Kg of Total aflatoxins. Also, AFB1 ranged between LOD-30.87 µg/Kg. and recorded a mean of 7.6102 µg/Kg (Table 3).

No aflatoxins (< LOD) were found in the palm wine samples.

9.4 Ochratoxins in *Pito* and palm wine

About 34% (17/50) of the *pito* samples tested positive which ranged between LOD- 68.57 µg/Kg and a mean of 14.933 µg/Kg. While 30% (15/50) palm wine samples tested positive and ranged between LOD-34.00 µg/Kg with a mean of 5.5 µg/Kg (Table 4).

Table 5 Evaluation of the Ochratoxin A (OTA) cancer risk associated with palm wine intake in the Ho municipality (Moderate Drinkers)

District	Age category	Estimated Daily Intake (EDI) (ng/kg bw/day)	MOE	Cancer risk (cases/10,000 persons/year)
Ho	Children (36mths-10yrs)	9.52	1.88	0.305
	Adolescents (11-17yrs)	5.35	3.34	0.171
	Adults (18-64yrs)	4.08	4.38	0.131

Mean ochratoxin—5500 ng/kg,

Av. Daily intake for Moderate Drinkers = 0.045 (0.020–0.070) kg/day was used for children, adolescents, and adults

Av.Potency = 17.86 ng ochratoxins/kg bw/day

Benchmark Dose Lower limit = 17.86 ng ochratoxins/kg bw/day

1000 ng = 1 µg, Margin of Exposure-MOE

9.5 Consumer risk assessment

9.5.1 Ochratoxin A (OTA)

9.5.1.1 Moderate drinkers of palm wine There were discrepancies in the outcomes of the risk assessments for ochratoxin A (OTA) levels brought on by the moderate consumption of the palm wine samples in the various zones of the Ho municipality. The documented Estimated Daily Intake (EDI) values for children, adolescents, and adults were 9.52, 5.35, and 4.08 ng/Kg bw/day, respectively. For the same age groups, the documented Margin of Exposure (MOE) values were 1.88, 3.34, and 4.38, respectively. Ochratoxins had an average potency of 17.86 ng/kg bw/day, resulting in cancer risks of 0.305, 0.171, and 0.131 Cases/10,000 persons/year respectively (Table 5).

Table 6 Evaluation of the Ochratoxin A (OTA) cancer risk associated with palm wine intake in the Ho municipality (Heavy Drinkers)

District	Age Category	Estimated Daily Intake (EDI) (ng/kg bw/day)	MOE	Cancer Risk(Cases/10,000 persons/year)
Zone A	Children (36mths-10yrs)	14.81	1.206	0.474
	Adolescents (11-17yrs)	8.32	2.150	0.266
	Adults (18-64yrs)	6.34	2.817	0.202

Mean ochratoxin A- 5500 ng/kg,

Av. Daily intake for Heavy Drinkers = 0.070 kg/day was used for children, adolescents, and adults

Benchmark Dose Lower limit = 17.86 ng ochratoxins/kg bw/day

1000 ng = 1 µg

Margin of Exposure-MOE

Table 7 Evaluation of the Ochratoxin A (OTA) cancer risk associated with *pito* intake in the Ho municipality (Moderate Drinkers)

District	Age category	Estimated daily intake (EDI) (ng/kg bw/day)	MOE	Cancer risk(cases/10,000 persons/year)
Ho	Children (36mths-10yrs)	25.840	0.69	0.826
	Adolescents (11-17yrs)	14.53	1.23	0.465
	Adults (18-64yrs)	11.06	1.07	0.353

Mean ochratoxin A = 14,930 ng/kg,

Av. Daily intake for Moderate Drinkers = 0.045 (0.020–0.070) kg/day was used for children, adolescents, and adults

Benchmark Dose Lower limit = 17.86 ng ochratoxins/kg bw/day

1000 ng = 1 µg, Margin of Exposure-MOE

Table 8 Evaluation of the Ochratoxin A (OTA) cancer risk associated with *pito* intake in the Ho municipality (Heavy Drinkers)

District	Age Category	Estimated Daily Intake (EDI) (ng/kg bw/day)	MOE	Cancer Risk(Cases/10,000 persons/year)
Ho	Children (36mths-10yrs)	40.20	0.444	1.286
	Adolescents (11-17yrs)	22.60	0.790	0.723
	Adults (18-64yrs)	17.22	1.04	0.551

Mean Ochratoxin A- 14,930 ng/kg

Av. Daily intake for Heavy Drinkers = 0.070 kg/day was used for children, adolescents, and adults

Benchmark Dose Lower limit = 17.86 ng ochratoxins/kg bw/day

1000 ng = 1 µg, Margin of Exposure-MOE

9.5.1.2 Heavy drinkers of palm wine Children, teenagers, and adults recorded EDI values of 14.81, 8.32, and 6.34 ng/Kg b.w/day, respectively. MOE values of 1.206, 2.150, and 2.817 were noted, respectively. While the cancer risks were 0.474, 0.266, and 0.202 Cases/10,000 persons/year, respectively, the average potency remained constant (Table 6).

9.5.1.3 Moderate drinkers of Pito The EDI values for *pito* were 25.84, 14.53, and 11.06 ng/Kg b.w/day for children, adolescents, and adults, respectively. The MOE measurements that were made were 0.69, 1.23, and 1.07. Cancer Risks were 0.826, 0.465, and 0.353 Cases/10,000 persons/year, although the average potency remained constant (Table 7).

9.5.1.4 Heavy drinkers of Pito Heavy drinkers of *pito* recorded higher levels (Table 8). Values of 40.20, 22.60, and 17.22 ng/Kg b.w/day as EDI were noted. MOE values are 0.44, 0.79, and 1.04. Cancer risks are 1.286, 0.723, and 0.551 Cases/10,000 persons/year.

There were differences in the outcomes of risk assessments for aflatoxins (AFTotal) levels brought on by the consumption of palm wine samples in the various zones of the Ho municipality. The established Estimated Daily Intake

Table 9 Evaluation of the Aflatoxins (AFTotal) cancer risk associated with palm wine intake in the Ho municipality (Moderate Drinkers)

District	Age category	Estimated daily intake (EDI) (ng/kg bw/day)	MOE	Cancer risk (cases/10,000 persons/year)
Ho	Children (36mths-10yrs)	18.51	21.61	0.700
	Adolescents (11-17yrs)	10.41	38.42	0.412
	Adults (18-64yrs)	7.93	50.44	0.314

Mean Aflatoxin Total = 10,700 ng/kg,

Av. Daily intake for Moderate Drinkers = 0.045 kg/day was used for children, adolescents, and adults

Benchmark Dose Lower limit = 400 ng Aflatoxins/kg bw/day

Average Potency AFB1- 0.0396 ng aflatoxins/kg bw/day

1000 ng = 1 µg, Margin of Exposure-MOE

Table 10 Evaluation of the Aflatoxin (AFTotal) cancer risk associated with palm wine intake in the Ho municipality (Heavy Drinkers)

District	Age category	Estimated daily intake (EDI) (ng/kg bw/day)	MOE	Cancer risk(cases/10,000 persons/year)
Ho	Children (36mths-10yrs)	28.81	13.88	1.156
	Adolescents (11-17yrs)	16.19	24.71	0.978
	Adults (18-64yrs)	12.34	32.41	0.489

Mean Aflatoxin Total = 10,700 ng/kg,

Av. Daily intake for Heavy Drinkers = 0.070 kg/day was used for children, adolescents, and adults

Benchmark Dose Lower limit = 400 ng Aflatoxins/kg bw/day

Average Potency AFB1- 0.0396 ng aflatoxins/kg bw/day

1000 ng = 1 µg, Margin of Exposure-MOE

(EDI) values for children, adolescents, and adults in Ho were in the range of 7.93–28.81 ng/Kg bw/day, respectively. For the same age groups, the documented Margin of Exposure (MOE) values were in the range of 13.88- 50.44. Aflatoxins had an average potency of 0.0396 ng/kg bw/day, resulting in cancer risks of range 0.314–1.156 Cases/10,000 persons/year (Tables 9 and 10).

10 Discussion

10.1 Fungal counts

In developing nations, fungus contamination of seeds, grains, and feed is a persistent issue because most tropical hot and humid conditions encourage the growth of these fungi in the field and storage systems. Due to their ability to survive in foodstuff and ingredients of many traditionally fermented alcoholic beverages, fungi can proliferate, dominate, and contaminate these drinks. There has been evidence of the growth of fungi in some traditionally fermented local alcoholic beverages in Ghana [6, 13, 66–68].

In the present work, fungal counts fell in the equivalent range of values of 2.098–4.23 $\log_{10}$ CFU/ml reported by [67]. Minamor et al. [68] recorded fungal counts of $< 10^4$ CFU/ml in "Pito" a cereal beverage of Sorghum were found to be within tolerable limits. Likewise, Akoma et al. [69] reported fungal counts of range 2.00–3.50 log10 CFU/ml in 'Kununzaki' samples in Nigeria. Recently, Kortei et al. [6] recorded fungal counts which ranged between 1.68 ± 0.8- 4.11 ± 0.9 log10 CFU/ml in "Solom" (beverage prepared from millet and sorghum) drinks in Ghana. Lyumugabe et al. [70] also reported counts of 4.12×10^4 (4.61 log10) CFU/ml in "Ikigage" a Rwandan traditional beer made from sorghum.

Contrarily, Ezekiel et al. [22] described greater fungal counts of range $3.4 \times 10^5 \pm 0.10$–$4.5 \times 10^6 \pm 0.10$ (5.53–6.65 $\log_{10}$) CFU/ml from "Otika" a cereal beverage of sorghum from Nigerian. In another interrelated study in India, a mean fungal population of 4.9×10^5 (5.69 $\log_{10}$) CFU/g in an amylase and alcohol-producing starters was recorded [71]. From Nigeria, in "Akamu" samples taken from dissimilar cereals, Popoola et al. [72] observed a range between 1.30×10^5 and 1.74×10^5 (5.11–5.23 log10) CFU/ml. Ayirezang et al. [13] also reported a range of fungal counts of 0.2×10^5–3.7×10^5 (4.30–5.57 log10) CFU/ml in *Pito* (locally brewed sorghum beer) in Northern Ghana. In Ethiopia, Tigist and Getnet [2] reported counts of 5.65×10^6 (6.75 log10) CFU/ml in "Bubugn" (a cereal beverage) samples.

Heavily contaminated traditional alcoholic beverages with mean fungal counts of 2.3×10^{16} (16.37 log10), 3.9×10^{16} (16.59 log10) and 7.0×10^{10} (10.85 log 10) CFU/ml respectively for "Mbege", "Mnazi" and "Komoni" were reported by [73] in Tanzania. Similarly, Bhardwaj et al. [74] noted a high density of 214.9×10^6 (8.33 log10) CFU/g of yeasts in the "Balma" beverage (an Indian traditionally fermented beverage). The microbiological counts that were recorded in this experiment fell within the acceptable (tolerable) and unsatisfactory ranges for ready-to-eat foods. The International Commission for Microbiological Specification of Foods (ICMSF)[65, 75] points out that higher colony counts $> 10^4$ are an indication of degradation caused by either a lack of cleanliness or the use of low-quality water and cereals in the beverage's production (Table 3). There haven't been many microbiological (mycological) studies on palm wine in Ghana.

10.2 Fungal species

Mycotoxins like aflatoxins and fumonisins are frequently connected to agricultural goods and are produced by the genera *Aspergillus, Fusarium*, and *Penicillium*. In corroboration with the findings of our study, Misihairabgwi et al. [4] detected certain mycotoxins in "Oshikundu", a millet-based beverage made in Namibia, which were suggestive of the presence of *Aspergillus, Fusarium, Penicillium, Alternaria*, and *Claviceps* fungi. In 100% of the sorghum malt samples used to make the traditional cloudy drink "Tobwa", which is used to make the traditional sorghum cloudy beer, researchers from Malawi [76] discovered AFs. This indicates that *Aspergillus spp*. was present in the samples of sorghum. Similarly, in Uganda, Byakika et al. [105] reported the fungi species of *A. niger, A. flavus, Fusarium spp.* as well as *Penicillium spp*. in "Obushera" (a fermented sorghum-millet beverage).

Again, our research's results on fungal species in *pito*, agreed favorably with those of [78], who likewise found *Saccharomyces cerevisiae*, *Candida krusei*, and *Aspergillus niger* in samples of "Burukutu" and "*pito*" that had been preserved and that had been taken fresh. Sefa-Dedeh et al. [66] identified several species of yeasts in *pito* samples in Ghana. Likewise, palm wine recorded a preponderance of yeasts which agreed with published findings of previous researchers such as Amoa-Awua et al. [79], Djeni et al. [5] and Atter et al. [80] who worked on palm wine and "Brukutu" samples.

Saccharomyces cerevisiae was also discovered by [68] to be the sole fungus connected to the fermentation of *pito* in Accra, Ghana. Despite the fact that there are few studies on the fungi that affect palm wine, some experts believe that yeasts, primarily *Saccharomyces cerevisiae*, are linked to the fermentation of palm wine [79].

Aspergillus, Fusarium, Penicillium, and *Rhodotorula spp*. were among the eleven additional fungal species from four different genera that were recovered from Ghana's "Asaana" (corn malted drink) in a different study [67]. Furthermore, Kortei et al. [6] discovered that there were fourteen (14) fungal species in total in "Solom" samples from Ghana which included *Aspergillus (A. niger, A. flavus, A. fumigatus, A. parasiticus, A. alutaceaus, and A. terreus), Rhizopus (R. stolonifer), Mucor (M. racemosus), Fusarium (F. oxysporum), Penicillium (P. digitatum, P. verucosum), Cladosporium (C. cladosporoides), Curvularia (C. lunata),* and *Rhodotorula sp*. Members of the genera *Aspergillus, Fusarium, Pennicillium, Macrophomena, Cercospora, Phoma, Rhizopus, Alternaria,* and *Curvularia* were discovered in Nigerian local beer samples [81].

The fungus *Aspergillus* and *Penicillium* have been connected to "Ogi," a fermented cereal porridge often made from millet, sorghum, or maize, according to reports from Nigeria and some parts of Benin [82]. The Nigerian cereal beverage "Kununzaaki" contained the well-known fungal *Penicillium digitatum, Aspergillus fumigatus, Rhizopus nigricans,* and *Mucor sitophila*, according to Elmahmood and Doughari [83]. According to [84], the prevalence of these fungi has been connected to beverage deterioration.

Using amylase and alcohol-producing starters, filamentous molds from seven different taxa—*Mucor, Aspergillus, Penicillium, Bjerkandera, Rhizopus, Trametes,* and *Cladosporium*—were isolated in India [71]. In contrast, a Burkinabe study found that samples of sorghum malt had incredibly high levels of *Aspergillus* [85]. Furthermore, in several of the local and international beverages they examined, [86] discovered signs of *Aspergillus flavus, Penicllium spp.,* and *Trichoderma viridae*. The prevalence of each of these organisms was 2 (2.2%) or below.

Due to the potential for them to contain mycotoxins, the presence of toxicogenic fungus in several cereals and drinks poses some public health issues. Mycotoxins are toxic, teratogenic, cancerous, and mutagenic substances [64, 87]. Some mycotoxins, particularly aflatoxins, and ochratoxins, have been linked to kidney, liver, and brain malignancies globally. If hepatocellular cancer already exists, prolonged exposure to these mycotoxins causes its onset or exacerbates its condition [88].

Additionally, a manufacturing plant's environment might be very unique and different, therefore it may be crucial to pinpoint the exact causes of contamination in each situation [97]. Microbiological contamination along the entire supply chain is unavoidable. Drinking beverages that contain pathogenic microbes and mycotoxins puts customers' health at risk and costs producers money [6]. Since many of these fungi are considered to be spoilage, there may be a chance that mycotoxin contamination will occur [22]. These fermented foods must always be produced under hygienic circumstances, including application. The implementation of good manufacturing practices (GMP) and good hygiene practices (GHP), which enable the contamination to be minimized to acceptable levels, lessens the impact of fungi in the food chain.

10.3 pH

The following environmental elements encourage the growth of fungi: moisture, pH, temperature, light, and others. The pH of a medium determines the kind of fungi present whether acidophilic, basophilic, neutrophilic, etc. Every sample that was tested was acidic, with *pito* having a pH of 4.29 and palm wine having a pH range of 4.35 to 4.5 on the pH scale. The range of pH values found in this study was slightly greater than pH values of range 3.2–3.6 in *pito* samples collected and stored for 56 days [13]. Likewise, Ellis et al. [89] and Osseyi et al. [90] also reported pH values in the range of 3.4–3.45 in pasteurized local sorghum beer which all disagreed with our results. Some researchers have noted the variety of acidity in cereal beverages and have attributed their sourness to the existence of lactic acid bacteria and yeasts throughout the fermentation process.

Our results corroborate with those of Frac et al. [91] and Hsu et al. [92], who stated that the ideal range for fungal growth was between 4.5 and 7.0. The pH range of 3.91–3.96 was found to be suitable for fungal development in samples of "Akamu" made from various grains in Nigeria, according to Popoola et al. [72]. Similarly, Anupma and Tamang [71] found that the average pH was 5.3. However, Yamanaka [93] observed a higher pH range of 7–9 for optimal fungus growth. These beverages' acidity tended to grow as the fermentation period increased, directly proportional to storage time, leading to deterioration. Furthermore, the various types of isolated microorganisms may be related to the low pH values, which may have induced the growth of fungus.

10.4 Occurrence of mycotoxins in traditionally fermented alcoholic beverages

Mycotoxins are common pollutants of cereals, and depending on the kind and country of origin of the raw material, products made out of these are promising to maintain noteworthy quantities of these venoms. Previous reports of mycotoxins in African and European beers have included aflatoxins, deoxynivalenol (DON), fumonisins, ochratoxins, and zearalenone (ZEN) [94, 95].

Although the incidence of ochratoxins (OTA) in local beer beverages has not been widely researched regarding traditionally fermented alcoholic beverages, pieces of evidence exist in the literature [4, 6, 22]. Odhav and Naicker [96] reported OTA levels of 1.5–2340 µg/L in traditional cereal beverages in South Africa. Lulamba et al. [97] reported trace levels of 0.07–0.081 µg/L OTA were found in cereal beer beverages. Furthermore, Juan et al. [98] reported a range of 1600–67002 µg/L of OTA in beer made from barley in Tunisia. According to Darwish et al. [99], the toxins found in cereals most frequently were AFs (43.8%) [69], followed by FBs (21.9%) [69], OTA (12.5%), ZEA (9.4%), and DON (6.3%). These poisons were discovered in ingredients used to make *pito* and other grain beverages, including maize, sorghum, barley, wheat, and teff.

Also, the average OTA contamination of palm wine in Ho Municipal was 2.77ug/kg. There aren't many reports on the occurrence of mycotoxin in palm wine, other than the exploratory work done in Ghana and Nigeria. Adbel-Hadi et al. [100] emphasized that the amount of mycotoxin produced depends on the microbe species, kind of raw material, temperature, water activity (aw), and meteorological spells. For instance, *A. flavus* grows best at 30–35 °C and 0.99 aw, although aflatoxin generation is optimal at 25–30 °C and 0.99 aw and 30–35 °C and 0.95 aw, according to [100]. This study recorded no amount of aflatoxins in palm wine samples collected from the various zones except for Zone C which recorded an amount of 1.468 µg/Kg of aflatoxin B1. In agreement with our findings, It was unanticipated to note that, in sorghum beers manufactured in Ouagadougou, Burkina Faso, from AFB1-contaminated malt, Bationo et al. [85] did not discover AFB1. Again, no aflatoxins were found in Botswana's traditional sorghum brews [101]. Lastly, Anthony et al. [102] reported no aflatoxin contamination in millet grains used for the production of *pito* in Nigeria.

Interestingly, there was an observed greater quantity of yeast species in the palm wine and *pito* samples which in turn, resulted in a low occurrence of aflatoxins in both samples. Presumably, the presence of yeasts and their unique ability to effectively inhibit the development of *Aspergillus flavus* and *A. parasiticus* impeded the formation of aflatoxins during the fermentation process [103, 104]. Because fermentation lowers mycotoxin levels, fermented foods often have lower mycotoxin levels than their raw counterparts. In corroboration with our findings, Byakika et al. [105] reported significantly lower aflatoxin levels in "Obushera" (a fermented sorghum-millet beverage) samples than in the raw materials used for its preparation in Uganda. Similar findings have been reported by several researchers [85, 101, 106, 107] in support of the observation of a significant reduction in mycotoxins (esp. aflatoxins) in fermented foods.

In contrast with our study, Ezekiel et al. [22] reported appreciable quantities of aflatoxins in the range of 2.00–3.5 µg/kg in *pito* samples in Nigeria. There have also been reports of aflatoxins in other fermented cereal drinks. Aflatoxin contamination was found in two traditionally made South African beers, "Isiqatha" (12 ppb) and "Utshwala" special (200 and 400 ppb), according to Odhav and Naicker [96]. Aflatoxin contamination (3.5–6.8 ppb) in opaque sorghum and millet beers was found by Okaru et al. [108] in Kenya. In Nigeria, millet contamination with AFB1 was observed by Daniel et al. [81] to be slightly more common but at lower levels (≤ 10 g/kg). In Burkina Faso, Bationo et al. [85] detected AFB1 and OTA (< 10 ppb) in local beers and sorghum malts. Warmer temperatures are more prone to generate mycotoxin-contaminated grains and, as a result, beers with harmful components, notably in Africa, according to research [109, 110]. Accordingly, a school of thought supported by the published results of [111–113] suggests that various industrial techniques used in local beer brewing, such as steeping, kilning, mashing, fermentation, and clarification that create the traditionally fermented alcoholic beverages, may have a momentous bearing on the amounts of toxicogenic fungi as well as mycotoxin content [112]. Additionally, some investigations [112, 114, 115] found that a lengthy fermentation process may increase mycotoxin level relocation from grain to malt and then to beer due to the superior heat stability.

10.5 Consumer risk assessment

According to the evidence now available, drinking African beverages contaminated with mycotoxins results in significant exposures and co-exposures, particularly to aflatoxins and fumonisins. Due to a lack of crucial and

country-specific information, such as consumption frequency, consumption amount, and actual body weight and age of users, determining mycotoxin exposure from consumption of traditionally processed beverages in many African countries is currently challenging. Even though these figures are only accessible for traditional beverages, like non-alcoholic types, they are more thorough in terms of how much alcohol/beer is consumed. As noted by Ezekiel et al. [22], few attempts have been made to estimate beverage exposures in South Africa as well as Malawi, all within Africa's southern portion.

Owing to the larger diversity of the toxicogenic fungi linked to these brews, habitual drinkers may be more exposed to the lethal properties of these fungal toxins [4, 6, 22]. Since all of the computed EDIs were above the PMTDI of OTA, which are 0.0143 and 0.01786 $\mu gkg^{-1}bwday^{-1}$ [28], we divided our individuals into heavy and moderate *pito* and palm wine drinkers. All age categories tested were at risk of the commencement or onset of hepatocellular cancer as a result. Likewise, all the calculated MOEs were less than 10,000. A similar trend was observed with the Total aflatoxins-contaminated brew.

Our results agreed favorably well with some risk assessment studies on mycotoxins in similar samples (beers) around the globe. According to Czerwiecki et al. [77], OTA was found in 79% of the beer samples they tested in Poland, with a mean level of 25.7 g/L, suggesting a negative risk to the local population's health.

The results of all these experiments supported the conclusion that ingesting these mycotoxins through drinks did not pose a toxicological concern as the exposure was far lower than the TDIs established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [28]. At 1.83 g/L, OTA was found in 80% of the beer with lemonade [97], but some studies on beer use and risk analyses found no adverse effects on health. However, some investigations found that beer samples had a greater OTA incidence. In Catalonia (Spain), beer samples had an OTA incidence of 89% according to Coronel et al. [116], and domestic beer samples had an OTA incidence of 48% according to Lasram et al. [117], with an average value of 0.12 g/L. Rubert et al. [118], who found OTA in 10% of their European beer samples at an average content of 3.2 g/L, observed modest incidences.

The majority of mycotoxicosis in humans, according to Bennett and Klich [119] is caused by consuming contaminated foods. It is worthy of note that both ochratoxin A (OTA) and Aflatoxins (AFs) recorded in this work were below the acceptable limits of 5 and 10 µg/kg prescribed by the Ghana Standards Authority (GSA). There is no known safe level of mycotoxin in the body of a human or an animal because some mycotoxins have longer-term chronic or cumulative effects on health, including the development of malignancies and immunological deterioration, among other things. The intensity of the symptoms is influenced by the type of mycotoxin, the amount and length of contact, as well as some inborn patient characteristics including age, sex, health, and nutritional status. Additionally, certain mycotoxins have immediate effects that might cause serious illness symptoms to appear overnight. It is advised that sorghum or millet be used instead of maize when processing beverages that may be prepared from a combination of grains, or that new beverages employing fewer mycotoxin-susceptible crops be created. This will encourage dietary variety and could lessen mycotoxins. According to research by Bandyopadhyay et al. [120], maize is substantially more likely than sorghum or millet to be infected by *Aspergillus spp*. which produces aflatoxin.

11 Conclusion

Generally, the mean fungal counts of palm wine samples in the Ho municipality were found to be unsatisfactory according to ICMSF guidelines. A total of 13 fungal species belonging to 8 genera namely: *Aspergillus, Fusarium, Trichoderma, Rhizopus, Penicillium, Rhodotorula, Mucor,* and *Yeast* were isolated from both samples. Again, there were no aflatoxins in palm wine while OTA was found in both palm wine and *pito*. Cancer risk assessments conducted revealed the consumption of palm wine and *Pito* could be threatening to the health of consumers in the Ho Municipality since all MOEs were less than 10, 000. Nevertheless, it is important to note that the conditions favorable for fungal growth in grains are not always conducive to mycotoxin production. Future research on beverages in Africa must therefore take exposure estimations into account.

Acknowledgements The technical team at the Department of Food Laboratory, the Food and Drug Administration, and the Council for Scientific and Industrial Research's Food Research Institute made a significant contribution to the mycotoxin analysis, for which the authors are quite thankful. For their assistance with the mycological analysis, we would also like to thank the technicians at the Microbiology Laboratory, School of Allied Health Sciences, UHAS.

Limitations of the study Although this study used a sample size which is statistically valid, a larger sample size of the traditionally fermented beverages would have given a better representation of the contamination levels in the geographical location of study.

Author contributions The performance of the experiments and the writing of the manuscripts were done by HNOL, NKK, GTO, and BKH. The determination of the mycotoxins (ochratoxin A and aflatoxin analysis) was done by LOA and VK-B. The conception of the experiments and manuscript was prepared by HNOL, NKK, BKH, SAR, FKM and SB-T. Furthermore, NKK, BKH, and GTO conceived the original study and EYB, NKK, and HNOL led the sampling, analysis, and study in Ghana. The final manuscript was read and approved by all authors.

Funding This study did not receive any funding.

Availability of data and materials The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

References

1. Debebe A, Redi-Abshiro M, Chandravanshi BS. Non-destructive determination of ethanol levels in fermented alcoholic beverages using Fourier transform mid-infrared spectroscopy. Chem Cent J. 2017;11(1):1–8.
2. Tigist M, Getnet B. the microbiological and physicochemical characteristics of bubugn, a traditional fermented ethiopian low alcoholic beverage.
3. Okoye J, Oni K. Promotion of indigenous food preservation and processing knowledge and the challenge of food security in Africa. J Food Secur. 2017;5(3):75–87.
4. Misihairabgwi J, Ezekiel C, Sulyok M, Shephard G, Krska R. Mycotoxin contamination of foods in Southern Africa: a 10-year review (2007–2016). Crit Rev Food Sci Nutr. 2019;59(1):43–58.
5. Djeni TN, Kouame KH, Ake FD, Amoikon LS, Dje MK, Jeyaram K. Microbial diversity and metabolite profiles of palm wine produced from three different palm tree species in Côte d'Ivoire. Sci Rep. 2020;10(1):1715.
6. Kortei NK, Asiedu P, Annan T, Deku JG, Boakye AA. Fungal diversity of "solom" a Ghanaian traditional beverage of millet (Pennisetum glaucum). Food Sci Nutr. 2021;9(2):811–21.
7. Sawadogo-Lingani H, Owusu-Kwarteng J, Glover R, Diawara B, Jakobsen M, Jespersen L. Sustainable production of African traditional beers with focus on dolo, a West African sorghum-based alcoholic beverage. Front Sustain Food Syst. 2021;5:672410.
8. Abegaz K. Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of borde, an Ethiopian cereal beverage. Afr J Biotechnol. 2007;6(12).
9. Marcos A, Serra-Majem L, Pérez-Jiménez F, Pascual V, Tinahones FJ, Estruch R. Moderate consumption of beer and its effects on cardiovascular and metabolic health: an updated review of recent scientific evidence. Nutrients. 2021;13(3):879.
10. Yin J, Winzenberg T, Quinn S, Giles G, Jones G. Beverage-specific alcohol intake and bone loss in older men and women: a longitudinal study. Eur J Clin Nutr. 2011;65(4):526–32.
11. WH Organization. Global status report on alcohol and health 2018. Geneva: World Health Organization; 2019.
12. Fernandez-Sola J. Cardiovascular risks and benefits of moderate and heavy alcohol consumption. Nat Rev Cardiol. 2015;12(10):576–87.
13. Ayirezang FA, Saba CKS, Amagloh FK, Gonu H. Shelf life improvement of sorghum beer (pito) through the addition of Moringa oleifera and pasteurization. Afr J Biotech. 2016;15(46):2627–36.
14. Zaukuu JLZ, Oduro I, Ellis WO. Processing methods and microbial assessment of pito (an African indigenous beer), at selected production sites in Ghana. J Inst Brew. 2016;122(4):736–44.
15. Adadi P, Kanwugu ON. Potential application of tetrapleura tetraptera and hibiscus sabdariffa (malvaceae) in designing highly flavoured and bioactive pito with functional properties. Beverages. 2020;6(2):22.
16. Sue Azam-Ali D. Toddy and Palm Wine. 2011.
17. Ramírez-Guzmán KN, Torres-León C, Martinez-Medina GA, de la Rosa O, Hernández-Almanza A, Alvarez-Perez OB, et al. Traditional fermented beverages in Mexico. In: fermented beverages. Amsterdam: Elsevier; 2019. p. 605–35.
18. Erukainure OL, Oyebode OA, Ijomone OM, Chukwuma CI, Koorbanally NA, Islam MS. Raffia palm (Raphia hookeri G. Mann & H. Wendl) wine modulates glucose homeostasis by enhancing insulin secretion and inhibiting redox imbalance in a rat model of diabetes induced by high fructose diet and streptozotocin. J Ethnopharmacol. 2019;237:159–70.

19. Lucky G, Cookey G, Ideriah T. Physicochemical and nutritional parameters in palm wine from oil palm tree (Elaies guineensis) and Raffia Palm (Raphia hookeri) in South-South Nigeria. Chem Res J. 2017;2(6):146–52.
20. Hernández A, Pérez-Nevado F, Ruiz-Moyano S, Serradilla M, Villalobos M, Martín A, et al. Spoilage yeasts: What are the sources of contamination of foods and beverages? Int J Food Microbiol. 2018;286:98–110.
21. Ashiq S. Natural occurrence of mycotoxins in food and feed: Pakistan perspective. Compr Rev Food Sci Food Saf. 2015;14(2):159–75.
22. Ezekiel CN, Ayeni KI, Misihairabgwi JM, Somorin YM, Chibuzor-Onyema IE, Oyedele OA, et al. Traditionally processed beverages in Africa: a review of the mycotoxin occurrence patterns and exposure assessment. Compr Rev Food Sci Food Saf. 2018;17(2):334–51.
23. Anal AK. Quality ingredients and safety concerns for traditional fermented foods and beverages from Asia: a review. Fermentation. 2019;5(1):8.
24. Haque MA, Wang Y, Shen Z, Li X, Saleemi MK, He C. Mycotoxin contamination and control strategy in human, domestic animal and poultry: a review. Microb Pathog. 2020;142:104095.
25. Reddy BN, Raghavender CR. Outbreaks of aflatoxicoses in India. Afr J Food, Agric, Nutr Dev. 2007;7(5):01.
26. Probst C, Njapau H, Cotty PJ. Outbreak of an acute aflatoxicosis in Kenya in 2004: identification of the causal agent. Appl Environ Microbiol. 2007;73(8):2762–4.
27. Kamala A, Shirima C, Jani B, Bakari M, Sillo H, Rusibamayila N, et al. Outbreak of an acute aflatoxicosis in Tanzania during 2016. World Mycotoxin J. 2018;11(3):311–20.
28. Authority EFS. Opinion of the scientific panel on contaminants in the food chain [CONTAM] related to ochratoxin A in food. EFSA J. 2006;4(6):365.
29. Ostry V, Malir F, Toman J, Grosse Y. Mycotoxins as human carcinogens—the IARC Monographs classification. Mycotoxin Res. 2017;33(1):65–73.
30. Lyon F. IARC monographs on the evaluation of carcinogenic risks to humans. World health organization, International agency for research on cancer availble at publication@ iarc fr. 2014.
31. Richard SA, Manaphraim NY, Kortei NK. The novel neurotoxic and neuroimmunotoxic capabilities of aflatoxin B1 on the nervous system: a review. Adv Biosci Clin Med. 2020;8(3):1–8.
32. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis. 2010;31(1):71–82.
33. Awuchi CG, Ondari EN, Nwozo S, Odongo GA, Eseoghene IJ, Twinomuhwezi H, et al. Mycotoxins' toxicological mechanisms involving humans, livestock and their associated health concerns: a review. Toxins. 2022;14(3):167.
34. Valery PC, Laversanne M, Clark PJ, Petrick JL, McGlynn KA, Bray F. Projections of primary liver cancer to 2030 in 30 countries worldwide. Hepatology. 2018;67(2):600–11.
35. Liu Y, Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environ Health Perspect. 2010;118(6):818–24.
36. Kew MC. Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. Liver Int. 2003;23(6):405–9.
37. McClune AC, Tong MJ. Chronic hepatitis B and hepatocellular carcinoma. Clin Liver Dis. 2010;14(3):461–76.
38. Service GS. 2010 Population & Housing Census Report: Urbanisation in Ghana: Ghana Statistical Service; 2014.
39. Kortei NK, Annan T, Quansah L, Aboagye G, Akonor P, Tettey C. Microbiological quality evaluation of ready-to-eat mixed vegetable salad, food ingredients and some water samples from a restaurant in ACCRA: a case study. Afr J Food Agric Nutr Dev. 2020;20(6):16669–88.
40. Kortei NK, Kumah G, Tettey CO, Agyemang AO, Annan T, Nortey NN, et al. Mycoflora, aflatoxins, and antimicrobial properties of some Ghanaian local spices and herbs. J Food Saf. 2022;42(5): e12996.
41. Odamtten G, Nartey L, Wiafe-Kwagyan M, Anyebuno G, Kyei-Baffour V. Resident microbial load, toxigenic potential and possible quality control measures of six imported seasoning powders on the Ghanaian market. J Nutr Health Food Eng. 2018;8(1):24–35.
42. Ezekiel C, Sulyok M, Frisvad JC, Somorin Y, Warth B, Houbraken J, et al. Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. Int J Food Microbiol. 2013;162(3):231–6.
43. Samson R, Hoekstra E, Frisvad J, Filtenborg O. Methods for the detection and isolation of food-borne fungi. Introduction to foodborne fungi. 1995:235–42.
44. Moss M. Samson RA, Van Reenen-Hoekstra ES, Introduction to Food-borne Fungi, Edit. 3, Centraalbureau voor Schimmelcultures, PO Box 273, 3740 AG Baarn, The Netherlands (1988), p. 299, ISBN 90–70351–16–1. Price Hfl. 42.50. Elsevier; 1989.
45. Samson RA, Hoekstra ES, Van Oorschot CA. Introduction to food-borne fungi: Centraalbureau voor Schimmelcultures. 1981.
46. Stroka J, Anklam E. New strategies for the screening and determination of aflatoxins and the detection of aflatoxin-producing moulds in food and feed. TrAC, Trends Anal Chem. 2002;21(2):90–5.
47. Chan D, MacDonald S, Boughtflower V, Brereton P. Simultaneous determination of aflatoxins and ochratoxin A in food using a fully automated immunoaffinity column clean-up and liquid chromatography–fluorescence detection. J Chromatogr A. 2004;1059(1–2):13–6.
48. dos Santos JS, Souza TM, Ono EYS, Hashimoto EH, Bassoi MC, de Miranda MZ, et al. Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products. Food Chem. 2013;138(1):90–5.
49. Chain EPoCitF, Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, et al. Risk assessment of aflatoxins in food. EFSA J. 2020;18(3): e06040.
50. Ogunro P. Plasma level of atherogenic and anti-atherogenic factors among palm wine drinkers of rural Southwest Nigeria. Afr J Med Med Sci. 2012;41(4):337–47.
51. EFSA Panel on Dietetic Products N, Allergies. Scientific Opinion on the appropriate age for introduction of complementary feeding of infants. EFSA J. 2009;7(12):1423.
52. Biritwum R, Gyapong J, Mensah G. The epidemiology of obesity in Ghana. Ghana Med J. 2005;39(3):82.
53. WH Organization. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: World Health Organization; 2006.
54. Afrifa-Anane E, Agyemang C, Codjoe SNA, Ogedegbe G, Aikins A-G. The association of physical activity, body mass index and the blood pressure levels among urban poor youth in Accra Ghana. BMC Pub Health. 2015;15(1):1–9.
55. Walpole SC, Prieto-Merino D, Edwards P, Cleland J, Stevens G, Roberts I. The weight of nations: an estimation of adult human biomass. BMC Pub Health. 2012;12(1):1–6.
56. F Joint Additives WECoF. Safety evaluation of certain mycotoxins in food. Rome: FAO; 2001.

57. Authority EFS. Outcome of a public consultation on the draft risk assessment of aflatoxins in food. Hoboken: Wiley Online Library; 2020. p. 2397–8325.
58. Groopman JD, Cain LG, Kensler TW, Harris CC. Aflatoxin exposure in human populations: measurements and relationship to cancer. CRC Crit Rev Toxicol. 1988;19(2):113–45.
59. Shephard GS. Risk assessment of aflatoxins in food in Africa. Food Addit Contam. 2008;25(10):1246–56.
60. Kortei NK, Annan T, Kyei-Baffour V, Essuman EK, Okyere H, Tettey CO. Exposure and risk characterizations of ochratoxins A and aflatoxins through maize (Zea mays) consumed in different agro-ecological zones of Ghana. Sci Rep. 2021;11(1):1–19.
61. F Joint Additives WECoF, Organization WH. Evaluation of certain food additives and contaminants: forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva: World Health Organization; 1999.
62. Abesig J, Chen Y, Wang H, Sompo FM, Wu IX. Prevalence of viral hepatitis B in Ghana between 2015 and 2019: a systematic review and meta-analysis. PLoS ONE. 2020;15(6): e0234348.
63. Adetunji MC, Alika OP, Awa NP, Atanda OO, Mwanza M. Microbiological quality and risk assessment for aflatoxins in groundnuts and roasted cashew nuts meant for human consumption. J Toxicol. 2018;2018:1.
64. Kortei NK, Ayiku PO, Nsor-Atindana J, Ansah LO, Wiafe-Kwagyan M, Kyei-Baffour V, et al. Toxicogenic fungal profile, Ochratoxin A exposure and cancer risk characterization through maize (Zea mays) consumed by different age populations in the Volta region of Ghana. Toxicon. 2023;226:107085.
65. Van Schothorst M, Zwietering M, Ross T, Buchanan R, Cole M, International Commission on Microbiological Specifications for Foods (ICMSF). , 2009. Relating microbiological criteria to food safety objectives and performance objectives. Food Control. 2009;20(11):967–79.
66. Sefa-Dedeh S, Sanni A, Tetteh G, Sakyi-Dawson E. Yeasts in the traditional brewing of pito in Ghana. World J Microbiol Biotechnol. 1999;15:593–7.
67. Aboagye G, Gbolonyo-Cass S, Kortei NK, Annan T. Microbial evaluation and some proposed good manufacturing practices of locally prepared malted corn drink ("asaana") and Hibiscus sabdarifa calyxes extract ("sobolo") beverages sold at a university cafeteria in Ghana. Sci Afr. 2020;8: e00330.
68. Minamor AA, Larteley AM, Laryea EN, Afutu E, Tetteh-Quarcoo PB. Microbiological quality of a locally brewed alcoholic beverage (pito) sold in a community within the greater accra region, Ghana. 2017.
69. Akoma O, Daniel A, Ajewole A, Nwodo P. Quality characteristics of kunun zaki (a Nigerian fermented cereal beverage) sold within bida metropolis. Glob Adv Res J Agric Sci. 2014;3(9):298–303.
70. Lyumugabe F, Kamaliza G, Bajyana E, Thonart P. Microbiological and physico-chemical characteristic of Rwandese traditional beer "Ikigage." Afr J Biotech. 2010;9(27):4241–6.
71. Anupma A, Tamang JP. Diversity of filamentous fungi isolated from some amylase and alcohol-producing starters of India. Front Microbiol. 2020;11:905.
72. Popoola O, Balogun D, Bello A. Microbiological quality of some selected akamu samples sold in some areas of Kano Metropolis (A case study of Hotoro, Tarauni and Mariri). Res J Food Sci Qual Control. 2019;5:8–11.
73. Mwambete D, Justin-Temu M, Mashurano M, Tenganamba O. Microbial quality of traditional alcoholic beverages consumed in Dar es Salaam, Tanzania East and Central African. J Pharm Sci. 2006;9(1):8–13.
74. Bhardwaj KN, Jain KK, Kumar S, Kuhad RC. Microbiological analyses of traditional alcoholic beverage (Chhang) and its starter (Balma) prepared by Bhotiya Tribe of Uttarakhand India. Ind J Microbiol. 2016;56:28–34.
75. Assessment ICoMSfFWGoMR. Potential application of risk assessment techniques to microbiological issues related to international trade in food and food products. J Food Prot. 1998;61(8):1075–86.
76. Matumba L, Monjerezi M, Khonga EB, Lakudzala DD. Aflatoxins in sorghum, sorghum malt and traditional opaque beer in southern Malawi. Food Control. 2011;22(2):266–8.
77. Czerwiecki L, Wilczyńska G, Kwiecień A. Ochratoxin A: an improvement clean-up and HPLC method used to investigate wine and grape juice on the Polish market. Food Addit Contam. 2005;22(2):158–62.
78. Fadahunsi I, Ogunbanwo S, Fawole A. Microbiological and nutritional assessment of burukutu and pito (indigenously fermented alcoholic beverages in West Africa) during storage. Nat Sci. 2013;11(4):98–103.
79. Amoa-Awua W, Sampson E, Tano-Debrah K. Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (Elaeis guineensis) in Ghana. J Appl Microbiol. 2007;102(2):599–606.
80. Atter A, Obiri-Danso K, Amoa-Awua W. Microbiological and chemical processes associated with the production of burukutu a traditional beer in Ghana. Int Food Res J. 2014;21(5):1769.
81. Daniel A, Ochi D, Adejumo A, Muhammad H, Saidu A, Atehnkeng J, et al. Mycotoxicological concerns with sorghum, millet and sesame in Northern Nigeria. 2016.
82. Phiri S, Schoustra SE, van den Heuvel J, Smid EJ, Shindano J, Linnemann AR. How processing methods affect the microbial community composition in a cereal-based fermented beverage. LWT. 2020;128:109451.
83. Elmahmood A, Doughari J. Microbial quality assessment of kunun-zaki beverage sold in Girei town of Adamawa State Nigeria. Afr J Food Sci. 2007;1(1):11–5.
84. Snyder AB, Churey JJ, Worobo RW. Association of fungal genera from spoiled processed foods with physicochemical food properties and processing conditions. Food Microbiol. 2019;83:211–8.
85. Bationo JF, Nikiéma PA, Koudougou K, Ouédraogo M, Bazié SR, Sanou E, et al. Assessment of aflatoxin B1 and ochratoxin A levels in sorghum malts and beer in Ouagadougou. Afr J Food Sci. 2015;9(7):417–20.
86. Salami Oluwafemi M, Onyemelukwe Ngozi F, Olowu Frederick A, Ibrahim Hadizat H, Tok PD. Detection of aflatoxins from foreign and locally made beer. IOSR J Environ Sci, Toxicol Food Technol. 2019;13(2):09–14.
87. Barac A. Mycotoxins and human disease. Clinically relevant mycoses: a practical approach. Cham: Springer International Publishing; 2019. p. 213–25.
88. Wu F. Global impacts of aflatoxin in maize: trade and human health. World Mycotoxin J. 2015;8(2):137–42.
89. Ellis W, Oduro I, Terkuu D. Preliminary studies on extension of the shelflife of pito. J Sci Technol (Ghana). 2005;25(1):11–5.

90. Osseyi E, Tagba P, Karou S, Ketevi A, Lamboni C. Stabilization of the traditional sorghum beer," tchoukoutou" using rustic wine-making method. Adv J Food Sci Technol. 2011;3(4):254–8.
91. Frąc M, Hannula SE, Bełka M, Jędryczka M. Fungal biodiversity and their role in soil health. Front Microbiol. 2018;9:707.
92. Hsu ST, Yang ST. Propionic acid fermentation of lactose by Propionibacterium acidipropionici: effects of pH. Biotechnol Bioeng. 1991;38(6):571–8.
93. Yamanaka T. The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. Mycologia. 2003;95(4):584–9.
94. Peters J, van Dam R, van Doorn R, Katerere D, Berthiller F, Haasnoot W, et al. Mycotoxin profiling of 1000 beer samples with a special focus on craft beer. PLoS ONE. 2017;12(10): e0185887.
95. Al-Jaal BA, Jaganjac M, Barcaru A, Horvatovich P, Latiff A. Aflatoxin, fumonisin, ochratoxin, zearalenone and deoxynivalenol biomarkers in human biological fluids: a systematic literature review, 2001–2018. Food Chem Toxicol. 2019;129:211–28.
96. Odhav B, Naicker V. Mycotoxins in South African traditionally brewed beers. Food Addit Contam. 2002;19(1):55–61.
97. Lulamba TE, Stafford RA, Njobeh PB. A sub-Saharan African perspective on mycotoxins in beer–a review. J Inst Brew. 2019;125(2):184–99.
98. Juan C, Berrada H, Mañes J, Oueslati S. Multi-mycotoxin determination in barley and derived products from Tunisia and estimation of their dietary intake. Food Chem Toxicol. 2017;103:148–56.
99. Darwish WS, Ikenaka Y, Nakayama SM, Ishizuka M. An overview on mycotoxin contamination of foods in Africa. J Vet Med Sci. 2014;76(6):789–97.
100. Abdel-Hadi A, Schmidt-Heydt M, Parra R, Geisen R, Magan N. A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by Aspergillus flavus. J R Soc Interface. 2012;9(69):757–67.
101. Nkwe DO, Taylor JE, Siame BA. Fungi, aflatoxins, fumonisin Bl and zearalenone contaminating sorghum-based traditional malt, wort and beer in Botswana. Mycopathologia. 2005;160(2):177.
102. Anthony MH, Ojochenemi AD, Yemi AHR, Tahir N, Okechukwu OJ, Saidu MA, et al. Determination of aflatoxins in sesame, rice, millet and acha from Nigeria using HPLC. Chem Sci Trans. 2014;3(4):1516–24.
103. Afzal N, Hassan SM, Mughal SS, Pando A, Rafiq A. Control of aflatoxins in poultry feed by using yeast. Am J Chem Biochem Eng. 2022;6(1):21–6.
104. Moradi M, Rohani M, Fani SR, Mosavian MTH, Probst C, Khodaygan P. Biocontrol potential of native yeast strains against Aspergillus flavus and aflatoxin production in pistachio. Food Addit Contam: Part A. 2020;37(11):1963–73.
105. Byakika S, Mukisa IM, Wacoo AP, Kort R, Byaruhanga YB, Muyanja C. Potential application of lactic acid starters in the reduction of aflatoxin contamination in fermented sorghum-millet beverages. Int J Food Contam. 2019;6(1):1–8.
106. Karlovsky P, Suman M, Berthiller F, De Meester J, Eisenbrand G, Perrin I, et al. Impact of food processing and detoxification treatments on mycotoxin contamination. Mycotoxin Res. 2016;32:179–205.
107. Ezekiel CN, Abia WA, Ogara IM, Sulyok M, Warth B, Krska R. Fate of mycotoxins in two popular traditional cereal-based beverages (kunu-zaki and pito) from rural Nigeria. LWT-Food Sci Technol. 2015;60(1):137–41.
108. Okaru AO, Abuga KO, Kibwage IO, Hausler T, Luy B, Kuballa T, et al. Aflatoxin contamination in unrecorded beers from Kenya–A health risk beyond ethanol. Food Control. 2017;79:344–8.
109. Nji QN, Babalola OO, Ekwomadu TI, Nleya N, Mwanza M. Six main contributing factors to high levels of mycotoxin contamination in African foods. Toxins. 2022;14(5):318.
110. Neme K, Mohammed A. Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A review. Food Control. 2017;78:412–25.
111. Hu L, Gastl M, Linkmeyer A, Hess M, Rychlik M. Fate of enniatins and beauvericin during the malting and brewing process determined by stable isotope dilution assays. LWT-Food Sci Technol. 2014;56(2):469–77.
112. Pascari X, Ramos AJ, Marín S, Sanchís V. Mycotoxins and beer. Impact of beer production process on mycotoxin contamination. A review. Food Res Int. 2018;103:121–9.
113. Inoue T, Nagatomi Y, Uyama A, Mochizuki N. Fate of mycotoxins during beer brewing and fermentation. Biosci Biotechnol Biochem. 2013;77(7):1410–5.
114. Rodriguez-Carrasco Y, Fattore M, Albrizio S, Berrada H, Manes J. Occurrence of Fusarium mycotoxins and their dietary intake through beer consumption by the European population. Food Chem. 2015;178:149–55.
115. Carballo D, Fernández-Franzón M, Ferrer E, Pallarés N, Berrada H. Dietary exposure to mycotoxins through alcoholic and non-alcoholic beverages in Valencia, Spain. Toxins. 2021;13(7):438.
116. Coronel MB, Marín S, Cano-Sancho G, Ramos A, Sanchis V. Exposure assessment to ochratoxin A in Catalonia (Spain) based on the consumption of cereals, nuts, coffee, wine, and beer. Food Addit Contam: Part A. 2012;29(6):979–93.
117. Lasram S, Oueslati S, Chebil S, Mliki A, Ghorbel A. Occurrence of ochratoxin A in domestic beers and wines from Tunisia by immunoaffinity clean-up and liquid chromatography. Food Addit Contam Part B. 2013;6(1):1–5.
118. Rubert J, Soler C, Marín R, James K, Mañes J. Mass spectrometry strategies for mycotoxins analysis in European beers. Food Control. 2013;30(1):122–8.
119. Bennett J, Klich M. Mycotoxins. Clin Microbiol Rev. 2003;16:497–516.
120. Bandyopadhyay R, Kumar M, Leslie JF. Relative severity of aflatoxin contamination of cereal crops in West Africa. Food Addit Contam. 2007;24(10):1109–14.