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Intestinal Parasites Associated with Tiger Nuts, *Cyperus esculentus* L. in Calabar, Nigeria: Implications for Public Health

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Original Research Article

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Abstract: Plant products usually eaten raw such as fruits and vegetables are known to play a role in transmission of fatal parasitic infections. In Nigeria, *Cyperus esculentus* is highly valued for its nutritional and medicinal value, including perceived aphrodisiac properties; but the sub-hygienic attitudes over its handling, preparation and consumption have aroused public health concern. This study aimed at determining parasitic contamination of tiger nuts as sold in the city of Calabar. 800 grams of dry and fresh tiger nuts purchased from four popular markets in the city were washed and subjected to parasitological analysis and examination using Formal ether sedimentation technique. Data were analysed using the SPSS. The Chi-square ($\chi 2$) statistics was used to determine association between parasite contamination and market source; and nut washing frequency. Parasitic organisms isolated were *Ascaris lumbricoides* (ova), 34%; *Trichuris trichiura* (ova), 9%; *Strongyloides stercoralis* (larva), 9%; and *Entamoeba histolytica* (cyst), 25%. Parasite eggs were the highest occurring parasitic forms (54%), followed by cyst (25%) and larva (21%). Nuts obtained from Ikot Ishie and Watt markets were the most contaminated (27.3% for each). Parasite prevalence had no association with market location ($\chi 2 = 11.467$; P-Value = 0.489). Fresh nuts were more contaminated (54.5%). This was statistically significant ($\chi 2 = 8.000$; P-Value = 0.238). Parasites were recovered most during the first wash of both dry and fresh nuts, with dry nuts yielding more parasites at first wash (70%); however, there was no association between parasite recovery and washing frequency ($\chi 2 = 2.000$; P-Value = 0.157). This study has identified contamination of tiger nuts sold in Calabar with parasites of public health importance. With increasing demand for tiger nut and its products, mass health education and monitoring on agricultural practices would help prevent imminent food-borne epidemics in our communities.

Keywords: Intestinal Parasites, Contamination, *Cyperus esculentus*, Food hygiene, Calabar.

INTRODUCTION

Parasitic infections are generally acquired either by active transmission by vectors, skin penetration or via the oral route with food and water contamination. Helminths, comprising cestodes, nematodes and trematodes, and a variety of protozoans are among the common food-borne parasites [1]. Food borne diseases constitute a growing public health problem globally [2]. Plant products usually eaten raw, such as fruits and vegetables, have been shown as sources of transmission for certain parasitic infections, owing principally to unhygienic handling, such as improper washing; and poor preparation, such as undercooking [3-8]. These include root vegetables and tubers like *Daucus carota* (carrot) [9], and *Cyperus esculentus* (Tiger nut), [10]. These food items are mostly contaminated at source due to methods and practice of cultivation, e.g. use of human or livestock waste as farm manure or raw untreated waste water for irrigation; unhygienic handling at sales point and exposure to flies or wind action during storage or sales in filthy market or home store surroundings [5,11,8].

Studies in different geographical locations have identified representatives of various classes of parasitic organisms as associated with vegetable contamination. These include Nematoda: *Ascaris lumbricoides, hookworm, Strongyloides stercoralis; Tricuris trichiura, Toxocara spp, Enterobius vermicularis;* Cestoda*: Taenia spp, Hymenolepis spp;* Trematoda*: Fasciola spp;* Protozoa*: Girdia intestinalis, Cryptosporidium spp* [12, 13, 9].

Cyperus esculentus is an annual or perennial tuber crop of the sedge family (Cyperaceae), also called tiger nut, chufa sedge, nut grass, yellow nutsedge, earth almond; found in the wild as weed or crop and cultivated for its edible tubers in many countries of the world [14, 15]. The edible tubers are 1-2 cm in diameter and vary in colour between yellow, brown and black.

Tiger nuts are slightly sweet with nutty flavor; consumed raw, roasted, dried or processed into a drink, popular as tiger nut milk in Nigeria (*Aya* in Hausa, *Ofio* in Yoruba and *Akihausa* in Igbo) or prepared as flour or oil [16-18].

Tiger nut is of high nutritional and medicinal value, informing the widely relishing consumption in various countries. It is reported to be rich in energy content, minerals, especially phosphorus and potassium, and vitamins E and C. It is said to be helpful in the prevention of heart disease and thrombosis and reduction in the risk of colon cancer; as well as its perceived aphrodisiac properties [19, 20, 10].

In Nigeria, tiger nut is principally sold and consumed dried or previously water-soaked, in public places including markets and motor parks, or hawked around virtually every cranny of major towns and communities. They are mostly exposed in wheel barrows and retailed in cellophane bags to buyers, most of who would consume without washing. The sub-hygienic handling of these highly relished nuts in Calabar aroused public health concern that prompted this study, which is aimed at determining parasitic contamination of the product as sold in the city.

METHODS

Study Site and Sample Collection

The study was conducted at the Biology laboratory of the Cross River University of Technology, Calabar, Nigeria. Calabar, a global tourists' destination in Southern, Nigeria, has been described elsewhere [21]. Tiger nuts were purchased from four popular markets in the city of Calabar, namely; Watt market, Ikot Ishie market, Marian market and CRUTECH Gate market. Two forms of the nuts were identified; dry nuts and 'fresh' nuts. The fresh forms are dry nuts soaked in water over a period of 24 to 48 hours to absorb water and appear farm-fresh. At each site, 100 grams of dry nuts and 100 grams of fresh nuts were obtained from different sellers. Hence a total of 800 grams of nuts were collected in clean cellophane bags and appropriately labeled against the respective sampling site, before taking to the laboratory.

Sample Analysis

100g of dry nuts were washed in 150ml of distilled water in a sterile plastic bottle by shaking the bottle intensely. The suspension was sieved and filtrate collected in a sterile beaker. Three washes were conducted to produce 3 sets of filtrate labeled as w1, w2 & w3, for each set of 100g dry nut from all four locations. The process was repeated with each set of 100g fresh nut samples from the four locations. Filtrates produced from the 3 washes of each set of samples were used for parasitological analysis and examination using Formal ether sedimentation technique [22, 10, 23].

Data Analysis

Data collected were analysed using the SPSS. The Chi-square ($\chi 2$) analysis was used to determine association between parasite contamination rate and source market of nuts; and rate of washing of nuts, at $p < 0.05$.

RESULTS

Laboratory investigations showed parasitic contamination of tiger nuts examined. A total of 44 parasitic organisms made up of 4 genera were isolated from 800 grams of nuts. Parasitic organisms isolated and identified occurred as ova, larva and cysts of helminths and protozoan, including *Ascaris lumbricoides* (ova), 34%; *Trichuris trichiura* (ova), 9%; *Strongyloides stercoralis* (larva), 9%; and *Entamoeba histolytica* (cyst), 11%, (Table 2).

The overall parasite prevalence showed *Ascaris lumbricoides* as the most occurring (34%); but helminths were generally more than protozoans, at 75% and 25%, respectively. Parasite eggs were the highest occurring parasitic forms (54%), followed by cyst (25%) and larva (21%).

Tiger nuts obtained from Ikot Ishie and Watt markets were the most contaminated (27.3% for each), while those from CRUTECH gate market had the least contamination (20.4%). Statistical analysis showed no association between parasite prevalence and source market location ($\chi 2 = 11.467$; P-Value = 0.489). Fresh tiger nuts were more contaminated, (54.5%), than dry nuts, (Table 1). This was statistically significant ($\chi 2 = 8.000$; P-Value = 0.238).

Parasites were recovered most during the first wash of both dry and fresh nuts, (Table 3). However, dry nuts yielded more parasites at first wash (70%) than fresh nuts (45.8%), (Figure 1). Parasite recovery from nuts was shown to be statistically not associated with frequency of wash ($\chi 2 = 2.000$; P-Value = 0.157).

Table-1: Parasite load on two forms of commercially available Tiger nuts in Calabar

Tiger nuts (100g)	Sampling Site (Markets)				Total (%)
	Marian	Ikot Ishie	Watt	CRUTECH Gate	
Dry nuts	5	4	6	5	20 (45.5)
Fresh Nuts	6	8	6	4	24 (54.5)
Total (%)	11	12	12	9	44

Table-2: Parasites recovered from Tiger nuts from markets in Calabar

Parasites	Sampling Site (Markets)				Total (%)
	Marian(%)	Ikot Ishie (%)	Watt(%)	CRUTECH Gate (%)	
Ascaris lumbricoides (Ova)	2 (18.2)	3 (25.0)	6 (50.0)	4 (44.4)	15(34.0)
Trichuris trichiura (Ova)	2 (18.2)	3 (25.0)	2 (16.7)	2 (22.2)	9 (20.5)
Strongyloides stercoralis(Larva)	3 (27.3)	3 (25.0)	1 (8.3)	2 (22.2)	9 (20.5)
Entamoeba histolytica (Cyst)	4 (36.3)	3 (25.0)	3 (25.0)	1 (11.1)	11 (25.0)
Total	11 (25.0)	12 (27.3)	12(27.3)	9 (20.4)	44

Table-3: Parasite recovery at various washes of Tiger nuts from different markets

Tiger nuts (100g)	Frequency of wash (% parasite recovery)			Total (%)
	w1	w2	w3	
Dry nuts	14 (70.0)	5 (25.0)	1(5.0)	20 (45.5)
Fresh Nuts	11 (45.8)	10 (41.6)	3 (12.5)	24 (54.5)
Total (%)	25 (56.8)	15 (34.1)	4 (9.1)	44

N/B: w1-w3 = 1st to 3rd wash

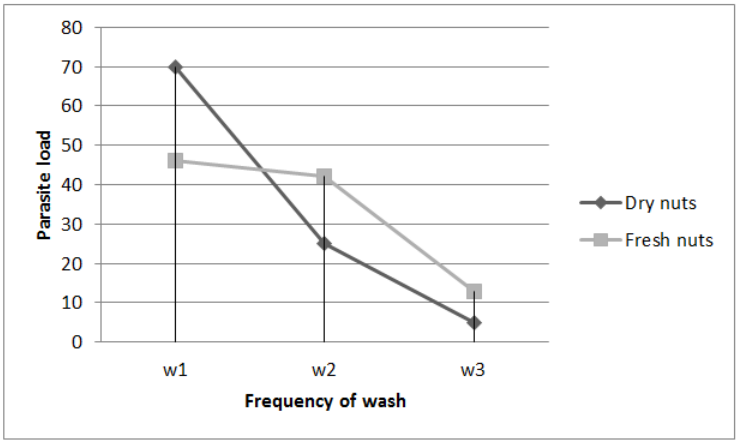

Fig-1: Comparison of parasite recovery from dry and fresh nuts at different washing frequency

DISCUSSION

Studies focused on parasitic contamination of fruits and vegetables usually eaten raw have been extensive, a few of which have been noted earlier in this work. However, literature on parasites associated with the contamination of *Cyperus esculentus* as a root vegetable is scarce. *Daucus carota* has been the commonly studied root vegetable in this regard in Nigeria [11, 24, 23]; while such information on Tiger nuts, which is equally as widely consumed as Carrot, is not available. This study is perhaps the first to report parasitic contamination of tiger nuts in Nigeria.

Within the limits of the designed protocol, this study has recorded parasitic contamination of tiger nuts sold in the city of Calabar. The parasites identified included nematodes and a protozoan, all of which human infection is linked to faecal contamination; and, except *Strongyloides stercoralis*, follow the faeco-oral route of transmission and are also associated with gastrointestinal infections [25, 26].

Findings from this study are consistent with results from a study in Ghana, a West African country, where tiger nuts are similarly extensively consumed; positing that tiger nuts could be a source of intestinal parasites infection [10]. However, of the four genera of parasites identified in this study (*Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, and *Entamoeba histolytica*), *only Strongyloides stercoralis* was common to both studies. The occurrence of soil-transmitted, intestinal nematodes and protozoan; *Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Entamoeba histolytica*, on fruits and root vegetables in the city of Calabar has been reported [11]. These parasites are implicated in common human intestinal infections with fatal pathological manifestations and mortality. The clinical features and pathology of ascariasis, trichuriasis, strongyloidiasis and intestinal

amoebiasis, which are infections associated with the isolated parasites, have been discussed elsewhere [25].

Based on established knowledge of the life cycle and transmission of the identified parasites [27], they have no metabolic relationship with the root vegetable; hence their occurrence on tiger nuts is purely a case of contamination. Various widely adduced factors responsible for parasitic contamination of fruits and vegetables include contamination with soil during harvest, use of contaminated water for irrigation, contaminated manure, runoff from livestock operations, unhygienic handling and poor personal hygiene level of sellers, etc. [28,29,10,23]. The sources of contamination of tiger nuts studied are certainly related to factors spanning from the farm, through transit to storage, preliminary processing and handling of the product for sale. As a tuber, tiger nuts in the farm are in constant direct contact with soil which might have been irrigated with faeces-contaminated water, or experienced runoff from faeces-polluted fields. Contamination with these helminth and protozoan forms could arise at the point of harvest. In an instance of improper post-harvest washing, the resistant stages like cysts and ova would persist on the tubers (nuts) through transit to storage and to the point of sales. The high prevalence of *Ascaris lumbricoides* ova (34%) on the studied nuts might be due to the resistant property of the eggs, which can remain infective for 3 years under appropriate conditions [25].

In Calabar markets tiger nuts are usually displayed and exposed in wheel barrows, either stationed in the open market or moved around the city by hawkers. During sampling, the points of sale in the open markets were characterized by proximity to solid waste dumps and clogged drainages. This could be a source of contamination of the nuts with cysts and ova from flies [11]. Both dry nuts and fresh nuts are so exposed for sale. The study showed fresh nuts bearing more parasites (54.5%). This was statistically significant; and it must be related to the pre-market processing practice by the sellers, who soak dry tiger nuts for about 24 to 48 hours to make it 'fresh'; and it is believed that the extent of freshness depends on the duration of soaking, the longer the period, the better. To keep the nuts constantly fresh, the sellers would sprinkle water over them intermittently while waiting for buyers. The quality of water used for soaking and sprinkling; and the duration of soaking obviously make the fresh tiger nuts more prone to contamination. This processing method could also cause cysts and eggs to stick firmly to the nuts as against what obtains with dry nuts. The results show that more parasites persisted on fresh nuts after the 3rd wash (Figure 1).

Washing of tiger nuts with distilled water could only dislodge parasites gradually. This was noticeable on the different forms of nuts. At the first wash, 70% of parasites found on the dry nuts were dislodged, this only reduced to 5% by the third wash. For the fresh nuts, 45.8% of the parasites could be washed off at the first wash, 41.6% by the second wash, and 12.5% of the parasites still persisted to be dislodged only by the 3rd wash (Table 3). The impact of washing on the whole was the recovery of 56.8% of parasites at first wash, 34.1% at second wash and 9.1% at third wash, (Table 3), showing that washing with water would not completely remove infective stages of parasites from the nuts, except it is done more than 3 times. Similar results were previously reported with leafy and root vegetables sold in Calabar [11]. Although there was statistically no association between rate of parasite recovery and frequency of wash, these findings pose implicating public health questions in a city where sellers do not wash the tiger nuts properly and most buyers oftentimes consume them directly after purchase without washing. These realities would cast uncertainties over the safety of Tiger nut milk, a purely water-based filtrate of blended tiger nuts, which is currently gaining prominence and heavy patronage amongst people in the city, especially owing to its perceived efficacy as an aphrodisiac.

CONCLUSION

The occurrence of helminth and protozoan parasites on tiger nuts studied was certainly due to superficial contamination most probably due to unhygienic handling by sellers. This can be controlled; and accordingly, the eventual health hazard due to contamination can also be prevented. Such measures of control and prevention would depend on conscious adjustment in the knowledge, attitude and practice of handling and processing of tiger nuts, post-harvest, during sales and before consumption. This study has shown that washing of nuts more than 3 times would eliminate parasitic contaminants. Proper pre-sales washing with potable water by sellers is recommended; and after-purchase washing by buyers is advised in order to protect the consumer. Washing with salt water has been suggested as a good decontamination measure [10]. With the increasing demand for tiger nut and its products, and the public health concern raised by this study, appropriate government authorities should begin to engage in mass health education and monitoring on agricultural practices as it concerns irrigation, nutrient enrichment of vegetable fields and food handling practices, especially for ready-to-eat plant products.

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