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Heavy metals, macro-minerals and human health risk assessment of spiced and unspiced tiger nut drink commercialized in Port Harcourt, Rivers State, Nigeria

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Abstract

The need for cheap, nutritional, refreshing and local content development has increased the demand and consumption of Tiger nut drink by residents of Port Harcourt and its environs in recent years. However, the inadequate nutritional information and without nutritional facts label has made it difficult for the Tiger nut consuming populace to ascertain its health benefits. Hence, the objective of this paper was to investigate the concentrations of selected heavy metals (Pb, Ni, Fe, Cu, and Zn), macro-minerals (Mg and Ca) and human health risk assessment of spiced and unspiced Tiger nut drink commercialized in Port Harcourt, Rivers State, Nigeria using standard analytical techniques after mixed acid digestion. The mineral elements were found in the order of Mg (79.87 mg/L) > Ca (50.79 mg/L) and Mg (96.82 mg/L) > Ca (68.18 mg/L) in unspiced and spiced tiger nut drinks respectively [< WHO: Mg (150 mg/L), Ca (200 mg/L)] while heavy metals concentrations in the samples analyzed were Fe (1.82 mg/L) > Zn (1.16 mg/L) > Cu (0.4160 mg/L) and Fe (2.12 mg/L) > Zn (1.51 mg/L) > Cu (0.45 mg/L) in unspiced and spiced tiger nut drinks respectively. Pb and Ni were undetected (< 0.00 mg/l) in both unspiced and spiced gingered tiger nut drinks studied. Only Fe and Cu concentrations exceeded WHO standards (0.3 and 0.02 mg/L). However, intake of these heavy metals is unlikely to pose any non-carcinogenic health risk to consumers (target hazard quotient (THQ) < 1, HI<1 for adults and children) in both spiced and unspiced tiger nut drink. Generally, results from this study showed higher concentrations of metals in spiced tigernut drink than in the unspiced tiger nut drink. This could be attributed to the combined bioaccumulated levels of heavy metals in the tiger nut tubers and in the spice used. Frequent consumption of spiced tiger nut drink may rapidly increase the levels of heavy metals in the human body and pose chronic risk to human health and also beneficially increase the levels of macrominerals in the body. Standard production processes and regulated spicing of tiger nut drink needs to be provided by regulatory agencies (NAFDAC) and made available to producers of tiger nut drink.

Keywords: Heavy Metals; Tiger Nut; Spiced**;** Gingered; Nutritional Facts Label; Health Risk assessment

1. Introduction

A heavy metal is any metallic chemical component with a comparatively high density that is lethal or hazardous at low doses [6]. Heavy metals contain substances like mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). Heavy metals are components of Earth's crust that naturally exist and cannot be removed or broken down in Nigeria or anywhere else in the world. Through our intake of food, water, and air, they occasionally enter our bodies. The body needs some heavy metals as trace elements to maintain a healthy metabolism, such as copper, selenium, and zinc. Conversely, they might cause harmfulness at greater volumes [9]. Heavy metal poisoning may be caused, for example, by drinking polluted water (such as high ambient air concentrations near emission sources, ingestion through food chain, or lead poisoning from lead pipes [3]. Heavy metals were dangerous because they regularly bioaccumulate

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in the human body, bioaccumulation is process by which chemical's concentration in biological organism upsurges over time in comparison to chemical's concentration in environment. When substances were taken up stored by living tissues faster than they were broken down (metabolized) or removed, an accumulation of those substances occurs [13]. Tiger nut crop was from sedge family that grown every area of the world. It was known as *Cyperus esculentus*, chufa, atadwe, yellow nutsedge, and earth almond. Most of Eastern Hemisphere contains it, as Middle East and Indian subcontinent, Southern Europe, Africa, and Madagascar. *Cyperus esculentus* is grown for its edible tubers, often known as earth almonds or tiger nuts, which are used snacks to make sweet drink horchata de chufa. The main threats to human health associated with exposure to heavy metals can be linked to carcinogens (lead, cadmium, mercury, and arsenic). Tiger nuts are rich in dietary fiber, which may be effective in the treatment and prevention of many diseases, including colon cancer [1], coronary heart disease [4], obesity, diabetes, gastrointestinal disorders [17]. The determinations of inorganic and organic contaminants in beverages are a matter of concern for both safety and nutritional aspects. Consequently, human health risk assessment of beverages contaminants has been investigated in several studies. Heavy metal poisoning can be cause by ingesting foods, drinks, medications, improperly sealed food containers, occupational exposure to heavy metals, contaminated air or water [5,7, 19, 20]. Heavy metals accumulate in body and affect function of vital organs and glands including heart, brain, kidneys, bone, liver, etc. They prevent the biological function of such minerals by displacing them from their original location. Heavy metals exist naturally and are also produced by anthropogenic activities like mining and industrial production, which are common in the environment. This is an issue in modern world. Due to their extreme toxicity, arsenic, cadmium, chromium, main, and mercury were among precedence metals of public health anxiety. These metallic features were characterized as systemic toxicants since they were known to affect various organs at low exposure stages [7]. Thus, the objective of this study is to determine the concentrations of selected heavy metals (Pb, Ni, Fe, Cu, and Zn), macro-minerals (Mg and Ca) and human health risk assessment of spiced and unspiced Tiger nut drink commercialized in Port Harcourt, Rivers State, Nigeria

2. Material and methods

2.1. Study Area

The Port Harcourt Metropolis, the fourth largest city in Nigeria after Lagos, Kano, and Ibadan [25]. The metropolis has an approximate area of about $369 km^2$. It is located between latitude 4^0 49’ 27’’N and longitude 7^0 2’ 1’’E. The metropolis is regarded as the most populous district in Rivers State with a population density of 7,865/km from a projection of 774,600 Population and covering an area of 98.49 km^2 [32].

Port Harcourt has attracted large population as the capital and most commercialized city in the state. The business of tiger nut drink production and sales is highly proliferating in Port Harcourt city has businesses have reported an open and on-demand market for the drink.

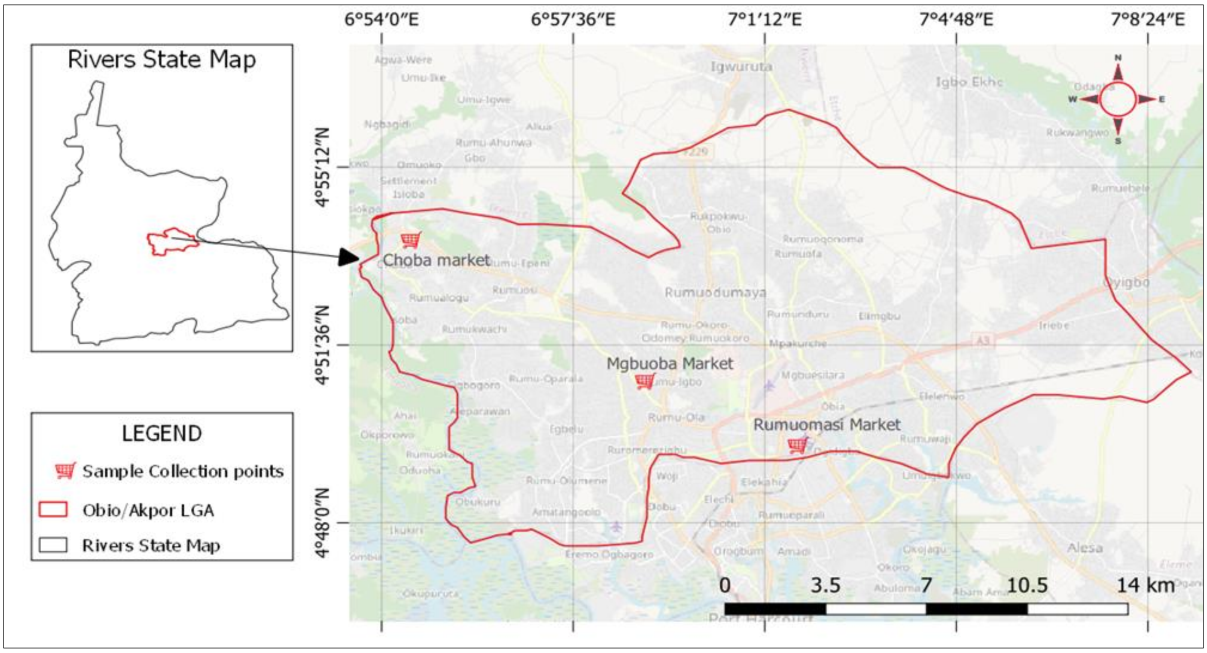

Figure 1 Map of Port Harcourt Metropolis Showing Areas of Sampling

2.2. Sample collection

The spiced and unspiced tiger nut drink samples used in this study were purchased from three vendors in Choba, Mgbuoba and Rumuomasi and made into composites, stored in an ice chest and transported to the laboratory for analysis.

2.3. Sample preparation and analysis

The liquid sample (tiger nut drink) was prepared using standard dry ashing method. 1.0 g of the sample was weighed into porcelain crucible and placed into a muffle furnace with a regulated temperature. The sample was then ashed for 3h at 500 ^{o}C. The dried sample was then removed and wetted with a small amount of distilled water followed by the addition 10 mL of 6N HCl and heated to near dryness on a hot plate. The ash was then dissolved with the addition of 10mL, 1N HCl to the crucible. All the samples were carefully transferred into 100 mL volumetric flasks and diluted to volume. Concentrations of heavy metals: iron, lead, nickel, zinc, copper and macrominerals: magnesium and calcium in the digested sample solution were determined using spectroscopic technique (Atomic Absorption Spectrophotometry).

2.4. Quality Assurance/Quality Control

During the research, appropriate quality assurance precautions and procedures were followed, field and laboratory methods were adjusted to ensure data quality. As a result, sample bottles were thoroughly cleaned with mild hydrochloric acid and they were then rinsed in the lab with de-ionised water. All the reagents were of analytical grade and glassware cleaned properly. The tigernut drink samples (Spiced and unspiced) were obtained within Port Harcourt metropolis. All laboratory tests for the quality of fresh tigernut drink were performed according to standard methods for the investigation of heavy metals and mineral elements using Perkin-Elmer atomic absorption spectrophotometer and Flame Photometer (FP Model 140). Deionized water was used throughout the study. For the heavy metals under consideration, samples were collected within Port Harcourt and examined independently. Spiced and unspiced tigernut drink samples were properly labelled and maintained in an ices chest container to ensure reliability of result and then transported to the lab for further analysis.

2.5. Human Health Risk Assessment for Tiger Nut Drinks Consumption

The non-carcinogenic health risks assessment associated with consumption of tiger nut drink was evaluated using estimated dietary intakes (EDI), target hazard quotient (THQ) and hazard index (HI) [14, 15, 16].

2.6. Estimation of Dietary Intake (EDI)

The estimated daily intakes (EDI) of Pb, Cu, Zn, Ni and Fe due to consumption of Tiger nut drink were assayed using the formula.

$$EDI(mgkg^{-1}d^{-1}) = \frac{CM_s * IR_v}{B_w} \dots\dots..(1)$$

Where IR_v is 0.245L/day. This represents the average volume of tiger nut drink ingested per day. It is converted from per capita consumption of non-alcoholic drinks in Nigeria [28], CM_S is the concentration of heavy metals in tiger nut drink and Bw represents body weight of 60 kg for adult and 24 kg for children.

2.7. Target Hazard Quotient (THQ)

Target hazard quotient (THQ) is calculated using the formula established by United States Environmental Protection Agency (USEPA, 2014). The equation used for estimating THQ was:

$$THQ = \frac{EDI}{RfD_o} = \frac{C * IR * EF * ED}{RfD_o * B_w * AT * 365days/yr} \dots.(2)$$

Where; THQ is the target hazard quotient, EDI is the estimated daily intake dosage

The oral reference dose (RfD_o) is an estimate of daily exposure to human population (including sensitive subgroup) that is likely to be without an appreciable risk of deleterious effect during life time. The oral reference dose (RFD_o) (mg/kg/day) used were Pb (1.0035), Cu (0.04), Zn (0.3), Fe (0.7) [29], chemical concentration in drink, C (mg/L), adult body weight, B_W (kg), averaging time, AT (30 yr-for noncarcinogens, equal to ED), exposure frequency, EF (350 days/yr), drink ingestion rate, IR (L/day).

2.8. Hazard Index (HI)

$$HI = THQ_{Pb} + THQ_{Cu} + THQ_{Zn} + THQ_{Fe} \ldots\ldots\ldots (3)$$

Where HI is the hazard index; THQ_{Pb}, THQ_{Cu}, THQ_{Zn}, THQ_{Fe} = the target hazard quotient due to intakes Pb, Cu, Zn and Fe respectively. The probability that cancer may arise in humans via consumption of tiger nut drink sampled from Port Harcourt metropolis was studied using state-of-the-art risk characterization indices developed by USEPA (1986). The indices allow for quantitation of the level of intake of individual potentially carcinogenic substances. The obtained values are then compared to standard health-based guidance values (HBGV).

2.9. Chronic Daily intake (CDI)

The estimated daily intakes (EDI) of heavy metals from drinking Tiger nut were estimated using the formula:

$$CDI = \frac{C \times IRw \times EF \times ED}{BW \times AT \times 365\ days/yr} \ldots\ldots\ldots (4)$$

Where: CDI= Chronic daily intake of individual carcinogenic substance (mg $kg^{-1}day^{-1}$)

Chemical concentration in drink, C (mg/L), IR_w = the average volume of tiger nut drink ingested per day, EF_r = exposure frequency for target population residents = 350 days per year ED_r = exposure duration for target population residents = 26 years, ATr= averaging time; the period over which the exposure is averaged = [EF_r x ED_{tot}] = [365years x 70years], EDtot = standard lifetime for carcinogenic effect: updated standard defaults exposure factors (USEPA, 2014).

2.10. Individual Lifetime Cancer Risk (ILCR)

$$ILCR = CDI \times CSF \ldots\ldots\ldots (5)$$

Where:
ILCR = Individual Lifetime Cancer Risk, CDI = Chronic daily intake, CSF = Cancer slope factor

The cancer slope factor (CSF) is a toxicity value developed by the [33]. It is an estimate of the probability that an individual may develop cancer due to exposure or consumption of a toxicant over a lifetime of pre-determined 70 years period.

An ILCR of 1.0×10^{-6} is considered the safe limit for cancer and represents the probability that one individual may develop cancer in every one million people (1:1,000,000), ILCR of 1.0×10^{-4} is the threshold risk limit and represents the probability of one individual developing cancer in every ten thousand people (1:10,000) and ILCR of 1.0×10^{-3} is the moderate risk level with a chance of one individual developing cancer in every one thousand people (1:1000), [26,27].

2.11. Cumulative Individual Cancer Risk (∑ILCR)

$$\sum ILCR = ILCR_1 + ILCR_2 + LCR_3 ----- + ILCR_n \ldots\ldots\ldots (6)$$

Where n = 1, 2, ----, n is the individual carcinogenic heavy meals in a given sample.

2.12. Statistical Analysis

The data generated from the laboratory was analysed using IBM SPSS software (version 23). One-way analysis of variance (ANOVA) was employed to determine if statistical significance exist between the studied samples at 95% confidence level. Microsoft Excel, Microsoft Word, and IBM SPSS was also used for all statistical analysis and calculations.

3. Results

The minerals and heavy metals content of unspiced and spiced tiger nut (*cyperus esculentus*) drink are presented as mean concentrations and standard deviations in Table 1. Results from the analytical findings showed that the levels of Ni and Pb in the unspiced and spiced tiger nut drinks were both below the 0.001 mg/L method detection limit (MDL). The mean Fe concentrations in unspiced and spiced tiger nut drinks ranged from 1.82 mg/L to 2.12 mg/L. In unspiced tiger nut drink, the mean Zn concentrations ranged from 1.156 mg/L to 1.5060 mg/L, while in spiced tiger nut drink, the mean Cu concentrations ranged from 0.431 mg/L to 0.446 mg/L. The findings showed that while the mean Ca concentration ranged from 50.786 mg/L in unspiced tiger nut drink to 68.180 mg/L in spiced tiger nut drink, the mean Mg concentration varied between 79.868 mg/L in unspiced tiger nut drink and 96.828 mg/L in spiced tiger nut drink. The findings showed that, with the exception of Ni and Pb, which could not be detected, the spiced tiger nut drink contains higher amounts of the studied heavy metals. Generally, the tiger nut drinks contain the following essential minerals in descending order: magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), and copper (Cu). Hence, both spiced and unspiced C*yperus esculentus* are very rich sources of Mg and Ca.

Variation in concentrations of Zn, Mg, and Ca showed statistically significant difference ($P<0.05$) between the unspiced and spiced tiger nut but Fe and Cu exhibited no significant differences ($P>0.05$). Hence the use of ginger in the drink contributes no significant amount of Fe and Cu. This may be due to low natural composition of Fe and Cu in the ginger tuber. This result agrees with a study conducted by [30] to evaluate food value of two (white and yellow)

Varieties of ginger (*zingiber officinale*) commonly consumed in Nigeria. The researchers reported that Cu had the least concentration (0.01 ppm) among the minerals analyzed (sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn)).

This study also supported earlier studies from [2] that found tiger nut milk to be abundant in minerals including phosphorus, calcium, and magnesium, iron, as well as vitamins C and E, all of which are necessary for healthy body growth and development. It is a particularly effective energy drink due to its high energy content (100 cal/100 g). The fact that it is lactose- and gluten-free makes it very beneficial and safe for human consumption [31].

Although metals and a few necessary minerals are naturally occurring in plants, the levels of these elements in tiger nut drink may increase due to contamination from production processes. For example, the use of water that is particularly rich in or contaminated with heavy metals. Rocks and soils are sources of water which commonly contain iron and other metals. Water seeps through soils and rocks containing metals during rainstorms or the melting of snow, and iron can dissolve in the water. Iron in water can result from corrosion of steel or iron water pipelines or well casings [12]. The main source of Copper in drinking water, according to [21] is corrosion of home plumbing, faucets, and water fixtures. As Copper corrodes from plumbing components including pipes, fittings, and brass faucets, water absorbs the Copper that is released.

Generally, the study's findings indicated that the metal concentrations in the spiced tiger nut drink were higher than those in the unspiced sample. The concentrations of heavy metals identified in this study's unspiced and spiced tiger nut drinks—lead (Pb), nickel (Ni), and other trace minerals like zinc (Zn), calcium (Ca), and magnesium (Mg) were all below the WHO/FAO permissible level. However, iron (Fe) and copper (Cu) exceeded regulatory limit by WHO.

Table 1 Heavy Metals/Mineral Concentration of Tiger Nut Drinks (*Cyperus Esculentus*) from Port Harcourt Metropolis

Metals	**Unspiced Tiger Nut Drink (mg/L)**	**Spiced Tiger Nut Drink (mg/L)**	**F**	**Significance of Variation**	**WHO/FAO Limit**
Iron	1.82 ± 0.52	2.12 ± 0.31	1.28	0.290	0.30
Lead	0.00 ± 0.00	0.00 ± 0.00	-	-	0.01
Nickel	0.00 ± 0.00	0.00 ± 0.00	-	-	0.02
Zinc	1.16 ± 0.17	1.51 ± 0.18	10.44	0.012	3.0
Copper	0.42 ± 0.06	0.45 ± 0.11	0.30	0.598	0.02
Magnesium	79.87 ± 7.59	96.83 ± 4.76	17.91	0.003	150
Calcium	50.79 ± 7.42	68.18 ± 2.50	24.65	0.001	200

Data are expressed as Mean ± Standard deviation

The results of this study supported [10] academic work on the examination of proximate and heavy metal concentrations in nuts and vegetables consumed in Port Harcourt, Nigeria. They found substantial heterogeneity among the trace mineral elements. A lack of iron can result in a nutritional issue because it has been reported to be essential for the formation of blood. It is necessary for the creation of the oxygen-transporting proteins myoglobin and hemoglobin. Additionally, compared to this study, [24] analysis of the nutritious and antinutritional components of residues on tiger nuts revealed minimal levels of heavy metal content. The significant antioxidant activity of tiger nut extracts was reported to exhibit an ameliorating effect on heavy metal-induced organ damage in wistar rats [11]. In addition, [8] confirmed that the addition of spices and dates enhance the nutritional composition which supporting the findings of this study on unspiced and spiced tiger nut drink.

Table 2 Estimated Daily Intake (EDI ($\boldsymbol{mgkg^{-1}d^{-1}}$)), oral reference dose (RfD_o) and mean concentrations (mg/L) of heavy metals in spiced and unspiced tiger nut drink, Port Harcourt metropolis

Metals	Unspiced Tiger Nut Drink		Spiced Tiger Nut Drink		RFD_o
	Adult	**Children**	**Adult**	**Children**	
Fe	0.007	0.019	0.009	0.022	0.7
Pb	-	-	-	-	0.0035
Ni	-	-	-	-	0.020
Zn	0.005	0.001	0.006	0.015	0.300
Cu	0.002	0.004	0.002	0.005	0.004

Table 3 Target Hazard Quotient (THQ) for Adult and Children

Metals	Unspiced Tiger Nut Drink		Spiced Tiger Nut Drink		USEPA (2014)
	Adult	Children	Adult	Children	
Fe	0.0102	0.027	0.012	0.031	1
Pb	-	-	-	-	1
Ni	-	-	-	-	1
Zn	0.016	0.035	0.021	0.051	1
Cu	0.043	0.111	0.049	0.120	1
THI	0.089	0.173	0.079	0.197	1

USEPA – United State Environmental Protection Agency (2014) **THI** – Total Hazard Index

4. Discussion

4.1. Health Risk Assessment

4.1.1. Estimated Daily Intake (EDI)

The results obtained showed that EDI ($mgkg^{-1}d^{-1}$) for adult and children population due to intake of Fe were 0.009 > 0.007 and 0.022 > 0.019 in spiced and unspiced tiger nut drinks respectively and less than the standard intake (RfDo) as recommended by USEPA (2014). Results obtained from the analysis of other metals Zn and Cu were found for adults and children population in the order of 0.006 > 0.005; 0.002 - 0.002 and 0.015 > 0.001; 0.005 > 0.004 in spiced and unspiced tiger nut drinks respectively. These intakes were also below the standard reference dose for Zn and Cu (0.3 and 0.004). The Estimated daily intake (EDI) obtained due to the consumption of unspiced and spiced tiger nut drink by children (aged 4 - 6 years) sampled from Port Harcourt Metropolis as shown in Table 2. These intakes were also below the standard reference dose for Zn and Cu (0.3 and 0.004). These results show that there may be no appreciable non-cancer risk to consumers of tiger nut drink in Port Harcourt metropolis. However, scientists have reported that high levels of Pb and Ni can be toxic to the human consuming tiger nut drink. Such toxic effect of Pb include: cerebral encephalopathy, inhibition of enzyme such as ferrochelatase and delta amino levulinic acid dehydratase (ALAD) synthase [18].

4.1.2. Target Hazard Quotient (THQ)

The target hazard quotient evaluated due to the ingestion of unspiced and spiced tiger nut drink by adults and children with different exposure duration in Port Harcourt metropolis are shown in Table 3. The results obtained showed that

THQ due to the dietary exposure were Fe 0.012; 0.010 and 0.027; 0.031 in spiced and unspiced tiger nut drink for adults and children population. Results obtained from the evaluation of other metals Zn and Cu were found in order of 0.021; 0.016 and 0.049; 0.043. The hazard index (HI) result for adults and children population who consumed tiger nut drink in Port Harcourt metropolis for spiced and unspiced are 0.079; 0.197 and 0.089; 0.173 respectively. Pb and Ni, are two known carcinogens heavy metals analyzed were below detectable limit. Mg and Ca are among the macro-minerals essential for human nutrition. Hence, they were not included in the risk assessment of this study. Results from the study showed THQ < 1 in both the spiced and unspiced tiger nut drink. Consequently, the consumption of Fe, Zn and Cu is likely to pose any acute or chronic noncancer risk to the health of consumers.

5. Conclusion

The result of the study provides information on the levels of heavy metals and minerals in ungingered and gingered, tiger nuts drinks consumed in Port Harcourt metropolis and the probable human health risk associated with the consumption of tiger nut drink. The Zn, Mg, and Ca concentrations varied considerably between the gingered and ungingered tiger nuts drink, but Fe and Cu exhibited no significant differences. The tiger nut drink is likely not to be carcinogenic and not harmful to human health because Pb and Ni values were below detection limit for both the gingered and ungingered tiger nut drinks. The iron level in the gingered and ungingered tiger nut drink above the permissible limit of WHO/FAO for iron concentration in drinks. The results of the study also showed no noncarcinogenic risk. However, high levels of Fe detected in the drinks may lead to hemochromatosis in human, hence the amount of tiger nut drink that people should consume daily should be controlled.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Statement of informed consent

All the authors have read and approved the paper for submission of publication.

Data Availability

The data that support the findings of this study are available on request from the corresponding author

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