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Selected heavy metals content and microbial quality in locally processed Locust beans samples retailed In Ibadan, Nigeria

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ABSTRACT

The influence of residual contaminant levels in processing water on the total heavy metal contents and microbial loads in fermented locust bean seeds popularly used as traditional spice was investigated. Twenty five domestically-prepared, fresh locust beans products (iru – Yoruba), produced in Ibadan, Nigeria were selected. Random samples of processing water and iru samples directly procured from each of 25 different local processors were respectively analyzed for microbial loads, total lead (Pb), cadmium (Cd), and arsenic (As) contents. Appropriate laboratory microbiological procedures for microbial assay and standard AOAC analytical methods for total heavy metals' contents were used to achieve this. The results showed that statistically significant differences were between the Mean total heavy metals contents (MTHMC) in mg/kg of the untreated water types with order of contamination as; Stream (0.77) > Spring (0.50) > Well 0.39) > Borehole (0.27). The MTHMC value in mg/kg for borehole water processed locust beans samples was a minimum of 0.14±0.04; and a maximum of 0.213±0.04 for stream water processed iru samples. The results of microbial analysis showed that ranges for TPC, TVC and TFC were respectively 7.43-7.92 log10cfu/g, 5.00-6.54 log10cfu/g and 6.56-6.91 log10cfu/g. The positive influence of processing water-purity on levels of heavy metals' contaminants in the final products was confirmed. The use of locust beans as a seasoning, often in little quantities suggests contaminants may not be hazardous to consumers on the long term.

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1. Introduction

Untreated water sources and harvested crops have often been consistently reported in several research reports to be possibly contaminated with toxic chemical contaminants, generally anthropogenic or industrial in origin, among which are toxic heavy metals, organohalide compounds, hydrocarbons and inorganic salts (1, 2). Toxic heavy metals especially Pb, Cd, Ni, As have been shown to readily contaminate all food crops and foodstuffs either by process of bioaccumulation during plant growth, or during food processing (3). The toxic heavy metals are extremely disruptive to living, with consequent neurotoxic, carcinogenic, mutagenic or teratogenic effects on critically important body organs and tissues (4).

The above trend has been confirmed in Nigeria too, as significant and widespread contamination of raw cooked and even processed dietary foodstuffs by toxic chemical and microbiological substances of all variety have been consistently extensively reported (5). Such reports confirm the susceptibility of virtually all local foodstuffs such as fish, meat, cereals, legumes,

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vegetables and fruits, cooked or not, to contamination (6). Spices and food condiments have been reported not to be exceptions to all possible kinds of contamination (7).

The fermented form of locust beans called "Iru" in Yoruba language is a popular, highly proteinous, and nutrients-rich culinary spice. Iru is processed at the domestic level from raw, dry seeds of the Parkia biglobosa tree through several steps that involved extensive use of water (8). Iru, widely consumed, is often added in small quantities to pepper stews, vegetable and draw soups in their preparation stages to add aroma and taste. Iru condiment is readily available in all the open food markets in most cities, towns and villages in Nigeria and is generally retailed by elderly women who often sell them in small leaf wrappings from wide calabash basins.

While microbial contamination of iru spices has been widely investigated and reported by (9, 10), there is no current report on possible toxic heavy metals' contamination of iru. Research findings showed that water samples used for "iru" processing were mostly from some untreated surface water sources (11, 12). This observation therefore strongly necessitates the need to investigate possible metallic contamination .Iru might be a potential dietary source of toxic heavy metals and needs to be investigated.

2. Materials and methods

2.1. Apparatus and Reagents

The apparatus required were: 50ml plastic bowls with lids, spatulas, porcelain crucibles, 100ml volumetric flasks, Whatman No. 595 filter paper, sample bottles, cuvettes. Aqua regia prepared by a mixture of ANALAR grade; 65% HNO3 and 37% HCl (ratio of 1:1), was used to soak all the glassware and porcelain crucibles were soaked overnight. They were then rinsed withdeionized water, and were air-dried for 12 hours prior to usage. The equipment and instruments used included; oven, muffle furnace and Flame AAS (Perkin Elmer Analyst 100).

2.2. Sampling procedure

The study which was limited in scope and preliminary in nature, analyzed twenty five randomly procured iru samples and their corresponding processing water samples for the most common of the toxic heavy metals which are lead (Pb) Cadmium (Cd), and Arsenic (As). Freshly prepared fermented iru-woro samples (20 grams), and the corresponding processing water samples were procured directly each of the twenty five different producers resident in from locations in Ibadan city, SW Nigeria. Each of these iru samples was transferred into each of twenty five (25) different (20ml) plastic sample bowls which were subsequently tightly covered by replacing their lids immediately. Each sample bowl was properly labeled for proper identification purposes and stored in a deep freezer. In addition, a stainless steel dipper was used to collect water samples from the sources of water used by iru producers for processing the dry raw seeds to finished iru products. Preliminary investigations revealed that these water sources consisted seven (7) streams, five (5) springs, eight (8) wells, and five (5) boreholes.

2.3. Chemical Analysis

2.3.1. Sample preparation for heavy metals analysis

The first step here involved the oven-drying of 10 grams all the twenty five iru- woro samples in porcelain crucibles at a temperature of 150^{o}C for two hours. Each of the oven-dried iru and seed samples were put in a muffle furnace maintained at a temperature of 350^{o}C for eight hours for it to ash, after which all the sample ashes were left to cool overnight.

2.3.2. Digestion of samples

All cool ashes were transferred into different 100ml volumetric flasks, and were each digested with 1.5ml of concentrated ANALAR grade 65% HNO3, after which 30ml of de-ionized water was added to each flask to dilute the digests. Filtration of each of the digested samples was achieved using Whatman No. 595 filter paper and collection of the filtrates in well washed, dry sample bottles. This procedure was likewise repeated for 10ml of the raw water samples that were collected from the producers' water sources.

2.3.3. Digestion of samples

Initial background correction for the analytical instrument was done with previously prepared sample blanks. These blanks were prepared just the experimental sample digests in the manner described above. Total Pb, Cd, and As contents in all the iru digests were measured by passing each of fifty digests through an air/acetylene Flame AAS (Perkin Elmer A Analyst 100); which has been calibrated with standard solutions for Pb, Cd and As (100 ppm). The reading for each test sample was taken thrice and was expressed in mg/kg wet weight.

2.4. Microbiological Analysis

This analysis was carried out on the freshly prepared fermented iru samples. The stages involved are described as follows;

a) Culture of freshly fermented iru samples

A small portion of about 2 grams of each freshly fermented twenty five iru samples were serially diluted in sterile distilled water and appropriate dilutions were plated on sterile media cultures. Potato dextrose agar (PDA) was used for isolation of fungi, Nutrient agar was used for total viable count and MacConkey Agar was used for isolation of coliform bacteria. The culture plates for isolation of bacteria were incubated for twenty four hours while those for fungal count were incubated for 72 hours at ambient temperature (30^{o}C).

b) Identification of microorganisms

The microorganisms isolated were purified by repeated streaking on solid sterile media mentioned above. Then the bacterial isolates were subjected to physiological and biochemical tests in addition to their cultural characteristics. The tests included sugar fermentation, catalase test, oxidase test, coagulase test, urease test etc. The identification of the bacterial isolates was confirmed by reference to Bergey's Manual of Systematic Bacteriology, (7th Edition) as edited by Nathan et al.,(1957) (19). The fungal isolates were identified by their cultural characteristics on solid media and morphological characteristics by lactophenol blue staining with reference to Biology of Soil Fungi. Additional tests like formation of ascospores and fruiting body, and sugar fermentation were performed which aided the identification of the fungal isolates.

2.5. Statistical Analysis

Student's t- tests were used to test if there were any significant differences between the results obtained for the bacterial isolates of each of the iru samples under consideration.

3. Results

From Table1, the streams which are surface based and flowing by in the neighborhood is relatively the most contaminated with heavy metals. The Mean Total Heavy Metal Contents± Standard deviation (MTHMC± SD) levels in mg/dL are in the order of stream (0.77±0.13) > springs (0.50±0.16) > Wells (0.39±0.11) > Boreholes (0.27± 0.12).

At the levels of $p < 0.05$, these MTHMC values for the iru samples were significantly different from each other suggesting a strong influence of source of water used by the processors on the contaminants' profile of the iru samples investigated. The summary of each of the MTHMC±SD values for the analyzed water and iru samples (see Tables 1 and 2) is shown in Table 3.

Regression analysis carried out on the relationship of MTHMC values of the processing water types and iru samples showed correlation coefficient r + 0. 47, that suggests that the contaminants in the untreated water sources obviously influenced the total heavy metal burdens in the analyzed iru samples.

The results of microbial analysis are presented in table 4 and this indicated that the total viable counts ranged between 7.43 and 7.92 log10cfu/g while that of coliform count ranged between 5.00 and 6.54 log10cfu/g. The fungal count ranged between 6.56 and 6.91 log10cfu/g. Students t-tests at the level of $p < 0.05$ showed that differences in processing water sources did not influence the final microbial load on the iru samples under investigation.

4. Discussion

As expected from previous findings (6), and from results presented in Tables 1 and 2 above, Lead (Pb) is the most predominant heavy metal contaminant in all the experimental samples. This is traceable to its extensive usage in industrial manufacturing processes and careless disposal strategies (13).These levels far exceed the permissible limits of these metals in processed foods/spices which are; 0.05 for lead, 0.02 for cadmium and 0.01 for arsenic (14). But because very little quantities of iru, usually about 20 grams and vary slightly, suggesting a not too significant contribution to the heavy metals' burden in the body tissues, when

Table 1. Mean ± Standard deviation (M±SD) for Lead (Pb), Arsenic (As), and Cadmium (Cd) contents (mg/L) in processing water used by producers

Sources (No)	Lead (Pb)	Arsenic (As)	Cadmium (Cd)	MTHMC±SD
Streams (4)	1.52± 0.03	0.36±0.11	0.48±0.05	0.77±0.13
Springs (3)	0.84± 0.05	0.29±0.04	0.38±0.07	0.50±0.16
Wells (15)	0.63±0.12	0.31±0.07	0.23±0.08	0.39±0.11
Boreholes (3)	0.47± 0.09	0.15±0.02	0.18±0.04	0.27±0.12

Table 2. Mean± Standard Deviation (M± SD) of heavy metals (mg/kg) in iru samples according to processing water source used by the iru produce.

Water source	St-WU	Sp-WU	We-WU	Bo-WU
HM				
Pb	0.36±0.04	0.28±0.02	0.28±0.04	0.26±08
As	0.11±0.06	0.07±0.01	0.06±0.03	0.04±02
Cd	0.17±0.02	0.15±0.02	0.09±0.02	0.11±02
Mean±SD	0.21±0.04	0.17±0.02	0.14±0.03	0.14±04

KEY: HM – Heavy metal, MTHMC – Mean Total Heavy Metal Contents. St-WU- Stream water users, Sp-WU- Spring water users, We-WU- Well water users, Bo-WU- Borehole Water, M_o - Overall mean

Table 3. Summary of the (MTHMC±SD) of the different experimental samples (water and iru beans) according to water type used for processing

Sample type	Water	Iru beans
Processing Water source		
Stream	0.77 ± 0.13	0.21± 0.04
Spring	0.50 ± 0.16	0.17± 0.12
Well	0.39 ± 0.11	0.14± 0.03
Borehole	0.27 ± 0.12	0.14± 0.04

Key: MTHMC – Mean Total Heavy Metal Contents, SD – standard deviation

Table 4. Total Microbial Count (TMC) (cfu/g) in the four batches of iru samples according to Water type used for processing

Water Type	St-WU	Sp-WU	We-Wu	Bo-WU
BCT				
TPC	7.43±0.01	7.92±0.00	7.69±0.02	7.73±0.04
TCC	5.64±0.02	6.46±0.03	5.00±0.01	6.54±0.05
TFC	6.59±0.02	6.91±0.01	6.59±0.02	6.56±0.01

KEY: BCT – Batch Count Type, TPC – TPC- Total plate count; TCC- Total coliform; TFC- Total Fungal Count, St-WU- Stream water users, Sp-WU- Spring water users, We-WU- Well water users, Bo-WU- Borehole Water

compared to other more significant sources like ingested foods and drinks. More so that the quantity often added to a soup or a sauce is subjective and is often determined by personal and socio-cultural choices. Despite that because of the extremely toxic and damaging effects to body systems, any contributor to the total burden of heavy metals in the tissues and organs cannot be ignored, suggesting that variations arose from exposure of the different water sources to pollutants of anthropogenic origin. Surface water sources like streams and springs are relatively more contaminated with the toxic heavy metals as compared to groundwater sources like wells and boreholes.

The bacterial isolates include Bacillus spp; Pseudomonas spp; Flavo bacterium spp, Micrococcus spp: and Staphylococcus spp: The isolated coliform bacteria were Aeromonas spp; Proteus spp and Enterobacter spp. The fungal isolates were Aspergillus spp; Rhizopus spp; Penicillium sp and Candida spp. Ladokun and Adejuwon reported the presence of similar organisms such as Aspergillus niger, Pseudomonas maltophila, Streptococcus faecalis and Aspergilus flavus (15). The authors concluded the presence of these organisms in iru samples indicated the unhygienic nature of iru processing. Several studies have implicated the members of Bacillus sp as the main organisms that carry out iru fermentation (12, 17,18). Just as in these previous studies, post fermentation microbial contamination is indicative of the unhygienic practices and exposure of iru condiments to contaminants from air, calabashes, and wrapping leaves used. This fact was buttressed further by statistical analysis by students't- tests on the mean values, which showed the differences in water purity/quality did not affect the microbial load of the investigated iru samples. It therefore explains why iru will be deteriorating after a few days to the extent of maggot infestation as earlier reported by 10). So iru should not be kept at room temperature because of the possibility of a geometric increase microbial flora population native on exposure for a few days at ambient temperature (16).

4. Conclusion

This study conclusively showed that the use of untreated water sources positively affected the toxic heavy metals' burdens in the experimental iru samples, while unhygienic post production practices were responsible for the observed significant microbial loads. Results however do not conclusively proved that these contaminants pose serious health hazards to habitual iru consumers, hence further studies using appropriate dietary survey instruments are suggested in order to determine the average daily per capita intake of iru.

Conflict of interest

The authors have no conflict of interest.

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