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AFLATOXINS CONTAMINATION IN NNAM OWONDO (A LOCAL GROUNDNUT-BASED FOOD), AND CONSUMERS' DIETARY EXPOSURES AND SAFETY LEVELS IN YAOUNDE, CENTRE REGION OF CAMEROON

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ABSTRACT: *This study aimed to assess total aflatoxins (AFT) dietary exposure levels and the associated health risk amongst 'Nnam owondo' (NO) consumers (adults and children) in Yaounde, Cameroon. A survey on NO consumption practices and consumers' knowledge of aflatoxins was conducted using a structured food frequency questionnaire. 'NO' samples together with 5 laboratory-simulated traditional (ST) and 6 simulated modified traditional (SMT) NO samples, were analyzed for AFT using an ELISA kit. The aflatoxins' health risk was determined using the Margin of Exposure (MOE) and quantitative liver cancer risk approach. Adults and children in this study consume on average 122.5 and 99.6 g of NO daily respectively, 2-3 times per week. All pooled samples were contaminated with AFT (mean: 17.2; range: 5.41-34.02 µg/Kg). Around 62.5% (20/32) of pooled NO samples had AFT levels that exceeded the regulatory limit of 10 µg/Kg established by the Food and Agriculture Organization for groundnut-based foods intended for direct human consumption. Mean daily exposures (MOE) of the pooled samples were 0.03 (13.02) and 0.04 (9.98) µg/Kg bw/day for adults and children respectively. A mean cancer risk range: 6 to 10 cancer cases per year per 100,000 populations were observed for children and adults in this study. Application of the SMT led to a 62.2 % reduction in AFT level when compared to ST, with a corresponding decrease in MOE to 0.011 (36.36) and 0.014 µg/Kg bw/day (28.57) for adults and children, respectively, although the AFT dietary exposure remains a public health treat.*

Keywords: *'Nnam owondo', groundnut, aflatoxins, AFs-dietary exposures, Margin of Exposure (MOE); liver cancer.*

1. Introduction

'Nnam owondo'(NO) is a cooked groundnut paste prepared either wrapped in leaves (banana and/or *Philodendron rojo* Congo commonly called "Congo leaves") or directly in pots. While NO is a traditional dish of the Beti tribe living in the Centre region of Cameroon, it is also produced and consumed by a large number of ethnic groups in Yaounde. NO is mainly prepared with groundnut that is susceptible to molds and aflatoxins contamination. Aflatoxins are toxic secondary metabolites produced

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primarily by *Aspergillus* species, mainly by the toxigenic strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* [1] when they contaminate agricultural commodities like groundnut. Aflatoxins $B_{1,}$ classified as a Group 1 human carcinogen, is the most prevalent and toxic fungal compound in tropical and sub-tropical countries [1, 2, 3, 4]. Aflatoxins predominantly contaminate agricultural commodities, particularly nuts (e.g., Groundnut) and cereals (e.g., Maize, Sorghum) and their food-based products. Previous studies from Cameroon have reported the occurrence of aflatoxins in groundnuts [5, 6, 7], groundnuts paste [7, 8], and groundnut soup and snacks such as kuru-kuru and Dagwa [7]. Meanwhile, the presence of aflatoxins has also been found in other crops including maize [9], cassava chips [6], and corn fufu [10]. The inevitable occurrence of aflatoxins in major food crops like groundnuts has resulted in constant exposure to a wide range of levels of aflatoxins daily in Cameroon [6, 7]. Aflatoxins pose serious health implications in consumer populations [6, 7, 8, 10, 11]. Chronic exposures have been linked to health effects like primary liver cancer (Hepatocellular carcinoma, HCC), immunosuppression, stunted growth, and low birth weight in children [11, 12, 13, 14]. Acute aflatoxin exposures cause aflatoxicosis, a life-threatening condition due to liver damage [15, 16]. Acute aflatoxicosis has been reported amongst humans in Kenya [17] which led to the death of children and re-occurred in 2004 [18]. Although several studies in Cameroon have reported the presence of aflatoxins in groundnut [6, 7, 8, 19], and some of its by-products [6, 7, 8]**,** there is so far no report on aflatoxins dietary exposures and health risks amongst NO consumers. Thus, this study reports on the dietary exposures and associated health risks amongst NO consumers in the Centre Region of Cameroon.

2. Methodology

2.1. Study Site and Target Population.

This study was carried out in Ekounou, Nkomo, and Nkoabang situated in Yaounde, Centre Region of Cameroon (Figure 1). Furthermore, the target populations for this study were 'Nnam owondo' producers and two of their children living in the same household and 'Nnam owondo' consumers in general.

2.2. Food Survey

A survey on NO production, consumption practices, storage methods, and consumers' knowledge of aflatoxins was conducted in the study locations from February 2021 to April 2021 using structured food frequency questionnaires.

2.3. Samples collection and preparation

A total of 96 NO samples were purchased and collected using a simple random sampling method from 32 households (3 samples per home) in the study locations. Each sample was placed in a properly labeled sterile polyethylene bag and stored in a deep freezer at -4 ℃ in the laboratory of Food Study and Quality Control of the Centre for Food, Food Security and Nutrition (CRASAN), Institute for Medical Research and Medicinal Plant Studies (IMPM) until aflatoxins analyses. Five (5) NO samples (hereafter referred to as Simulated Traditional, ST) were produced by simulating the traditional NO preparation method (Figure 2). Additionally, 6 NO samples (hereafter referred to as Simulated Modified Traditional, SMT) were produced by the modified traditional NO preparation method (Figure 3). These ST and SMT NO samples were stored in the same conditions as the purchased NO samples.

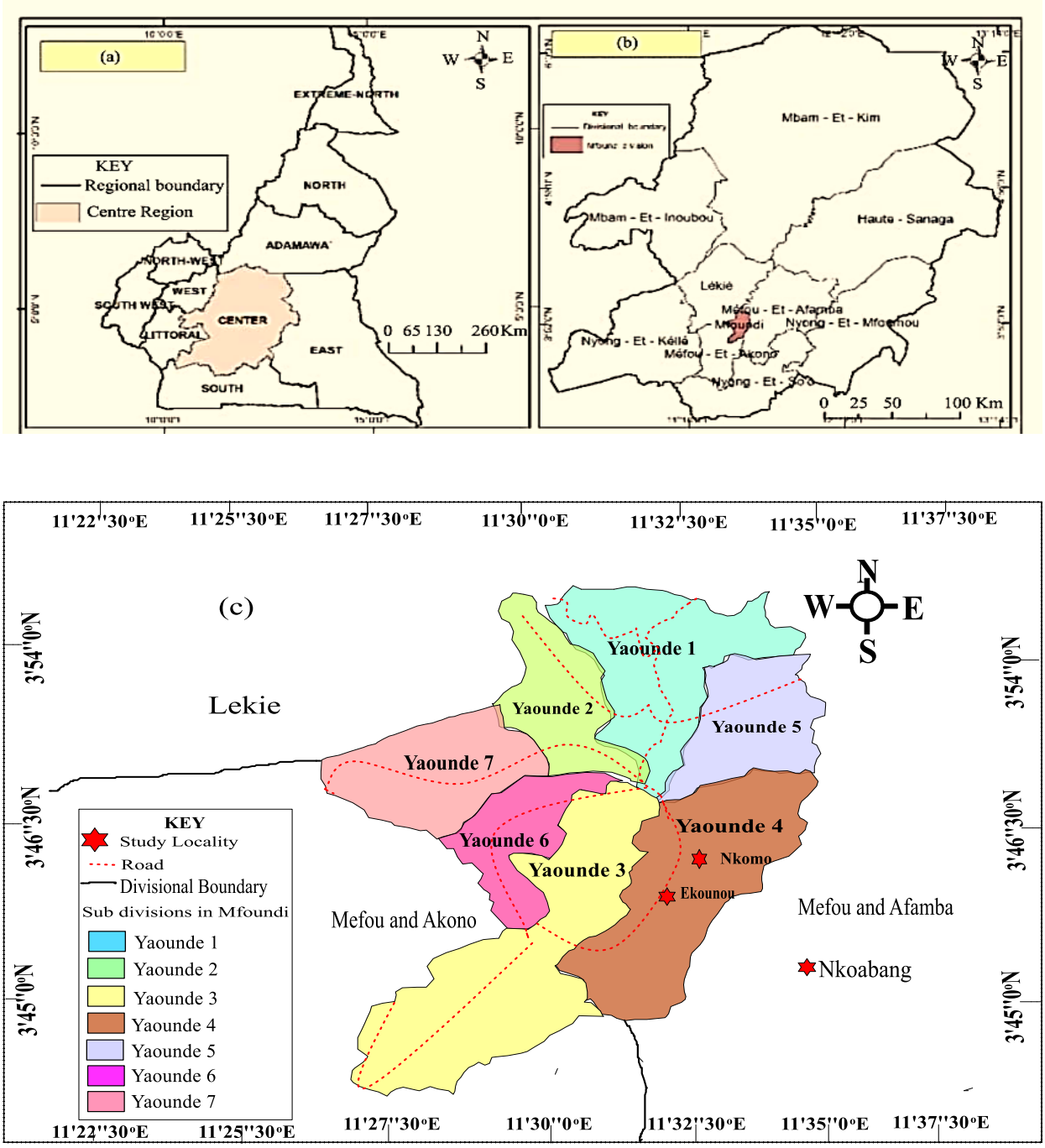

Figure 1. Map indicating the study areas in Yaounde. Modified (star) from Tiafack, O et al. [20]. (a) Centre region of Cameroon; (b) Mfoundi and Mefou and Afamba Divisions in the centre region; (c) Ekounou and Nkomo study area in Yaounde IV Subdivision, Mfoundi Division and Nkoabang in Nkolafamba subdivision, Mefou, and Afamba Division.

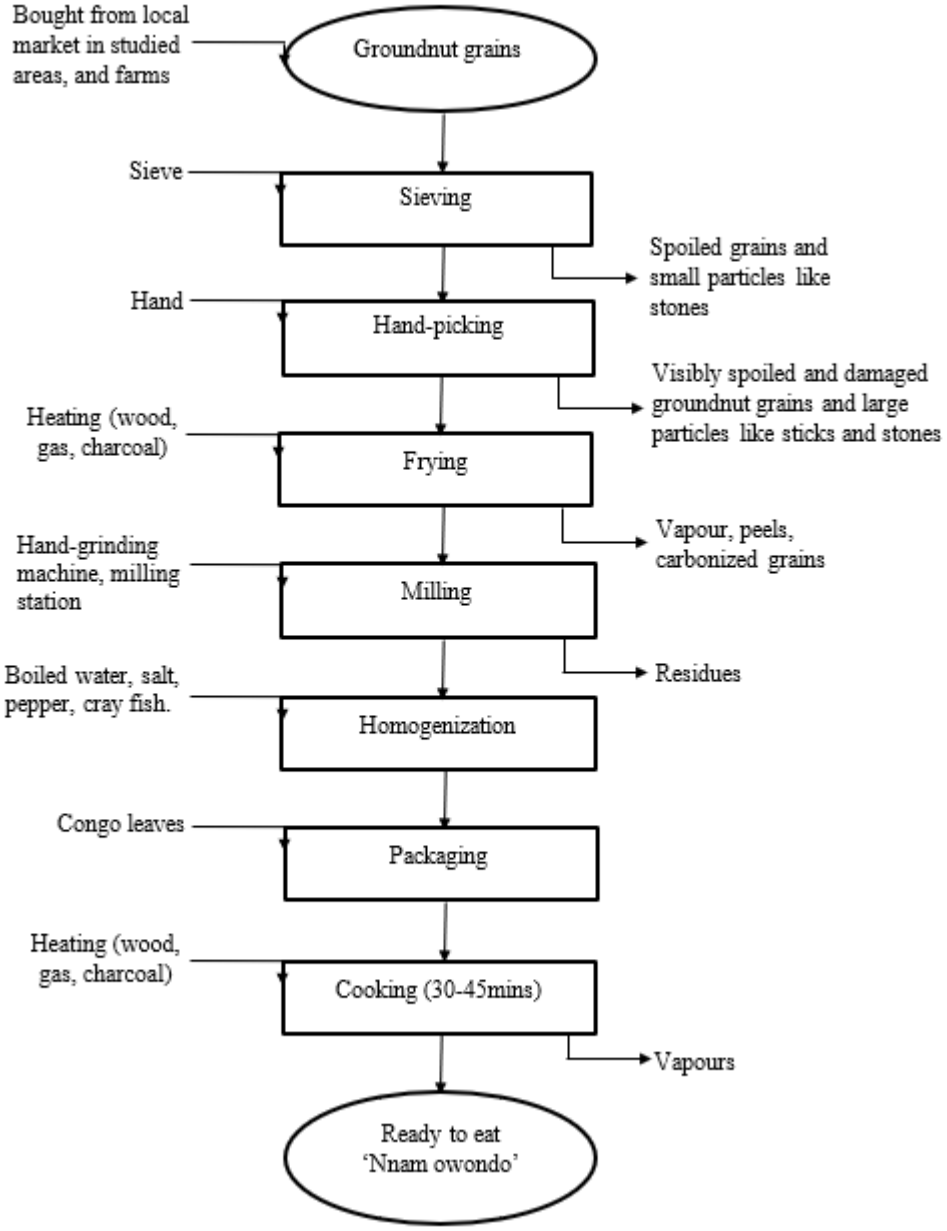

Figure 2. Flow chart of the simulated tradition method of 'Nnam owondo' preparation (source: from 'Nnam owondo' producers in this study)

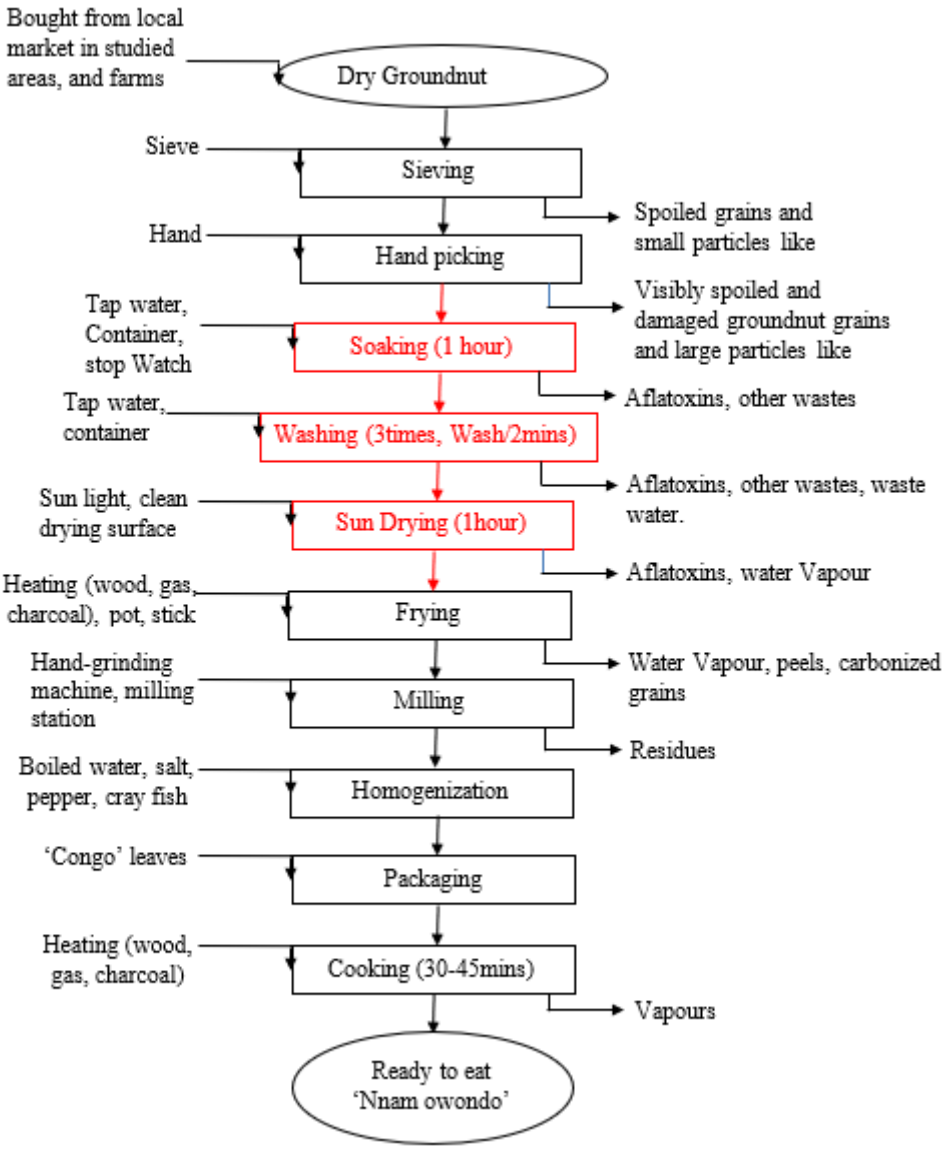

Figure 3. A flow diagram of the proposed modified traditional-simulated method of 'Nnam owondo' preparation

2.4. Measurement of aflatoxins total (AFT) levels in 'Nnam owondo' samples

The total 43 studied 'NO' samples for this study; 32 pooled samples, 5 simulated traditional samples, and 6 simulated modified traditional samples were analyzed for AFT levels using a direct competitive Enzyme-Linked Immunosorbent Assay (ELISA) kit (PriboLab Pte. Ltd. Singapore, EKT-011-48/96T) according to the manufacturer's protocol. Methanol of analytical grade was purchased from Sigma, Germany, for sample extraction.

2.4.1 Sample processing and extraction of aflatoxins

All the 'NO' samples were brought to room temperature (25±1°C). The packaging leaves were removed, and 5 g of each sample was carefully taken from several spots following the recommended methods [21, 22], and weighed using a graduated balance into a 50 mL Falcon tube. Approximately 25 mL of the extraction solution (Methanol–Distilled water; v/v; 70:30) was added into each Falcon tube and mixed using a vortex mixer (Thermo Scientific, Maxi Mix™ II Vortex Mixer, Manchester, United Kingdom) for 10 minutes. Thereafter, the mixture was centrifuged at 4000 rpm for 20 minutes using a centrifuge machine (Centrifuge; ROTOFIX 32 A, Tuttlingen, Germany). After centrifugation, the supernatant was collected and the bottom was discarded. Then, 200 µL of each of the supernatant (extract solution) was transferred into a fresh Eppendorf tube (1.5 mL) through a micropipette. Subsequently, 300 µL of sample diluent solution was added into each of the Eppendorf tubes bringing the volume to 500 µL.

2.4.2. Quantification of aflatoxins total (AFT) levels in prepared 'Nnam owondo' ('NO') samples

To quantify AFT levels in prepared samples, according to the ELISA kit manufacturers' instruction, the standards, reagents, and extract solutions were brought to room temperature 60 minutes before the experiment. In the protocol, a microplate of 96 wells was filled with 50 µL samples and 50 µL of the standards at different concentrations (0, 0.25, 0.5, 1.0, and 2.0 ng/mL). This was immediately followed by the addition of 50 µL of AFT enzyme solution and 50 µL of AFT antibody solution into each well (standards and samples). The microtiter plate was then covered with aluminum foil, and mixed gently by rocking the plate manually for approximately 1 minute and thereafter incubated for 20 minutes at room temperature (25±1°C) in the dark. The contents of the wells on the microtiter plate were discarded, then washed with 1x washing buffer (approximately 300 µL /well) for 4~5 times at 10-second intervals and the microplate was taped on an absorbent paper towel to dry the wells. An aliquot of 150 µL of substrate solution was later added into each well and mixed gently by rocking the plate manually. The 96-well plate containing the solutions was then incubated at room temperature (37 °C) for 10 minutes in the dark. Thereafter, 50 µL of stop solution was added into each well, and mixed gently by rocking the plate manually. Then, the absorbance of each microwell was read on an ELISA microplate reader ('EL 800 Biotek Instruments Inco, Winooski, VT, VS') at 450 nm wavelength. Standard solutions of AFT at different concentrations (0, 0.25, 0.5, 1, and 2 ng/mL) were used to plot a calibration curve which

was used to calculate the AFT content of each sample.

2.5. Assessment of Aflatoxins Dietary Exposure, Health risk and safety concerns

The aflatoxins dietary exposure assessment in this study was performed using the dietary intake of AFT for each population (adults and children) of study participants following 'NO' consumption. It was calculated as an individual (In.) estimated daily exposure (EDI) using equation (eq) 1 below:

$$\text{Daily exposure} = \frac{\text{Av.Daily intake of NO (Kg)x AFT levels in individual pooled NO sample } \left(\mu\frac{g}{Kg}\right)}{\text{Individual Body Weight (Kg)}} \quad (1)$$

The risk assessment of aflatoxins (genotoxic carcinogens) associated with the 'NO' consumption was calculated in this study based on two basic well-known approaches: the Margin of Exposure (MOE) approach proposed by the European Food Safety Authority, EFSA [23] and a quantitative liver cancer risk approach proposed by the Joint FAO/WHO Expert Committee on Food Additives, (JECFA) at its 48th meeting [14].

In this study, the health risk of AFB_1 was equated to AFT as assumed by the European Food Safety Authority (EFSA), the Scientific Panel on Contaminants in Food Chain, CONTAM [24, 25], and the JECFA [14] when assessing aflatoxins in food. The calculation of individual MOE was done using equation (eq) 2 below:

$$\text{MOE} = \frac{\text{Benchmark dose lower limit 10\% (BMDL10)in rodents}}{\text{Individual Estimated Daily exposure (EDI)}} \quad (2)$$

When the MOE value is less than 10,000, the toxic compound may be considered a public health concern. A MOE value between 10,000 and 1,000,000, means the compound is unlikely to be a health concern. When the MOE is greater than 1,000,000, it implies the toxic compound is highly unlikely to be a concern [24, 25, 26]. In this study, quantitative liver cancer risk resulting from aflatoxins (total) exposure via NO consumption by both populations of the study (adult and children) was calculated using the quantitative liver risk approach proposed by the JECFA, [14]. The carcinogenic potency factors for genotoxic carcinogens (like aflatoxins) provided by JECFA, [14] for HBsAg-negative individuals, 0.01 per 100,000 person-years per ng/kg bw per day and HBsAg-positive individuals, 0.3 per 100,000 person-years per ng/kg bw per day were used**.** The overall hepatitis B virus infection prevalence of 12.6% of the blood donors of the Yaounde Central Hospital, Centre region of Cameroon reported by Ankouane et al. [26] was used to determine the cancer risk in this study. The population of Yaounde in 2022 which is 4,336,670 was also taken into account **(**Worldo meters). The formula used was:

$$\text{Pcancer}=[(\text{PHBsAg+})\text{x (pop. HBsAg+)}]+[(\text{PHBsAg-})\text{x (pop. HBsAg-)}] \quad (3)$$

$$\text{Cancer risk}= P_{cancer} \text{ x Estimated Daily Exposure} \quad (4)$$

Where:

P_{cancer} = Carcinogenic potency

PHBsAg+ = 0.3 cancers/year/100,000 individuals ng/AFT/kg/bw/day

PHBsAg- = 0.01 cancers/year/100,000 individuals ng/AFT/kg/bw/day

Pop. HBsAg+ = fraction of population with Hepatitis B

Pop. HBsAg- = fraction of population without Hepatitis B

This formula takes into account only the estimated number of liver cancer cases in a single year. To estimate the risk for a lifetime exposure, the average life expectancy considered for a population is multiplied by the carcinogenic potency calculated for that population. However, in

relation to the safe margin of this approach, there exist no national or international accords on the number of acceptable cancer cases to be considered of no concern to health. But for risks associated with food, a level of one (1) million-extra risk upon lifetime exposure is frequently used in judging the exposure to a genotoxic carcinogen. Likewise, the effectiveness of the three aflatoxins reducing steps (soaking, washing, and sun drying) introduced in the 'NO' simulated modified method was evaluated by preparing from the same dry groundnut samples 5 and 6 'NO' samples through the simulated traditional and simulated modified traditional method respectively. The percentage reduction of AFs levels in the modified 'NO' samples was determined using the equation (eq) 5 below:

$$\% \text{ Red.} = \frac{\text{AFT levels in ST, NO } \left(\mu\frac{g}{Kg}\right) \text{-AFT levels in SMT,NO } \left(\mu\frac{g}{Kg}\right)}{\text{Mean AFT levels in ST, NO}} X100 \quad (5)$$

Where:

% Red = Percentage Reduction

ST = Simulated traditional 'Nnam owondo' samples.

SMT = Simulated modified traditional 'Nnam owondo' samples.

AFT = Aflatoxins total.

2.7 Statistical analysis.

Data collected from the investigation were edited in Microsoft Excel 2013 and descriptive statistics were applied to summarize data as frequencies and percentages. The statistical software Stat Graphic Centurion XV version 16.1.18 (Stat Point Technologies, Inc., Virginia, USA) was used to perform an analysis of variance on the AFT contents of samples.

3. Results

3.1 Data from Food Frequency Survey

3.1.1 Socio-demographic variables of adult participants, 'Nnam owondo' ('NO') processing information and consumption pattern in study areas.

Based on this study, the studied 'Nnam owondo' ('NO') samples were exclusively produced by women (100 %) with a majority being small business individuals (62.5 %) and most of the studied participants had attained the secondary level of education (65.6 %; Table 1).

Table 1.

Socio-demographic variables of adult participants.

Parameters	Number	Percentage (%)
a) Sex:		
Female	32	100
b) Occupation:		
Small business individuals	20	62.5
Farmers	9	28.1
Teachers	3	9.4
c) Level of Education :		
Primary	7	21.9
Secondary	21	65.6
Undergraduate	4	12.5

3.1.2 'Nnam owondo' processing information and consumption pattern in study areas.

Groundnuts for 'NO' processing were generally stored in bags (59 %) for a maximum of 1 month (50 %). Children like adults generally consume 'NO' 2-3 days per week (81.3 % and 46.9 % respectively) prepared and served in the same pots within the same homes. Adults and children consume on average 122.5 g and 99.6 g of 'NO' per day respectively. The prepared 'NO' were stored in pots (62.5 %), but more in refrigerators (90 %) and generally between 1 (38 %) or 2 days (28 %). Milling of fried groundnut to paste was mostly carried out by participants in public milling stations (25/32, 78.1 %) than at home with a hand grinding machine (9/32, 28.1 %; Table 2).

Table 2.

'Nnam owondo' processing information and consumption pattern in study areas.

Parameters	**Number**	**Percentage (%)**
i. Storage Methods:		
Bags	19	59.4
Containers	15	46.9
ii. Storage Duration:		
3-6 months	1	3.1
2-3 months	6	18.8
1-2 months	11	34.4
Less than 1 month	16	50
a. Frequency of consumption of 'NO'		
i. Children (girls and boys)		
Number of intakes in days per week		
1 day/week	24	75
2-3 days/week	26	81.3
3-5 days/week	4	12.5
5-6 days/week	2	6.3
7 days/week	2	6.3
ii. Adults (male and female)		
Number of intakes in days per week		
1 day/week	11	34.4
2-3 days/week	15	46.9
3-5 days/week	1	3.1
5-6 days/week	1	3.1
7 days/week	4	12.5
b. Storage methods and duration of 'NO'		
i. Storage Methods:		
Pots	20	62.5
Cover in plates	5	15.6
Refrigerator	29	90.6
ii. Storage Duration		
Above 5 days	6	18.8
2 days	9	28.1
1 day	12	37.5
Less than 1 day	5	15.6
c. Estimated average serving sizes of 'NO'		
i. Adults (male and female)	122.5 g	
ii. Children (boys and girls)	99.6 g	

3.1. Mean levels of aflatoxins total (AFT) in Studied 'Nnam owondo' ('NO') samples; Simulated traditional and simulated modified traditional 'NO' samples.

3.1.1 Mean levels of aflatoxins total (AFT) in simulated traditional 'Nnam owondo' samples collected from study participants

AFT was detected in all the NO samples collected from study participants (100 %, 32/32). The overall mean of AFT was 17.2, ranging from 5.41 to 34.02 µg/Kg with 'NO' samples from Ekounou being the most contaminated with an AFT mean of 18.8 and a range of 5.41 to 34.02 µg/Kg (Table 3). Around 62.5 % (20/32) of the 'NO' samples collected from study participants had levels of AFT that exceeded the Codex Alimentarius Commission regulatory limit of 10 µg/Kg fixed for groundnut-based food destined for direct human consumption [28]. Based on the study locations, 72.2 % (13/18), 44.4% (4/9), and 60% (3/5) of the samples collected from study participants in Ekounou, Nkoabang, and Nkomo respectively had levels of AFT greater than the CAC regulatory limit (10 µg/Kg) established for groundnut-based food intended for direct human consumption [28].

Table 3.

Mean levels of aflatoxins total (AFT) in pooled, simulated traditional, and simulated modified traditional 'NO' samples.

Study area	Frequency (%) positive	Range (Min-Max) µg/Kg	Total mean AFT level (µg/Kg) ± SD	% Mean AFT level (µg/Kg) > CAC regulatory limit (10 µg/Kg) [28]
Ekounou (n=18)	18 (100)	5.41-34.02	18.8 ± 10.5	72.2 % (13)
Nkoabang (n=9)	9 (100)	5.41-28.08	15.6 ± 9.4	44.4 % (4)
Nkomo (n=5)	5 (100)	5.85-23.24	13.9 ± 7.4	60 % (3)
Grand total mean (range) AFT level (µg/Kg) ± SD	na	*5*.41-*34.02*	17.2 ± 9.8	Na
Grand total % (N°)	Na	na	na	62.5 % (20)

na: not applicable; SD: Standard Deviation ; % : Percentage; > : Greater than; AFT: Aflatoxins total; CAC: Codex Alimentarius Commission; min: minimum; max: maximum.

3.1.2 Mean levels of aflatoxins total (AFT) in simulated traditional and simulated modified traditional 'Nnam owondo' samples produced in the laboratory.

AFT was detected in all simulated traditional (100%, 5/5) and simulated modified traditional (100%, 6/6) NO samples produced in the laboratory from the same groundnut sample. The overall mean (range) AFT for simulated traditional and simulated modified traditional 'NO' samples was 15.6 (11.6-21.7) and 5.9 (5.9) µg/Kg respectively (Table 4). All simulated traditional 'NO' samples had AFT levels that exceeded the norm (10 µg/Kg) of Codex Alimentarius Commission (CAC) set for groundnut-based destined for direct human consumption whereas the simulated modified traditional 'NO' samples were all found to be below the regulatory limit set by CAC [28].

Table 4.
Mean levels of aflatoxins total (AFT) in pooled, simulated traditional, and simulated modified traditional 'NO' samples.

Study area	Frequency (%) positive	Range (min-max) µg/Kg	Total mean AFT level (µg/Kg)	% Mean AFT level (µg/Kg) > CAC regulatory limit (10 µg/Kg) [28]
A. Laboratory				
i. Simulated Traditional 'NO' samples (n=5)	5 (100)	11.6-21.7	15.6	100 (5)
ii. Simulated modified Traditional 'NO' samples (n=6)	6 (100)	5.9	5.9	0 (0)

%: Percentage; >: Greater than; AFT: Aflatoxins total; CAC: Codex Alimentarius Commission; min: minimum; max: maximum.

3.1. Dietary Exposure Levels, Margin of Exposure, and quantitative liver cancer risk

The average estimated daily exposure (EDE) for adults and children was 31.84 µg/Kg bw/day and 46.24 µg/Kg bw/day of AFT, respectively. When considering the Benchmark dose lower confident limit, 10 % ($BMDL_{10}$) of 0.4 ng/Kg bw/day (0.004 µg/Kg bw/day) for rodents, the Margin of Exposure (MOE) of adults and children was estimated to be 0.000022 and 0.0000087, respectively.

Table 5.
Mean Daily Exposure and Margin of Exposure for children (12-17yrs) and adults (18yrs and above) in this study

Category	Study location	No. of Households	Av. Body weight (Kg)	Av. Daily intake of 'NO' (g)	Total mean AFT levels (µg/Kg)	Av. Daily exposure µg/Kg bw/day	Av. *MOE
Children (n=57)	Ekounou (n=33	18	41.61	106.52	18.8	0.05	13.36
	Nkoabang (n=17)	9	43.18	91.46	15.6	0.04	21.87
	Nkomo (n=7)	5	42.14	86.91	13.6	0.02	22.87
Total mean	**na**	**na**	**42.14**	**99.6**	**17.2**	**0.04**	**9.98**
Adults (n=32)	Ekounou (n=18)	18	67.3	123.9	18.8	0.04	18.7
	Nkoabang (n=9)	9	71	105.2	15.6	0.03	30.04
	Nkomo (n=5)	5	68	148.72	13.6	0.03	18.50
Total mean	**na**	**na**	**68.44**	**122.5**	**17.2**	**0.03**	**13.02**

*MOE: Margin of Exposure (this was calculated using $BMDL_{10}$ for rodents: 0.4 µg/Kg bw/day); µg/Kg bw/day (calculated using daily intakes of 'NO' in Kg by dividing values in gram by 1000): Microgram per kilogram per body weight per day; ng/Kg/bw/day: nanogram per kilogram per body weight per day. na: not applicable NB. The total mean value row is obtained from the addition of all the different values divided by the number of items in this study and not from adding the mean and dividing by 3. This applies to adults and children.

Table 6.

Quantitative liver cancer assessment of study participants (adults and children)

Category	Study Area	PHBsAg+ (cancers/yr/100,000 pops ng/ AFT/kg/ bw/day)	PHBsAg- (cancers/ yr/100,000 pops ng/ AFT/kg/ bw/day	Pop. HBsAg+	Pop. HBsAg-	P_{cancer} /1000 (cancers/ yr/ 100,000 / µg AFT kg/bw/day)	Av. Daily exposure µg/Kg bw/day	Cancer risk (cancers/yr / 100,000 pop.
Children	Ek	0.3	0.01	546420	3790249	201.8	0.05	10.1
	Nkb	0.3	0.01	546420	3790249	201.8	0.04	8.1
	Nkm	0.3	0.01	546420	3790249	201.8	0.02	4.0
Total mean	**na**	**0.3**	**0.01**	**546420**	**3790249**	**201.8**	**0.04**	**8.1**
Adults	Ek	0.3	0.01	546420	3790249	201.8	0.04	8.1
	Nkb	0.3	0.01	546420	3790249	201.8	0.03	6.1
	Nkm	0.3	0.01	546420	3790249	201.8	0.03	6.1
Total mean	**na**	**0.3**	**0.01**	**546420**	**3790249**	**201.8**	**0.03**	**6.1**

* P_{cancer}: Carcinogenic potency/ cancer potency (this was calculated, and the answer was divided by 1000 to convert the unit of measurement from nanogram). *Pop. HBsAg+: Fraction of population with Hepatitis B; Pop. HBsAg-: Fraction of population without Hepatitis B; P_{cancer}: Carcinogenic potency/ cancer potency; $HBsAg^-$: Hepatitis B virus negative persons; HBsAg+: Hepatitis B virus-positive persons; NO: 'Nnam owondo'; Ek: Ekounou; Nkm: Nkomo; Nkb: Nkoabang; na: not applicable; pops: Populations. *The overall hepatitis B virus infection prevalence of 12.6% of the blood donors of the Yaounde Central Hospital, Centre region of Cameroon reported by Ankouane et al. [27] was used to determine the cancer risk in this study.*The population of Yaounde in 2022 which is 4,336,670 was also taken into account (Worldo meters).

3.2. Safety improvement of 'Nnam owondo' production process; average Estimated Daily exposure and Margin of Exposure of 'Nnam owondo' samples produced through stimulated modified traditional method compared to the simulated traditional method.

Table 5 provides data on total mean AFT levels, daily exposure, and Margin of Exposure (MOE) for adult and children NO consumers. 'NO' processed through the simulated-traditional method was more contaminated with AFT when compared with 'NO' produced through the traditional modified method. A 62.2 % reduction of AFT levels was observed in 'NO' samples produced through the simulated modified traditional method when compared to 'NO' samples produced through the simulated traditional method after laboratory analysis and calculation of percentage reduction. The estimated daily exposure of adults and children 'NO' consumers also decreased by 60.7 and 62.2 % respectively when considering their average daily intake of 'NO' and mean body weight. This subsequently leads to a reduction in the Margin of exposure and as well the level of public health concerns for both adults and children 'NO' consumers in this study.

Table 7.

Comparison of aflatoxin total (AFT) levels and Margin of Exposure (MOE) in simulated traditional (n=5) and modified simulated modified traditional (n=6) 'NO' samples.

'Nnam owondo' preparation method	**Average daily intake of 'NO' (g)**		**Mean body weight (Kg)**		**Total mean AFT levels (µg/Kg)**	**Total mean Daily exposure µg/Kg bw/day**		**Total mean *MOE**	
	Adults	Children	Adults	Children		Adults	Children	Adults	Children
Simulated Traditional method (n=5)	122.5	99.6	68.44	42.14	15.6	0.028	0.037	15.531	12.026
Simulated modified traditional method (n=6)	122.5	99.6	68.44	42.14	5.9	0.011	0.014	36.364	28.571
% Reduction of AFT	na	na	na	Na	62.2	60.7	62.2	na	na

*MOE: Margin of Exposure (this was calculated using $BMDL_{10}$ for rodents: 0.4 µg/Kg bw/day); µg/Kg bw/day (calculated using daily intakes of NO in Kg): Microgram per kilogram per body weight per day; ng/Kg/bw/day: nanogram per kilogram per body weight per day

4. Discussion

The focus of this study was to determine the AFs dietary exposure levels and associated health risks amongst 'NO' consumers in Yaounde, Centre Region of Cameroon. The NO was exclusively produced by women (100%) with a majority being small business individuals (62.5 %) and most of the studied participants had a secondary level of education (65.6 %) who sometimes sell to the population to generate income for their families. This traditional delicacy was produced by study participants across all study locations through the processing steps of sieving, hand-picking, frying (dry-frying), milling, homogenization, packaging, and cooking (Figure 2), a finding which was in accordance with that reported by Ponka et al. [29]. However, when considering the presence of well-known aflatoxins-reducing steps of sieving [30], and hand-picking [31] involved in 'NO' production in our study, which are absent in that of Ponka et al. [29]. One may conclude that the processing steps presented in this work are more appropriate. The steps of sieving and hand-picking which are known to be vital for the reduction of aflatoxin levels in foods were implemented by some but not all participants in this study. Since over 80% of participants did not know about AFs contamination of crops, besides culture it is in part obvious why these vital AFs-reducing steps (sieving and hand-picking) were left out. In addition, this can be attributed to the lack of knowledge by study participants on well-known aflatoxin mitigation methods such as sieving and hand-picking applied during food production.

The observed 100 % AFT contamination levels in all samples collected from study participants (n=32) were not unexpected when considering groundnut as one of the most susceptible agricultural commodities to AF contamination. This finding corroborates with the report from Abia *et al.* [7] which revealed 93, 97 and 100 % AF contamination levels in groundnut soup, groundnut, and kuru-kuru respectively as well as Njobeh et al. [6] who observed levels of Afs, 6.5 (0,1-13) µg/Kg in groundnut from Yaounde. The overall mean

(range) AFT level in all 'NO' samples (N=32) collected from study participants in this present study was 17.2 (5.41-34.02) µg/Kg. This was low when compared to AF levels revealed by Abia et al. [7] in groundnut, and other groundnut-based foods like groundnut soup, and kuru-kuru (mean: 47, range: <LOQ-210 µg/Kg) [6]. The overall mean of AFT in 'NO' samples from Ekounou (overall mean: 18.8 µg/Kg) was higher than all the other study areas (Nkomo: overall mean: 13.9 µg/Kg and Nkoabang: overall mean: 15.6 µg/Kg). This might partially be due to the large sample size of Ekounou (n=18) which doubles that of Nkoabang (n=9) and is three times more than that of Nkomo (n=5). In addition, this can be as well due to the presence of the sample with the highest AFT level (34.02 µg/Kg) across all study areas from Ekounou. The overall mean level of AFT in samples (n=32) collected from participants (17.2 µg/Kg) in this study as well as the individual mean of all study areas; Ekounou (18.8 µg/Kg), Nkomo (13.6 µg/Kg) and Nkoabang µg/Kg) were observed to exceed the AFT-regulatory limit of 10 µg/Kg set by Codex Alimentarius Commission [28]. However, 62.5 % (20/32) of samples collected from study participants had AFT levels more than the Codex Alimentarius Commission (CAC) regulatory limit of 10 µg/Kg fixed for groundnut-based foods intended for direct human consumption like 'NO' [28]. All simulated traditional modified 'NO' samples were also found to be contaminated with AFT with an overall mean of 5.9 µg/Kg. The simulated traditional modified 'NO' samples though contaminated with AFs revealed a 62.2 % reduction in AFT levels when compared with simulated traditional 'NO' samples produced with the same groundnut sample. This reduction can be explained by the introduction of three well-known AFs reducing steps; soaking, washing, and sun drying between steps 2 and 3 of the simulated traditional method of 'NO' preparation. However, the observed relatively low AFT and overall mean levels in modified processed 'NO' samples were all (100 %) lower than the global regulatory limit of 10 µg/Kg set by codex Alimentarius Commission [28] but greater than those of the European Union (4 µg/Kg) set for groundnut-based foods intended for direct human consumption [22].

The mean daily exposure for adults from samples collected from participants was 0.031 µg/Kg bw/day and for children, 0.040 µg/Kg bw/day. The MOE of 13.018 and 9.980 was obtained for both adults and children respectively when considering the daily exposure values and the above $BMDL_{10}$. 'NO' samples produced through simulated modified traditional method revealed 60.7 and 62.2 % reduction in AFs dietary exposures after analysis with daily exposure µg/Kg bw/day (MOE) values of 0.011 (36.364) and 0.014 µg/Kg bw/day (28.571) for adults and children, respectively. Both MOE values in this study imply AFs dietary exposures are a public health concern.

For samples collected from studied participants, the Margin of Exposure values of 13.018 and 9.980 were obtained for both adults and children respectively when considering the mean daily exposure of 0.031 µg/Kg bw/day for adults and 0.040 µg/Kg bw/day for children together with the $BMDL_{10}$. For simulated modified traditional 'NO' samples a lower mean daily exposure of 0.011 µg/Kg bw/day and 0.014 µg/Kg bw/day were calculated for adults and children respectively. A safer MOE value of 36.364 and 28.571 were obtained for adults and children respectively based on daily exposure values of 0.011 µg/Kg bw/day for adults and 0.014 µg/Kg bw/day for children and the $BMDL_{10}$ value. Both MOE values for adults and children for simulated traditional 'NO' samples and simulated traditional modified samples in this study imply AFs dietary exposures are a public health concern. This

indicates that the consumption of 'NO' contaminated with aflatoxins a toxic and carcinogenic compound should be considered a food safety and public health concern in our study areas. This MOE value of AFT may be aggravated in the study population as they consume other groundnut-based foods like groundnut soup, ndole, okok, and maize-based foods such as Ekomba vulnerable to aflatoxins reported in Cameroon [6, 7]. A mean cancer risk of range: 6 to 10 primary liver cancer cases per year per 100,000 populations were observed for children and adults in this study. This can be explained by the high average aflatoxins daily exposure observed for children and adults through 'NO' consumption across the different study locations. This was found to be lower than that reported in foods in Africa [32]. The high levels of AFT reported in groundnut a staple in Cameroon as well as its by-products consumed daily, justify the urgent need for more awareness raising, sensitization education on AFs and our health, as well as the need for national legislation towards AFT control in groundnut and other foodstuffs.

5. Conclusions

NO is a cooked groundnut-based paste commonly produced in the study locations using the following processing steps sieving, hand-picking, frying, milling, homogenization (mixing), packaging, and cooking. All the studied NO samples were contaminated with AFs irrespective of the treatment. Around 62.5 % (20/32) of samples collected from participants had AF levels exceeding the Codex Alimentarius Commission (CAC) regulatory limit of 10 µg/Kg fixed for groundnut-based foods destined for direct human consumption. All the simulated traditional processed samples (n=5, 100 %) had AF levels greater than the CAC regulatory limit while none of the simulated modified traditional samples (n=6, 0%) exceeded the CAC regulatory limit. About 62.2% reduction in AFT levels was achieved in simulated modified traditional samples when compared with simulated traditional samples. The calculated estimated Margin of Exposure (MOE) was less than 10, 000 irrespective of sample treatment, suggesting that the studied 'NO' samples are not safe for consumption irrespective of consumer category (children and adults).

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