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Dietary exposure to aflatoxin B1 and ethanol from homemade and industrial fermented beverages in South Kivu, Democratic Republic of the Congo

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Abstract

BACKGROUND: The prevalence of AFB1 contamination in agricultural products used to produce fermented alcoholic beverages is increasing, raising concerns for human health.

AIM: The aim of this study was to investigate dietary exposure to AFB1 and ethanol through homemade and industrial fermented beverages commonly consumed in South Kivu, DR Congo.

METHOD: AFB1 and ethanol were measured using reverse-phase HPLC with a fluorescence detector and a refractive index detector, respectively. Data on fermented beverage consumption were collected from 847 adults using a food frequency questionnaire (FFQ).

RESULTS: The findings revealed that industrial sample Man 8 had the highest exposure to AFB1 (77.8±45.0 ng/kg b.w/day) and Man 4 had the highest ethanol exposure (4.83±2.40 mg/kg b.w/day); while among homemade samples, Kasiksi had the highest exposure to both AFB1 (8.8±6.6 ng/kg b.w/day) and ethanol (2.46±1.85 mg/kg b.w/day). The margin of exposure (MOE) for AFB1 was 1011.7 or less, and for ethanol, it was 818.2 or less. Men are more likely to be exposed.

CONCLUSION: Increased consumption of homemade and industrial fermented beverages raises the risk of developing hepatocellular cancer (HCC) because the levels of AFB1 and ethanol MOE drop below the safe limit of 10000. Further research is needed to investigate the connection between AFB1, ethanol, and HCC, especially in regions where alcohol misuse is common.

Introduction

In Sub-Saharan Africa, indigenous fermented beverage production is part of the tradition and uses different methods, styles, local ingredients, and flavors. The spontaneous fermentation of the wort gives taste and aroma varying from one brew to another. Fermented beverages reflect ethnic identity and are often drunk communally [1].

In the Democratic Republic of the Congo (DR Congo), fermented beverages are prepared and consumed daily by the population, they are present in various sociocultural and religious ceremonies such as birth, baptism, marriage, rituals, and mourning; and also have their place in diet schedule and daily budget. Homemade fermented beverages "i.e., home-produced" are brewed to generate income for households and are also much cheaper. The general trend toward greater availability of fermented beverages and commercialized production of European-style beverages "i.e., industrial fermented beverages" has been reported [2]. Recorded consumption of fermented beverages per capita (15 years and over) was 60% and 39% for homemade and industrial fermented beverages, respectively, and <1% for wines and spirits [3].

Aflatoxins generally coexist in crops and diverse diets [4]; for example, aflatoxins have been reported in samples of cassava, maize, sorghum, soybeans, and their processed products collected from local markets in eastern DR Congo, where South-Kivu is located [5] and also in maize samples from North Kivu (Beni, Goma, and Butembo) [6]. Throughout the supply chain in DR Congo (between the city store and the distribution system at the market), the aflatoxin content of maize samples was found to increase significantly [7]. Aflatoxin contaminations have been reported in some cereals, tubers, fruits, and spices linked to mold growth during pre-harvest and post-harvest conditions [8] due to fungal contamination (mainly *Aspergillus flavus*, *A. parasiticus,* and *A. nomius*).

Consumption of aflatoxin leads to acute aflatoxicosis resulting in nausea, vomiting, abdominal pain, pulmonary or cerebral edema, necrosis, fatty liver,

depression, diarrhea, photosensitivity, lethargy, and jaundice [9]. The International Agency for Research on Cancer has categorized aflatoxins as Group I human carcinogens [10]. Chronic aflatoxin exposure may be the cause of 4.6–28.2% of all global hepatocellular carcinoma (HCC) cases; the majority of cases occur in Sub-Saharan Africa (40%), Southeast Asia and China (27%), the Western Pacific (20%), the Eastern Mediterranean (10%), and Latin America (3%), where dietary exposure is not well controlled [11]. The international approach to address this issue involves using the margin of exposure (MOE) approach, which includes estimating the amount of exposure through diet to calculate the MOE. The MOE is the ratio of the lowest dosage at which cancer may occur to the estimated human exposure [12]. A MOE of <10,000 is generally considered a health risk [13].

A considerable proportion of fermented beverages in the South Kivu region of the DR Congo is derived from cereals (such as sorghum, maize, millet, and rice), fruits (primarily bananas), and tubers (including cassava and sweet potatoes). The fermentation process of beverages leads to a wide variety of flavors. It is worth noting that traditional fermented beverages do not have clear labeling, which makes it challenging to determine their alcohol content. At present, there is no existing research on the examination of aflatoxin and ethanol levels in homemade and industrial fermented beverages in South Kivu, DR Congo. However, limited studies have been conducted to investigate the presence of aflatoxin in specific agricultural products in the DR Congo.

Studies also have shown that ethanol can increase the metabolism of aflatoxin, which requires activation by certain enzymes to cause toxic and cancerous effects [14]. The differential effects of aflatoxin poisoning in different regions may not solely be due to genetic differences but could also be influenced by social alcohol habits [15].

Therefore, the objective of this study is to fill the existing gaps by analyzing the levels of AFB1 and ethanol, as well as assessing the dietary exposure to aflatoxin and ethanol resulting from the consumption of fermented beverages in South Kivu, DR Congo.

Methods

Study setting

The present research was conducted in seven zones (Ibanda, Kadutu, Katana, Lemera, Miti-Murhesa, Nyatende, and Walungu) in the DR Congo as shown in Figure 1 [16]. The samples were taken in May 2022. South Kivu is in the eastern part of the DR Congo. It is bounded to the east by Rwanda, Burundi, and Tanzania, to the north by North Kivu, to the west by Maniema, and the south by Maniema and Katanga. The province has an equatorial climate with year-round rainfall in certain areas and a tropical mountain climate with a 9-month rainy season (September–May) and a 3-month dry season (June–August) in others. On average, annual temperatures range from 11°C to 25°C [17].

Consumer survey

A cross-sectional study was conducted to gather information about the frequency of fermented beverage consumption. The participants were selected using a multistage, stratified sampling method, where three out of the five South Kivu districts were randomly chosen in the first stage, followed by randomly selecting zones within each district in the second stage (giving seven zones). Finally, a sample of adult fermented beverage consumers from various locations such as homes, marketplaces, cabarets, and bars was gradually chosen until a convenient sample was reached. The study used a food frequency questionnaire (FFQ) [18] and involved 847 adults who were 18 years or older, including 535 men and 312 women and had consumed at least one of the fermented beverages.

The participants' consumption of fermented beverages was recorded in the FFQ. In addition, the participants' body weight was measured using Gibson procedures. Before measuring the participants' body weight, the reader was calibrated daily to ensure accuracy. The participants' weight was measured to the closest "100 g" while they were wearing light clothing and no shoes. They were instructed to remove bulky belts, empty their pockets, stand with one foot on each side of the scale, and stay still with their backs facing the scale until instructed to step off [19].

Sample collection

Eight different open markets were visited in each selected region to collect every type of homemade beverage. To ensure random and representative sampling, equal amounts of each type of fermented beverage from every area were pooled in a single sample. A total of 26 pooled homemade samples were obtained and used for analysis. Homemade samples included eight different varieties of beverages: "Kabamba" and "Kasiksi", both made from banana and sorghum, "Kanyanga" made from maize and cassava, "Lungwela" made from corn, "Mandale" made from maize and millet, "Muhama" made from sorghum and soybean, "Musululu" made from corn and sweet potatoes, and "Ntole" made from sorghum and soybean. On the other hand, each industrial sample was collected in the different open markets and gathered in one pool. A total of eight pooled industrial samples were obtained and used for analysis. Industrial samples included eight different brands made from rice or barley. Man 1, 2, 3, 4, 5, 6, 7, and 8 were used to identify industrial samples.

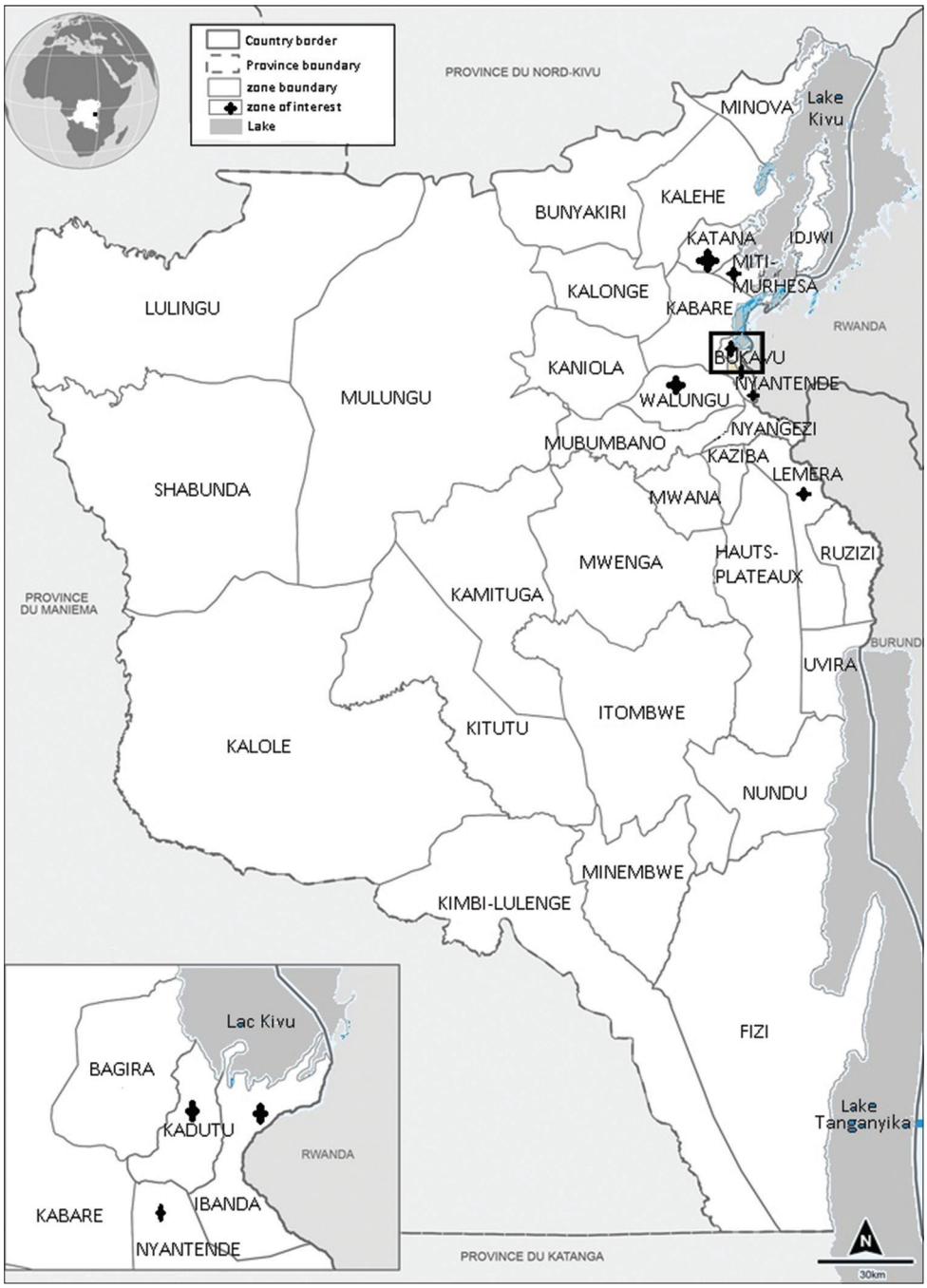

Figure 1: Map of the zones of interest located in the province of South Kivu, DR Congo

Instrumentation and chemicals

The equipment included HPLC, UltiMate 3000, Dionex UltiMate 3000; RS Fluorescence Detector (ThermoFisher Scientific, USA); Rotary Evaporator, Scilogex RE100-Pro (Rocky Hill, USA); Refractive Index Detector, Shodex RI-101 (Lexington, USA), Whatman® qualitative filter paper, grade 1, circles, diameter 90 mm from Merck KGaA (Darmstadt, Germany), and Syringe filters polytetrafluoroethylene (PTFE) hydrophobic, diameter 25 mm, pore size 0.22 mm (Chromtech, China), Ultrasonic Cleaners, WUCD06H (Daihan Scientific, Korea).

The solvents acetonitrile, dichloromethane, hexane, methanol, ethanol, water, and hexane, all HPLC grade, were purchased from Merck KGaA (Darmstadt, Germany). A standard solution of AFB1 in acetonitrile B1 (2 μg/mL) and sodium chloride were both obtained from Sigma-Aldrich (St Louis, MO, USA). Trifluoroacetic acid and chloroform were purchased from Loba Chemie Pvt. Ltd (Mumbai, India).

Sample analysis

Aflatoxin B1 analysis

The AFB1 present in the fermented alcoholic beverage sample was determined using the method established by the Association of Official Analytical

Chemists (AOAC), with minor adjustments [20], [21]. Each 25 mL of sample, after being degassed by sonication for 10 min, was mixed with 100 ml of a methanol-water solution (80:20) for 2 min. Afterward, 50 mL of the filtrate was transferred to a 500 mL separator funnel, along with 20 mL of a 10% sodium chloride solution and 25 mL of chloroform. The mixture was gently shaken and allowed to separate into different layers. The lower aqueous layer was collected and transferred to another separatory funnel, where 25 mL of chloroform was added. The layers were shaken again for 1 min and allowed to separate. The bottom layer was drained through anhydrous sodium sulfate in a 250 mL bottle. Other portions of the aqueous layer were washed with 25 mL of chloroform. The chloroform was then evaporated using a rotary evaporator at 30 rpm and 62°C until dry. The remaining flask residue was cleaned with 2 mL of dichloromethane, followed by evaporation using nitrogen gas until completely dry. After removing the dichloromethane, the aflatoxins were reconstituted in a solution of acetonitrile and water (1-9) and vortexed and then passed through syringe filters before being subjected to HPLC analysis [22].

The analysis was performed using a reverse-phase HPLC with a fluorescence detector at wavelengths of 360 nm and 440 nm for excitation and emission, respectively. The separation was carried out on an ODS Hypersil-C18 column, and the mobile phase consisted of a mixture of water, acetonitrile, and methanol (57:17:26 v/v) at a flow rate of 1 mL/min; the injection volume was 50 μl. The data obtained from the analysis were processed using the Chromeleon software© Dionex Version 7.1.2.1478 Chromatography Data System (CDS).

To guarantee accurate findings, the method's accuracy and precision were examined according to the performance criteria established by Commission Regulation (EC) N-401/2006 [23].

Aflatoxin-spiked samples of uncontaminated fermented beverages were analyzed to determine the recovery rates. The concentrations of aflatoxins in the samples were adjusted based on the recovery values. By testing different concentrations of aflatoxins and creating a standard curve, the linearity of the method was confirmed. The limit of detection ranged from 0.4 to 2.5 μg/L and the limit of quantification ranged from 1.45 to 7.6 μg/L. The method showed satisfactory recovery percentages of 68.3–77.2%. The results align with the criteria established by the European Regulatory Commission [24].

Ethanol analysis

The approach utilized by Gabriella *et al*. and Sharma *et al*. was applied to determine the ethanol concentration in the sample of the fermented beverage [25], [26] with minor modifications. After being degassed by sonication for 10 min, each 20 mL sample was filtered with Whatman filter paper. The samples were then centrifuged at 4000 rpm for 5 min before being passed through 0.22 mm polytetrafluoroethylene syringe filters for cleaning. A reverse-phase HPLC with a refractive index detector was used for the analysis. The separation was carried out on an ODS Hypersil-C18 analytical column (diameter 250 × 4.6 mm, particle size 5-micron). The system includes a binary gradient solvent pump to control the mobile phase flow rate as well as an autosampler for automatic injection, a vacuum degasser, a column oven (30°C), and refractive index measurement. The mobile phase is HPLC-grade water with a flow rate of 1 mL/min and an injection volume of 30 μl.

Estimation of dietary exposure of consumers to AFB1 and ethanol

The dietary exposure to AFB1 or ethanol was assessed using the formula of El Tawila [27]. This involved multiplying the levels of AFB1 or ethanol present in the sample by the daily intake of these beverages and then dividing by the respondent's body weight

$$Ei = \frac{\sum Qik \times Cik}{BWi \times 7}$$

Where:

- Ei is the dietary aflatoxin exposure of consumer i (ng/kg bw/day)
- Qik is the amount of fermented beverages k consumed by consumer i in a week
- Cik is the aflatoxin concentration in the fermented beverage k (ng/kg)
- BWi is the body weight of the consumer i (kg)
- Σ is the sum overall fermented beverages consumed by respondent i.
- The result of the equation was divided by 7 to obtain the daily aflatoxin exposure.

Risk assessment of aflatoxin exposure

The European Food Safety Authority uses the Margin of Exposure (MOE) method to assess health risks. This involves comparing the dose-response curve observed in animal studies to the estimated levels of consumption in humans [28]. The benchmark dose ($BMDL_{10}$) for AFB1 and ethanol, which cause an increase in HCC in male rats, was found to be 400 ng/kg bw/day [28] and 700 mg/kg bw/day, respectively [29], [30]. The MOE was determined using a specific formula [12]:

- MOE for mean exposure = BML_{10} value/Mean exposure
- MOE for higher exposure= BML_{10} value/95^{th} percentile

Table 1: Dietary exposure to Aflatoxin B1 from homemade and industrial beverage's consumption

Sample	AFB1 (ng/L), mean ± SD	AFB1 (ng/kg b.w/day)		Contribution (%)	MOE (mean)	MOE (P95th)
		Mean ± SD	Percentile 95th			
Homemade fermented beverages						
Kabamba	1373 ± 20	6.8^{b,d} ± 4.7	12.66	19 (2.24)	58.9	31.6
Kanyanga	910 ± 60	0.4^{ac,e} ± 0.3	0.99	139 (16.41)	1011.7	402.9
Kasiksi	2240 ± 50	8.7^{b,d,e} ± 6.5	22.01	368 (43.45)	46.0	18.2
Mandale	200 ± 130	0.8^{a,c,e} ± 0.6	1.90	85 (10.04)	511.2	210.5
Musululu	920 ± 190	4.4 ± 2.5	10.49	39 (4.60)	91.9	38.1
Ntole	910 ± 60	3.7^{b,c,d} ± 2.6	8.65	63 (7.44)	107.6	46.3
Industrial fermented beverages						
Man 3	2088 ± 70	15.4^{b,c,d} ± 6.5	27.02	33 (3.90)	26.0	14.8
Man 4	994 ± 120	7.6^{a,d} ± 3.4	12.69	23 (2.72)	52.4	31.5
Man 6	1151 ± 80	13.8^{a,d} ± 8.8	32.46	23 (2.72)	29.0	12.3
Man 8	10070 ± 340	77.8^{a,b,c} ± 45.0	139.33	80 (9.45)	5.1	2.9

Homemade sample (significant with): [a]Kabamba, [b]Kanyanga, [c]Kasiksi, [d]Mandale, eNtole, Industrial sample (significant with): [a]Man 3, [b]Man 4, [c]Man 6, [d]Man 8. b.w: Body weight superscript letters that follow numbers in the same column indicate that there are significant differences between the values, with a significance level of $p < 0.05$. SD: Standard deviation, MOE: Margin of exposure, AFB1: Aflatoxin B1.

A MOE of <10,000 is generally considered a health risk [13].

Statistical analysis

The collected data were analyzed using the IBM SPSS software package. Descriptive statistics (number and percent) for qualitative analysis were used to assess the level of contamination of fermented beverages and level of dietary exposure as well as the calculation of percentiles (P50 and P95), mean and standard deviation, for quantitative analysis. The Shapiro–Wilk test was used to evaluate if the distribution was normal or not. The results were considered significant at the 5% level. An independent t-test was used to compare the means of two unrelated groups for the normal distribution of fermented beverage intake. A one-way ANOVA test was used to compare the means of more than two unrelated groups. For variables that were not normally distributed, the Kruskal–Wallis test was used to compare more than two groups, and the *post hoc* Dunn's multiple comparison test was used for pairwise comparisons [31].

Results

Dietary exposure to AFB1 from homemade and industrial fermented beverages

According to the information in Table 1, six out of the eight homemade samples were found to be contaminated with AFB1. Similarly, four out of the eight industrial samples were also contaminated. One homemade sample and two industrial samples had levels of AFB1 that was higher than the European Union's limit of 2000 ng/L. There is a significant difference ($p < 0.0001$) in the amount of AFB1 exposure through different fermented beverages. Man 8 had the highest average exposure (77.8 ± 45.0 ng/kg b.w/day) among industrial samples, affecting 9.45% of consumers. Kasiksi had the highest average exposure (8.7 ± 6.5 ng/kg b.w/day) among homemade samples, affecting 43.45% of consumers. The maximum exposure levels to AFB1 were 139.33 ng/kg b.w/day for Man 8 and 22.01 ng/kg b.w/day for Kasiksi, affecting 5% of consumers for each. The calculated MOE values were below the safe threshold limit of 10000 or higher.

Dietary exposure to ethanol from homemade and industrial fermented beverages

The data from Table 2 show that the levels of ethanol in homemade samples varied between 26.2 ± 0.8% v/v and 2.3 ± 0.1% v/v, with Kanyanga having the highest concentration (26.2 ± 0.8% v/v). Among industrial beverages, the levels of ethanol varied between 6.7 ± 0.3% v/v and 5.1 ± 0.1% v/v. There was a significant difference ($p < 0.0001$) in dietary exposure to ethanol between different samples. The industrial sample Man 6 had the highest average exposure (4.93 ± 3.14 mg/kg b.w/day), affecting 2.72% of consumers. Among homemade samples, Kasiksi had the highest average exposure (2.42 ± 1.82 mg/kg b.w/day), affecting 43.45% of consumers. The maximum levels of ethanol exposure were 11.58 mg/kg b.w/day for the

Table 2: Dietary exposure to ethanol from homemade and industrial fermented beverages

Sample	Ethanol (%v/v)	Ethanol (mg/kg b.w/day)		Contribution, n (%)	MOE (mean)	MOE (P95th)
		Mean ± SD	Percentile 95th			
Homemade fermented beverages						
Kabamba	3.6 ± 0.1	1.40^{c,d} ± 1.0	2.62	19 (2.24)	499.2	267.2
Kanyanga	26.2 ± 0.8	0.9^{c,d} ± 0.7	2.26	139 (16.41)	778.0	309.8
Kasiksi	7.9 ± 0.3	2.42$^{a,-f}$ ± 1.82	6.13	368 (43.45)	289.4	114.2
Mandale	7.0 ± 0.3	2.16^{a,b,d,e} ± 1.73	5.25	85 (10.04)	323.8	133.3
Musululu	2.3 ± 0.1	0.86^{c} ± 0.49	2.06	39 (4.60)	818.2	339.6
Ntole	4.0 ± 0.2	1.30^{b,c} ± 0.91	3.02	63 (7.44)	538.4	231.6
Industrial fermented beverages						
Man 3	5.2 ± 0.2	3.0^{b,d} ± 1.26	5.27	33 (3.90)	233.1	132.7
Man 4	5.7 ± 0.3	3.46^{a,c} ± 1.53	5.74	23 (2.72)	202.6	122.0
Man 6	5.1 ± 0.1	4.93^{b,d} ± 3.14	11.58	23 (2.72)	142.1	60.5
Man 8	6.7 ± 0.3	4.10^{a,c} ± 2.37	7.34	80 (9.45)	170.8	95.4

Homemade sample (significant with): [a]Kabamba, [b]Kanyanga, [c]Kasiksi, [d]Mandale, [e]Musululu, [f]Ntole, Industrial sample (significant with): [a]Man 3, [b]Man 4, [c]Man 6, [d]Man 8. b.w: Body weight superscript letters that follow numbers in the same column indicate that there are significant differences between the values, with a significance level of $p < 0.05$. SD: Standard deviation, MOE: Margin of exposure.

industrial sample Man 6 and 6.13 mg/kg b.w/day for the homemade sample Kasiksi, affecting 5% of consumers for each.

The calculated MOE values for both the average and P95 levels of ethanol intake ranged from 818.2 to 231.6 for homemade samples and 233.1–60.5 for industrial samples. These calculated MOE values were lower than the acceptable limit safe threshold of 10000 or higher.

Dietary exposure to AFB1 and ethanol from homemade and industrial fermented based on the gender

The data from Table 3 reveal that men and women consume significantly different amounts of AFB1 and ethanol from fermented beverages ($p < 0.0001$). Men are more likely than women to be exposed to the greatest levels of ethanol through the intake of homemade and industrial fermented beverages. The results reveal that men are more exposure than women for both homemade samples (5.73 ± 6.13 versus 5.34 ± 6.07 ng.kg^{-1} bw day^{-1}) and industrial samples (49.71 ± 44 vs. 44.79 ± 46.27 ng.kg^{-1} bw day^{-1}).

Table 3: Dietary exposure to Aflatoxin B1 and ethanol according to the gender

Parameters	Homemade		Industrial	
	Men	Woman	Men	Woman
AFB1 (ng/kg b.w/day)				
Mean ± SD	5.73[b,c] ± 6.13	5.34[b,c] ± 6.07	49.71[a,c] ± 44.00	44.79[a] ± 46.27
P95	20.14	19.64	115.09	120.6
MOE (mean)	69.9	74.9	8.0	8.9
MOE (P95)	19.9	20.4	3.5	3.3
Ethanol (mg/kg b.w/day)				
Mean ± SD	2.00[b,c] ± 1.70	1.68[b,c] ± 1.53	4.12[a,c] ± 2.42	3.28[a,c] ± 1.76
P95	5.56	5.34	7.36	6.21
MOE (mean)	349.3	416.2	169.9	213.6
MOE (P95)	125.9	131.1	95.1	112.6

[a]Significant with the group of men consuming homemade samples, [b]Significant with the group of men consuming industrial samples, [c]Significant with the group of women consuming industrial samples. b.w: Body weight superscript letters that follow numbers in the same column indicate that there are significant differences between the values, with a significance level of $p < 0.05$. SD: Standard deviation, MOE: Margin of exposure, AFB1: Aflatoxin B1.

Men and women MOEs calculated for the average AFB1 were 69.9 versus 74.9 for a homemade sample and 8.0 versus 8.9 for an industrial sample. The calculated MOE values were lower than the acceptable limit.

Dietary exposure to AFB1 and ethanol from homemade and industrial fermented based on the area

Table 4 reveals that respondents consumed significantly different amounts of AFB1 and ethanol from industrial fermented beverages ($p < 0.0001$).

The average exposure to AFB1 in rural and urban areas was higher for industrial samples (44.8 ± 46.3 vs 48.7 ± 44.1 ng.kg^{-1} b.w. day^{-1}) than homemade samples (5.4 ± 6.2 ng.kg^{-1} b.w. day^{-1} vs. 5.3±5.7 ng.kg^{-1} b.w. day^{-1}). In rural and urban areas, participants had the MOEs calculated for the average AFB1 of 73.5 versus 75.6 for homemade samples and 19.4 versus 17.9 for industrial samples.

Table 4: Dietary exposure to Aflatoxin B1 and ethanol according to the area

Parameters	Homemade		Industrial	
	Rural	Urban	Rural	Urban
AFB1 (ng/kg b.w/day)				
Mean ± SD	5.4[b,c] ± 6.2	5.3[b,c] ± 5.7	44.8[a,c] ± 46.3	48.7[a] ± 44.1
P95	19.64	19.35	118.9	122.28
MOE (mean)	73.5	75.6	8.9	8.2
MOE (P95)	20.4	20.7	3.4	3.3
Ethanol (mg/kg b.w/day)				
Mean ± SD	1.91[b] ± 1.67	1.78[b] ± 1.56	3.92[a] ± 2.41	4.12[a] ± 1.81
P95	5.52	5.39	7.09	7.08
MOE (mean)	365.8	392.2	178.7	169.9
MOE (P95)	126.8	130	98.7	98.9

[a]Significant with the group of men consuming homemade sample, [b]Significant with the group of men consuming industrial sample, [c]Significant with the group of women consuming industrial sample. b.w: Body weight superscript letters that follow numbers in the same column indicate that there are significant differences between the values, with a significance level of $p < 0.05$. SD: standard deviation, MOE: Margin of exposure, AFB1: Aflatoxin B1.

Discussion

The consumption of homemade and industrial fermented beverages can pose health risks due to the presence of AFB1 and ethanol, as well as the frequency and quantity of consumption reported by the majority of respondents. The margin of exposure (MOE) for AFB1 and ethanol was found to be significantly below 10000, indicating potential health risks. Homemade fermented beverages, which are both inexpensive and high in ethanol content, contribute to excessive drinking. The affordability factor increases the likelihood of individuals consuming larger quantities without being aware of the AFB1 and alcohol content. In addition, homemade fermented beverages often lack proper labeling, making it difficult for consumers to know how much they are consuming [32]. According to the World Health Organization (WHO), men are more likely to be current drinkers of fermented beverages compared to women, with a ratio ranging from 1.3 to 3.8. When women do drink, they consume less on average and participate in heavy drinking less frequently [2], [33]. Women are more likely to be former drinkers than men. A survey in 20 African countries found that the majority of women choose to abstain from alcoholic beverages [34]. A study conducted in several European Union countries along with Finland, Germany, Netherlands, and Switzerland found that men are more likely than women to consume alcoholic fermented beverages [35]. The results of this study further indicate that women are also prone to being subjected to significant levels of AFB1 and alcohol through the consumption of both homemade and industrial fermented beverages ($p < 0.0001$). Women in certain locations are becoming increasingly self-sufficient, following professional paths and lifestyles like men. They are also starting to use beer in similar ways. A study conducted in Italy observed a rise in the number of people consuming alcohol, with a significant increase

noted among women, particularly young women [36]. A study conducted in Brazil has found that consuming large amounts of beer increases the risk of exposure to cancer-causing aflatoxins and ethanol. The study focused on the MOE for two types of fermented beverages and found that this posed a greater health concern for men compared to women [37]. The risk of being exposed to high levels of AFB1 and ethanol was shown to be higher among urban consumers of industrial fermented beverages. This is most probable because industrial fermented beverages are more popular. A study has shown that the rise in popularity of industrial fermented beverages in Africa can be attributed to the efforts of the beer industry. These efforts include supporting charitable causes, organizing policy-related events, increasing advertising, introducing new products, and forming partnerships. The goal is to promote favorable policies for the industry and make these fermented beverages more easily accessible [38]. Another study found that various factors such as family, culture, personal traits, and the promotion of industrial fermented beverages can influence drinking patterns. The study revealed that being exposed to its marketing can lead to early initiation of consumption and higher levels of drinking [39]. A survey done in Germany discovered that a large percentage of people begin drinking alcoholic fermented beverages at a young age, with rural areas having greater rates (93.7%) than metropolitan ones (89.1%) [40].

A significant number of liver cancer cases in Sub-Saharan Africa remain unexplained, despite the region's high exposure to aflatoxins [41]. Many factors have been identified as increasing the risk of developing HCC, among them exposure to toxins such as aflatoxin and lifestyle choices such as alcohol consumption [42]. A meta-analysis has indicated that there is a correlation between aflatoxin exposure and an increased risk of liver cirrhosis [41]. A study found strong evidence that consuming AFB1 and ethanol through diet increases the risk of HCC later in life [43], [44]. These findings emphasize the importance of dietary aflatoxin and ethanol exposure in the high rates of HCC in Asia and Sub-Saharan Africa [44]. The number of new cases of HCC is increasing in many countries, with over 500,000 estimated cases each year. HCC is the leading type of liver cancer and is causing a growing number of deaths, particularly in developing countries in Asia and Africa [33]. Aflatoxin contamination has been reported in cereals, as well as processed products such as wheat flour and beer derived from cereal crops such as corn and sorghum in several African countries, including Benin, Ethiopia, Malawi, Nigeria, South Africa, RD Congo, Togo, and Uganda [45].

A recent study examined the MOE for different types of alcoholic beverages and levels of contamination in homemade and industrial fermented beverages; the study found that ethanol had the lowest MOEs, suggesting a higher risk of cancer and a direct association between alcohol consumption and cancer risk [30]. In 2016, alcohol consumption was the seventh highest cause of both deaths and disability-adjusted life-years (DALYs), making up 2.2% of female deaths and 6.8% of male deaths [46].

According to the World Health Organization (WHO), culture, community, and religion play a role in how acceptable, accessible, and affordable alcoholic beverages are [47].

Conclusions

The study evaluated the level of exposure to AFB1 and ethanol through the consumption of homemade and industrial fermented beverages. The average exposure resulted in an MOE below or equal to 1011.7 (for AFB1) and 818.2 (for Alcohol) indicating potential negative health effects, including a higher risk of liver cancer. The study found that men are more likely than women to be exposed to higher levels of AFB1 and ethanol from fermented beverages.

Public health authorities in DR Congo should address the issue of fermented alcoholic beverage consumption as a significant health concern. They should implement various strategies to assist individuals in reducing their alcohol consumption and enhancing their well-being. These measures include monitoring AFB1 and alcohol levels, labeling homemade fermented beverages, increasing taxes, limiting alcohol marketing, and addressing unrecorded alcohol consumption.

Farmers and manufacturers should be educated on how to prevent fungal growth by reducing seed moisture content and implementing mitigation strategies. They should also explore detoxification strategies such as hot water treatment, ozonation, and probiotics during fermentation. In addition, the implementation of the hazard analysis critical control point (HACCP) system in the factories should ensure that the products are free from aflatoxin.

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