



## Assessing food safety of tigernut (*Cyperus esculentus* L.) juice through entomotoxicology in parts of Port Harcourt, Nigeria

Maduamaka C Abajue\*, Akpan Uduak Obong

Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Nigeria

### Abstract

Heavy metals are excessively released into the environment because of technological development and urbanization. This has resulted in contaminating groundwater and water bodies (ponds, streams, rivers) hence, are becoming threats to human health. Thus, assessment of heavy metals, was carried out on tigernut juice samples sold in some parts of Port Harcourt, Nigeria. Its food risk was assessed with blow fly maggots, as toxicological specimens. Fresh Tigernut juice samples were purchased from vendors in Port Harcourt, and a manually processed sample in a Laboratory at University of Port Harcourt, were used for the study. The method described by Belewu and Abodunrin (2008), was adopted to process the tigernut juice sample. The samples were filtered after decantation and subjected to atomic absorption spectrophotometer for heavy metals assessment. Blow fly maggots cultured on cow liver-tigernut juice samples, were used for toxicological assessment by adopting the method of Abajue and Ewuim (2020). Heavy metals (arsenic, cadmium, chromium, copper, cobalt, lead, mercury and nickel) assessed in the tigernut juice samples, showed that nickel and arsenic were negative in all the samples irrespective of their source of collection. Except for mercury that was slightly higher than few permissible limits, other heavy metals were lower than the permissible limits of national and international regulatory organizations. Entomotoxicology assessment showed that the heavy metals which were present in the tigernut samples, were absent in the blow fly maggots. The food safety of tigernut juice was discussed, while transient appreciation for posing no food risk was as well highlighted.

**Keywords:** tigernut, food safety, blow fly, toxicology, assessment

### Introduction

Diversity of vegetation in the tropics has been a source of life, as man depends on them for food, medicine, shelter, trade, and commerce. Among the plants, tigernut (*Cyperus esculentus* L.) belongs to the group that can offer man food and other beneficial services. It is a small root tuber of a nutsedge plant which has been cultivated widely in the countries of Africa, Asia, and Europe (Boydston and Prosser, 2008; Arranz *et al.* 2006; Yeboah *et al.* 2012) <sup>[10, 7, 28]</sup>. Tigernut tubers have about three varieties that can be easily identified, based on the colour (yellow, brown and black) of their tubers. Yellow and brown colours, are common variety seen in most markets in Nigeria. In Western Africa, the yellow (large or small) variety is cultivated, processed, distributed, and sold in Nigeria, Niger, Mali, and Ghana (Pascual *et al.* 2000) <sup>[22]</sup>. It has been known to be a nutritious crop mainly used to supplement diets because of its richness in iron and calcium content (Ogunka-Nnoka *et al.* 2020) <sup>[20]</sup>.

In food industry, tigernut tuber is used in the production of vegetable oil, flour, confectioneries, food additive, and roasted for snack. Its use is also extended to pharmaceutical/medicinal industry, agro-allied industry, cosmetic, and biofuel industries (Barminas *et al.* 2001; Arafat *et al.* 2009) <sup>[8, 6]</sup>. Currently, the most popular and preferable use of tigernut is in the making of juice, popularly known as tigernut milk in Nigeria. This juicy beverage is comparatively less expensive than dairy milk and highly preferable by lactose intolerant individuals. Because of the inexpensive cost of tigernut tubers, many low income earners have resulted to processing the tubers to tigernut juice in commercial scale, which is in high demand

in semi and urban cities of Nigeria. Tigernut juice is processed by sorting and washing the tubers, grinding, sieving, pasteurizing, homogenizing, bottling, and cooling (Adejuyitan 2011) <sup>[3]</sup>. During washing and sieving processes, a lot of water is used. Therefore, there is intrinsic suspicion that the water used by the low income earners in processing tigernut tubers into juice, would likely be contaminated with heavy metals. Water is very essential in man's life and play vital roles in cities and communities (Waziri *et al.*, 2018) <sup>[25]</sup>, hence, its importance in tigernut juice production. However, the promise of tigernut juice in food security is suspiciously predisposing man to food risk through heavy metal consumption.

Heavy metal poisoning and risk occur due to accumulation of heavy metal(s) over time in the human body mainly through drinking water. Heavy Metals affect the nervous system, damage the liver and kidney, and even skin and bones (Sankhla *et al.* 2016) <sup>[23]</sup>. Heavy metals in water, refer to the heavy, dense, metallic elements that occur in trace quantities but are highly toxic and tend to accumulate over time. Major sources of heavy metals in water include discharge of industrial wastes, exploration of natural resource activities, acid rain runoff from roads, and others. Due to industrialization, these heavy metals (lead, mercury, arsenic, tin, chromium, nickel, cadmium, and others) have been excessively released into the environment which calls for a global attention (Ngah and Hanafiah, 2007) <sup>[19]</sup> especially in water sources. The leading factor to this, is the quest for technological development and urbanization which have resulted in activities that continually contaminate groundwater and other water bodies (Boateng *et al.*, 2015; Momodu and Anyakora, 2010) <sup>[9, 18]</sup> In Nigeria, studies on

heavy metals in water sources are abundant (Ite *et al.* 2016; Chika and Prince, 2020) [14, 12] while the food risk of tigernut juice in Nigeria was reported by (Maduka and Ire, 2017) [17], but no specific study in relation to heavy metals in tigernut juice which contains about 85% of water has been carried out so far. Therefore, this study is geared towards assessing heavy metals in tigernut juice sold in some parts of Port Harcourt, Nigeria. The study will also assess heavy meals in blow fly maggots which will be cultured with the tigernut juice samples so as to ascertain the food safety of the tigernut juice.

## Materials and Methods

### Study Area

The study was carried out at Trans Amadi, an industrial area with a diverse residential community, in the city of Port Harcourt. Trans Amadi is located at 4°48'53" N latitude and 7°2'14" E longitude, with majority of the industrial workers living within the neighborhood of the industrial area. Trans Amadi stands as a thousand-hectare (2,500-acre) with a diverse residential neighborhood, that supports a strong manufacturing sector and is considered to be a major industrial zone in Port Harcourt. Materials such as glass bottles, tires, aluminum, and papers have production plants in the area (Wikimapia, 2014) [26]. It is the most popular districts in Port Harcourt and the center of the oil business in Nigeria, since it houses the biggest and multinational industries in the country. By June 2003, there are 248 new and completed residential units existing with previous estimates of the total number of dwellings in the neighborhood (All Africa.com 2003) [4].

Port Harcourt as the capital and largest city in Rivers State, Nigeria, lies along the Bonny River and is situated in the Niger Delta. In 2016, the Port Harcourt urban area had an estimated population of 1,865,000 inhabitants against the 1,382,592 of 2006 (Macrotrends.net, 2021; Britannica.com 2022) [16, 11]. The population of the city area of Port Harcourt is almost twice its urban area population with a 2021 United Nations estimate of 3,171,076 (worldpopulationreview.com, 2021) [27]. The main city of Port Harcourt is the Port Harcourt City in the Port Harcourt local government area, consisting of the former European quarters now called Old GRA and New Layout areas. The urban area (Port Harcourt metropolis), on the other hand, is made up of the local government area itself and parts of Obio-Akpor an Eleme accordingly. The Greater Port Harcourt region, spans into eight local government areas that include Port Harcourt, Okrika, Obio-Akpor, Ikwerre, Oyigbo, Ogu-Bolo, Etche and Eleme.

### Tigernut Juice Sample Collection and Fresh Tigernut Preparation

Fresh Tigernut juice samples were bought once in the months of May, June and July, 2021 from local vendors at Trans Amadi area, denoted as TAS, and from one of the biggest shopping mall, denoted as MS, in (Rumudara) Port Harcourt City. Another sample was processed in the Laboratory of Animal and Environmental Biology, University of Port Harcourt, Nigeria as a control, denoted as CS. The control sample was processed using fresh tigernut tubers bought at Choba mini market close to University of Port Harcourt, in the month of May. The processing method described by Belew and Abodunrin (2008) was adopted. The tigernut tubers were carefully selected to get rid of any stone, spoilt tubers and other debris and washed thoroughly to remove sands on the tubers. About 1 kg of the tubers,

were grinded manually with 2 litres of distilled water until it was finely slurred. It was filtered afterwards with a muslin cloth by applying a gentle pressure to hasten the required liquid extraction. The filtrate was allowed to stay for about an hour to settle out the starch fraction with subsequent decantation of the top liquid portion. The concentrated liquid was put in a sterile bottle and refrigerated.

### Assessment of Heavy Metals in the Tigernut Juice and Entomotoxicology

The tigernut samples (TAS, MS, and CS) were filtered separately with a filter funnel into a 250 ml sterile screw bottles and labelled accordingly. They were transported in an ice-packed cooler to preserve the shelf-life of the samples to Project Development Institute (PRODA), Enugu, Nigeria for heavy metal analysis. The labelled samples were subjected to atomic absorption spectrophotometer (AAS) according to the method of (APHA 1995) [5], for heavy metals (Arsenic, Cadmium, Chromium, Copper, Cobalt, Lead, Mercury and Nickel) analysis.

After the AAS analysis of the tigernut juice samples, third generation of blow fly maggots, cultured on cow liver in a mosquito cage were used for toxicological assessment to validate the heavy metals that were positive in the tigernut samples. About 100 ml of the tigernut juice samples that were positive for heavy metals, were ground with a 50 g of cow liver to form a feed source for blow fly maggots. About 50 second instars of blow fly maggots from the maggot culture, were introduced into the ground feed source. After feeding on the source for 4 days, about 1 g of the fed maggots were collected, washed with a distilled water and digested in a fume cupboard at the Biochemistry Laboratory of the Department of Biochemistry, University of Port Harcourt, Nigeria. The method described by Abajue and Ewuim (2020) [2] was adopted to process the maggot samples from the ground feed source for heavy metal assessment with AAS.

### Results

Out of the 8 heavy metals assessed in the tigernut juice samples, nickel and arsenic meals were completely absent in all the samples irrespective of the sampling locations (Trans Amadi, Shopping Mall, and Control). At Trans Amadi, cadmium, chromium, copper, lead, mercury, and cobalt were all present in the tigernut juice samples. But at the shopping mall and in the control, cobalt was the only metal present in the tigernut juice samples.

Comparatively with permissible limits of national and international standard organizations (World Health Organization (WHO), European Union (EU), Nigerian Industrial Standards (NIS), National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON)), as shown in (Table 1) revealed that the quantity of the metals in milligram per litre vary in the tigernut juice samples. Thus, at Trans Amadi (TAS), cadmium (0.001±0.000 mg/l), chromium (0.012±0.007 mg/l), copper (0.003±0.007 mg/l), lead (0.002±0.007 mg/l), mercury (0.002±0.007 mg/l), and cobalt (0.012±0.000 mg/l) were found in the tigernut juice samples at different quantities. Among these heavy metals, mercury was higher than the permissible limits of WHO and SON (0.001) respectively while cobalt was higher than the permissible limit of WHO (0.004) only. Hence, the remaining four metals (cadmium, chromium, copper, and lead) assessed in the tigernut juice sample, were lower than the permissible limits of all the standard organizations.

**Table 1:** Heavy metals contained in the tiger nut juice sold at Port Harcourt, Nigeria and permissible limits of some local and international regulatory bodies

Metals	Locations			Regulatory Organizations				
	TAS	MS	CS	WHO	EU	NIS	NAFDAC	SON
Cd	0.001±0.000	0.000±0.000	0.000±0.000	0.003	0.005	0.003	0.005	0.003
Cr	0.012±0.007	0.000±0.000	0.000±0.000	0.050	0.050	0.050	0.100	-
Cu	0.003±0.007	0.000±0.000	0.000±0.000	2.000	2.000	1.000	-	1.000
Ni	0.000±0.000	0.000±0.000	0.000±0.000	0.010	0.002	0.020	-	-
As	0.000±0.000	0.000±0.000	0.000±0.000	0.010	-	-	0.005	-
Pb	0.002±0.007	0.000±0.000	0.000±0.000	0.010	0.010	0.010	0.015	0.010
Hg	0.002±0.007*	0.000±0.000	0.000±0.000	0.001	-	-	0.050	0.001
Co	0.012±0.000*	0.001±0.007	0.004±0.002	0.004	-	-	-	-

**Note:** Cd = cadmium, Cr = chromium, Cu = copper, Ni = nickel, As = arsenic, Pb = lead, Hg = mercury, Co = cobalt, TAS = Trans Amadi sample, MS = super market sample, CS = control sample, WHO = World Health Organization, EU = European Union, NIS = Nigerian Industrial Standard, NAFDAC = National Agency for Food and Drug Administration and Control, SON = Standard Organization of Nigeria, \* = metal that exceeds permissible limit(s)

From the shopping mall (MS) at Rumudara, all the metals were absent in the tigernut juice samples except cobalt (0.001±0.007), which was lower than all the standard permissible limits. Similarly, from the control samples (MS), all the metals were absent in the tigernut juice samples except cobalt (0.004±0.002), which was lower than all the standard permissible limits but equivalent to WHO permissible standard.

For entomotoxicology analysis, it showed that cadmium, chromium, copper, lead, mercury, and cobalt which were positive in the tigernut samples were negative on the blow fly maggots that were fed with ground cow liver and the heavy metal contaminated tigernut juice.

**Discussion**

Heavy metals may directly or indirectly get into man’s body system and would cause health challenges, as bioaccumulation level increases over time. It is likely that the heavy metals which were assessed, got into the tigernut juice through the water used in the processing and production. It would be linked to the environment where the processing took place, which was a major predisposing factor. The evidence was shown, as 6 (cadmium, chromium, copper, lead, mercury, and cobalt) out of the 8 heavy metals, were positive in the tigernut juice samples.

The tigernut juice sample from a shopping mall (MS), was positive for cobalt only but was below the permissible limit of WHO which suggests its high level of food safety. However, Dogara *et al.* (2021) [13] reported a cobalt in drinking water at Kaduna, Nigeria which was slightly higher than WHO permissible limit. The presence of cobalt in the MS tigernut sample, may be attributed to the tigernut tuber itself and not from the water used in the processing of the juice. This is in agreement with the report of Ogunka-Nnoka *et al.* (2020) [20], that cobalt was already present in tigernut tubers prior to processing. Similarly, tigernut juice for control sample (CS), was also positive for cobalt. Again, it was lower than the permissible limits of WHO, NIS, SON, EU and NAFDAC. The presence of cobalt in the tigernut juice sample for CS, may also be attributed to its presence in the tigernut tubers according to Ogunka-Nnoka *et al.* (2020) [20].

Cadmium, chromium, copper, lead, mercury, and cobalt were found in the tigernut juice sample at Trans Amadi (TAS) but only mercury was slightly higher than the WHO and SON standards respectively. This report contradicts Ite *et al.* (2016) [14] that mercury in the groundwater at Okrika

Rivers State, Nigeria was below the permissible limits of regulatory organizations. The contradiction, may be because of the industrial activities at TAS which predisposed the groundwater for mercury contamination against the pristine community at Okrika. Mercury is a non-essential metal and therefore, is biologically needless at any quantity to plants and animals. Its source to aquatic environment, is from atmospheric depositions mainly through rainfall, snow and dry particles based on 2014 National Emissions Inventory (Leclerc *et al.* 2019) [15]. The industrial activity at TAS with many production plants and direct discharge of industrial wastes within the area, would be the reason for mercury contamination of groundwater which the tigernut tuber vendors used in processing the tigernut juice. Other heavy metals (cadmium, chromium, copper, and lead) that were positive in the tigernut juice, were lower than the permissible limit of the regulatory organizations. The finding concurs with the reports of Ogunlaja *et al.* (2019) [21], that these heavy metals in drinking water at Lagos, Nigeria were lower than permissible limits of regulatory organizations.

The tigernut juice sold in some parts of Port Harcourt, Nigeria pose no food risk to consumers as correlated to the finding of entomotoxicological assessment. Entomotoxicology is a practice of using insects, as specimen for toxicological assessment. The 6 heavy metals (cadmium, chromium, copper, lead, mercury, and cobalt) that were positive in the tigernut samples, were negative on the blow fly maggots which were fed with cow liver, ground with heavy metal contaminated tigernut juice samples. The observation was similar to Abajue and Ewuim (2018) [1] that the growth rate of blow fly (maggots), that fed on a zinc contaminated carcass was not affected. There is no biochemical explanation of how the maggots metabolized the heavy metals, beyond detection with AAS. However, we assume that because the heavy metals were in traces in the tigernut juice samples, hence they were not able to diffuse completely in the cow liver or probably, that the maggots metabolized them with specific enzymes which would have been rid of their body, during the larval instar’s shedding of skin (moulting).

## Conclusion

An entomotoxicological assessment of tigernut juice samples sold in some parts of Port Harcourt, Nigeria, revealed that no serious health threat is inherent, especially the ones that are hygienically processed with good drinking water. Its food safety, is still an ephemeral appreciation that no food risk is associated with it, provided that the source of water used in processing it is known. However, food risk evaluation with a higher animal exposure to heavy metals-contaminated tigernut juice, would be necessary for its food safety confirmation.

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