Assessment of Arsenic levels in rice brands sold in Kampala. An experimental study to demonstrate the modifying effect of boiling, soaking and washing

Joshua Nfambi
Makerere University College of Health Sciences

Allan Lugaajju
Makerere University College of Health Sciences

Agnes Namaganda
Makerere University College of Health Sciences

Ester L. Acen
Makerere University College of Health Sciences

Robert Kalyesubula
Makerere University College of Health Sciences

Lawrence F. Sembajwe (larryfeds@gmail.com)
Makerere University College of Health Sciences

Research Article

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Abstract

**Background:** Arsenic is one of the major toxic metals in the environment that is also known to be carcinogenic with several other health side effects in human beings. It can cause both acute and chronic toxicity depending on the doses available or duration of exposure in the human body. The major sources of arsenic exposure to humans include; via contaminated water, food and inhalation of arsenic dust from polluted air from car-fumes or industrial wastes and mining/ smelting grounds. Due to the fact that high levels of arsenic have been reported in rice from major rice-growing countries in Asia and rice being a major staple food in Uganda, some of which is imported, this study aimed at determining the concentration of total arsenic in various rice brands sold in Uganda.

**Methods:** Various rice brands were sampled from different supermarkets and grocery shops, taken to the laboratory and analyzed for arsenic using atomic absorption spectrometry. The arsenic concentration in the rice were determined following boiling or overnight soaking in plenty of water to see if these two approaches can help in significantly reducing the arsenic in the rice. The concentration of arsenic in the various brands of rice were compared using an unpaired t-test after setting a p-value of \( \leq 0.05 \) as significant.

**Results:** The York taste rice brand had the lowest arsenic levels of 1.4±0.0002 ppm and Zhong Yi rice brand had the highest levels of 2.4±0.004 ppm. The tap water used to boil, soak and wash the rice brands had much higher arsenic levels of 3.5±0.000003 ppm, which caused increased retention of the heavy metal in the rice.

**Conclusion:** Rice brands sold in Kampala city seem to have higher than acceptable arsenic levels than what is recommended by WHO or American food and drug authority. The high levels of arsenic in tap water used in preparing the rice causes increased retention of the metal on boiling, soaking and washing. It is therefore, necessary to routinely monitor for arsenic levels in the rice on the market and domestic water sources so as to protect the general public from arsenic toxicity.

Introduction

Arsenic is one of the toxic heavy metals along others that include Mercury (Hg) and Lead (Pb), associated with toxicity via water, human food and animal feeds. Arsenic exists both in organic and inorganic forms, with the later being more toxic than the former \((1, 2)\). This metal is naturally found to be bivalent, either as Ars- III (arsenite) or as Ars-V (arsenate) \((3)\).

Most of the arsenic contamination in the environment is of geological origin from underlying rocks, but there is also a significant contribution from industrial production or pollution \((4)\). Arsenic of industrial origin enters the environment in form of additives to pesticides (or insecticides), herbicides, cosmetics and herbal remedies \((5, 6)\), which end up polluting water bodies \((1, 7)\). The subsequent use of polluted water in irrigation of crops, then introduces arsenic into the food chain leading to potential intoxication of human beings and animals \((8, 9)\). Due to the fact that plants have the potential to absorb and
accumulate arsenic from various soil types or environment, there is always a possibility of finding either some trace amounts or significant amounts of arsenic in the harvested and processed food stuffs (10). Moreover, food crops such as rice have been reported to assimilate and accumulate 10 times as much arsenic as compared to other similar crops (3, 11). Thus, the regulatory bodies such as WHO and the American food and drug authority (FDA), set acceptable levels or limits of arsenic in food or water, not exceeding 50 parts per billion (ppb) / (or 0.05 parts per million [ppm]) or 50µg/l, to prevent toxicity in humans and animals (1, 12).

Arsenic toxicity in human population can occur mainly through direct intake of excessive amounts of this heavy metal as a poison or indirectly through chronic/prolonged consumption of aliquot amounts via food and water over a long period of time (8, 13). This results into either acute or chronic toxicity among the human victims (1). In addition, some people get arsenic exposure and toxicity by inhalation of arsenic fumes from industrial pollution or via direct skin contact with arsenic compounds (13). Acute arsenic toxicity manifests with symptoms including excessive salivation, vomiting, excessive watery diarrhea (bloody diarrhea) and rapid dehydration with consequent cardiovascular collapse (2). Chronic arsenic toxicity on the other hand, manifests with wide-ranging dysfunction and malignancy in several body systems or organs including the heart, liver, kidney, nervous system, urinary bladder and spleen (1, 2).

In order to prevent arsenic toxicity, it is therefore important to know or have an idea about the amount of this heavy metal or its residues deposited in the environment, in the food chain and in water resources. In this study, we therefore, aimed at profiling the arsenic levels in the major rice brands being sold in various grocery shops in Kampala city, as rice is one of the staple foods in urban areas of Uganda. In addition, we also attempted to determine if there is a modifying effect of boiling, soaking or washing on the arsenic concentration in the rice.

**Materials And Methods**

**Study setting:**

This was a laboratory based experimental study for determining Arsenic levels in rice. The various rice brands were consecutively/conveniently sampled from grocery stores around Kampala city and transported to the Nutrition Unit in Department of Medical Physiology Laboratory for boiling, soaking or washing. Then, the prepared rice samples were taken for determining their arsenic concentrations using an Agilent Atomic Absorption spectrometer in the Department of Chemistry at Makerere University.

**Sampling procedure**

The rice samples were picked randomly from selected supermarkets and grocery shops around Kampala and transported to the Department of Medical Physiology Laboratory for processing.
Sample storage:

The collected rice samples were protected from contamination and loss of the undesired metal content during the analysis. After subjecting the rice to boiling, overnight soaking or washing, 25 milliliters of the water used in each case were collected in sterile Falcon conical centrifuge tubes and kept at -20 °C until analysis for arsenic content. The solid rice samples were stored in sterile laboratory sample bags at room temperature (around 25 °C) until preparation for further analysis.

Sample preparation before analysis

Five hundred grams (500g) of each collected rice brand were prepared by subjecting it to either overnight soaking or washing and boiling using tap water for at least 45 minutes until when ready. Fifteen milliliters (15ml) of the water in which each sample is soaked or washed and boiled were stored in sterile falcon containers for further analysis. The 500g of the prepared samples were heated in an oven until dry. The rice samples were homogenized to fine powder using a grinder, while the liquid samples were analyzed for arsenic directly.

Analyzing for Arsenic: All samples were prepared for analysis using a modified ‘wet ashing’ procedure as described by Adeloju, 1989 (14). Briefly, the analysis was done in two experiments or duplicates of samples. For all the solid rice samples, 1.25g of the dried sample (dried in the oven for 24 hours at 103 °C) was weighed and transferred to the destruction tube; 25ml of 65% nitric acid (HNO₃) was then be added with three boiling chips and a funnel placed on top of the destruction tube. The tube was then heated to 100 °C and maintained for 1hour, heat was increased to 125 °C, 150 °C, 175 °C and 200 °C maintaining the temperatures for 15 minutes at each change. The remaining volume was concentrated to 5ml and left to cool. After cooling, 1ml of 30% hydrogen peroxide (H₂O₂) was added and destructed for 10 minutes. This destruction was repeated once. Then after cooling again, 3ml of 30% H₂O₂ was added and destructed for 10minutes. Twenty-five (25ml) of deionized water was then be added, mixed and heated till boiling. The solution was cooled and transferred to a 250ml volumetric flask, filled up to the mark, mixed and left to settle for 15 hours. The absorbance of the supernatant was then measured by Atomic Absorption Spectrometry (AAS) using an Agilent 240AA spectrometer series machine (Agilent Technologies, Santa Clara, California, USA) following standard procedures.

Quality control: All reagents used in this study were of high-quality fitting laboratory standards and all the equipment used in the laboratory procedures were pre-calibrated by highly qualified laboratory-technicians. The study was reviewed and approved by Makerere University, School of Biomedical Sciences Research and Ethics committee (Reference number: SBS-2022-138).

Data management and analysis
All the data collected was cleaned before summarizing in Excel spread sheets. This was followed by data analysis for the variations in the arsenic content between the various brands of rice by performing an unpaired t-test and plotting the relevant graphical presentation of the results using GraphPad prism (version 8) software. For statistical significance, p-values of \( \leq 0.05 \) was considered.

Results

Table 1: Shows a summary of the comparison p-values following an un-paired t-test statistical analysis of the respective rice sample treatment groups
<table>
<thead>
<tr>
<th>Sample</th>
<th>raw SWT Ravi</th>
<th>raw SWT 1</th>
<th>raw Zhong Yi</th>
<th>raw Turkey</th>
<th>raw Numa</th>
<th>raw Kibimba</th>
<th>raw York Taste</th>
<th>raw Ordinary</th>
</tr>
</thead>
<tbody>
<tr>
<td>washed SWT Ravi</td>
<td>0.3333</td>
<td>&gt;0.9999</td>
<td>0.3333</td>
<td>0.3333</td>
<td>&gt;0.9999</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>washed SWT 1</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.6667</td>
<td>0.3333</td>
<td>&gt;0.9999</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>washed Zhong Yi</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>washed Turkey</td>
<td></td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
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<tr>
<td>washed Numa</td>
<td></td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>washed Kibimba</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
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<tr>
<td>washed York Taste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>washed Ordinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>Overnight soaked SWT Ravi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>overnight soaked SWT 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>overnight soaked Zhong Yi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>overnight soaked Turkey</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>overnight soaked Numa</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>overnight soaked Kibimba</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>overnight soaked York Taste</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
<tr>
<td>overnight soaked Ordinary</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.3333</td>
</tr>
</tbody>
</table>
Discussion

Rice being one of the major foods consumed in Uganda, we tried to profile the arsenic levels in various rice brands sold in Kampala city as well as investigating if there was any modifying effect of boiling, overnight soaking and washing of the rice during food preparation. From our findings we observed that the local rice brands such as Zhong Yi (grown in the Lwera wetland near Masaka city) and Kibimba (grown in Bugiri district in Eastern Uganda) had slightly higher levels of arsenic than all the other brands on the market. This might suggest that the soils and water resources in which these particular rice brands are grown already carry or contain a high concentration of arsenic. It might also be an indicator of presence of mining activity in the vicinity or proximity of the agricultural sites, that results into the pollution of the soil and water with higher levels of arsenic than they would contain, if there was no any mining taking place. Indeed, the increased mining of sand in the Lwera wet land that has been previously highlighted (15, 16), may be contributing to the increased release of arsenic into the water used in cultivating of rice in that area. Our results show that all the rice brands included in this study have arsenic levels ranging from an average of 1.4 parts per million (ppm) (for York Taste = Punjabi Indian Rice brand, with long grains) to 2.4 ppm (for Zhong Yi = rice brand Grown and packaged in Lwera-Lukaya, Kalungu district near Masaka city of Uganda) (figure 1, panel A), which are well above the acceptable levels of

<table>
<thead>
<tr>
<th>Boiled Rice Brand</th>
<th>Arsenic Level 1</th>
<th>Arsenic Level 2</th>
<th>Arsenic Level 3</th>
<th>Arsenic Level 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiled SWT1</td>
<td>0.3333</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled SWT ravi</td>
<td>0.3333</td>
<td>0.3333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled Zhong Yi</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td></td>
</tr>
<tr>
<td>Boiled Turkey</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>Boiled Numa</td>
<td>&gt;0.9999</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>Boiled Kibimba</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>Boiled York Taste</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>Boiled Ordinary</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
</tbody>
</table>

N.B: SWT Ravi = rice brand, Milled processed and packaged in Pakistan; SWT1 = Long grain white rice brand, milled processed and packaged in Uganda; Zhong Yi = rice brand Grown and packaged in Lwera-Lukaya, Kalungu district near Masaka city of Uganda; Turkey = An Indian Basmati Rice brand imported to Uganda; Numa = a rice brand, Packed and processed in Kabwohe-Sheema District of Uganda; Kibimba = a rice brand, Grown and Packaged in Kibimba, Bugiri district, along Kampala-Malaba Highway in Uganda; York Taste = Punjabi Indian Rice brand, with long grains; Ordinary = a rice brand, Grown in the swampy areas of Kayunga District of Uganda.
0.05ppm set by the American FDA and WHO as the highest limit of arsenic to be tolerated in food or water (1, 12, 17). This may suggest that continued consumption of these rice brands on a daily basis and for a long time might expose the general population to the toxic effects of arsenic in the body (1). These relatively high arsenic levels in the rice brands sold in Kampala city might even increase over the years in case there is no government intervention to test and constantly monitor the amount of arsenic in the foods sold to the general public.

We attempted to investigate the modifying effect of boiling, overnight soaking and washing of the rice during food preparation as possible methods of reducing arsenic that might be contained in the raw rice brands. We tried as much as possible to mimic the general method used in preparing a rice meal locally by boiling, soaking and washing of the rice in tap water as relied on by majority of the population in Kampala for cooking food. However, to our surprise the tap water seems to contain a higher concentration of arsenic than that found in the raw rice brands as shown in figure-3 (panel B) and figure-1 (panel A). This means that everything we did in trying to reduce the arsenic concentration of the raw rice brands by boiling, soaking or washing did not produce the desired effect, instead caused increased retention of arsenic as indicated in figure-1 (panel B), figure-2 and figure-3 (panel A). This suggests that the water sources used in food preparation in Uganda's capital city Kampala, might be actually contaminating the food (in this case rice) with higher levels of arsenic than what is originally found in the raw rice. It has been previously reported that methods such as soaking and washing can help in reducing the arsenic levels in rice before cooking during food preparation (18–20). Our results seem to contradict this assertion mainly because we used tap water containing a higher concentration of arsenic than what was found in the raw rice brands investigated in our study. On the other hand, our findings seem to support a report published by Ujjal Mandal et al., which indicated that boiling of rice using arsenic-rich underground water in West Bengal – India, caused increased retention of arsenic in the food consumed by the local population of that region (21). Therefore, there is a need for constant monitoring of the arsenic concentration in the rice brands sold to the general population, as well as the levels of arsenic in the water used for domestic purposes in Uganda.

**Limitations to the Study**

In this study we assessed for arsenic levels in rice brands boiled, soaked or washed only with tap water as used by the local population to prepare food using tap water and not with other forms of water. We wanted to mimic the local methods of preparing a rice meal as appropriately as possible, but for experimental purposes, it would have been more informative to include an assessment of arsenic levels in some rice brands boiled, soaked or washed in deionized water. This would have helped to rule out the confounding effect of the additional arsenic already found in the tap water used in food preparation by the general population.

**Conclusion**
Our study shows that the rice brands sold in Kampala city have abnormally higher levels of arsenic than the acceptable limit set by the WHO or the American FDA. Our attempts to study the modifying effect of boiling, soaking and washing as means of reducing the arsenic levels in the rice were not successful because the tap water used had almost twice as much arsenic as that of the raw rice brands.

**Abbreviations**

AAS = atomic absorption spectrometry

Ars-III = arsenite

Ars-V = arsenate

FDA = food and drug authority

Hg = mercury

H\(_2\)O\(_2\) = hydrogen peroxide

HNO\(_3\) = nitric acid

Pb = lead

Ppb = parts per billion

Ppm = parts per million

WHO = world health organization

**Declarations**

**Ethics approval and consent to participate**

Not applicable because they study did not involve use of human subjects or human body parts

**Consent for publication**

Not applicable

**Availability of data and materials**

All the data and materials used to arrive at the conclusion are readily available in the manuscript

**Competing interests**

The authors have no competing interests / conflict of interest to declare
Funding

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Authors' contributions:

JN = was involved in study design, data collection and obtaining the funding; AL = was involved in data analysis and manuscript writing; AN = was involved in manuscript writing and editing; ELA = was involved in manuscript writing and editing; RK = was involved in manuscript writing and editing and LFS = was involved in conceptualizing of the study, manuscript writing, editing and submission.

Acknowledgements

We are very grateful to the technical staff in the chemistry laboratory for their effort in calibrating all the equipment and securing the necessary reagents. We are grateful for the funding that enabled this research work, received from the Fogarty International Center of the National Institutes of Health (NIH), U.S. Department of State’s Office of the U.S. Global AIDS Coordinator and Health Diplomacy (S/GAC), and President’s Emergency Plan for AIDS Relief (PEPFAR) under award number: IR25TWO11213. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Authors’ information (optional)

References


**Figures**

**Figure 1**

Shows levels of arsenic in raw rice brands (panel A); a comparison of arsenic levels between raw and boiled rice brands (panel B). Bars represent mean ± SEM of two separate measurements of arsenic levels. ppm = parts per million; SWT Ravi = rice brand, Milled processed and packaged in Pakistan; SWT1 = Long grain white rice brand, milled processed and packaged in Uganda; Zhong Yi = rice brand Grown and packaged in Lwera - Lukaya, Kalungu district near Masaka city of Uganda; Turkey = An Indian Basmati Rice brand imported to Uganda; Numa = a rice brand, Packed and processed in Kabwohe- Sheema District of Uganda; Kibimba = a rice brand, Grown and Packaged in Kibimba, Bugiri district, along Kampala-Malaba Highway in Uganda; York Taste = Punjabi Indian Rice brand, with long grains; Ordinary = a rice brand, Grown in the swampy areas of Kayunga District of Uganda. All comparisons were done using an unpaired t-test between each raw and the respectively treated rice brand, the p-values were all insignificant (see table 1).
Figure 2

Shows comparison of arsenic levels between raw and overnight-soaked rice brands (panel A); a comparison of arsenic levels between raw and overnight-soaked & boiled rice brands (panel B). Bars represent mean ± SEM of two separate measurements of arsenic levels. ppm = parts per million; os = overnight-soaked; SWT Ravi = rice brand, Milled processed and packaged in Pakistan; SWT1 = Long grain white rice brand, milled processed and packaged in Uganda; Zhong Yi = rice brand Grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda; Turkey = An Indian Basmati Rice brand imported to Uganda; Numa = a rice brand, Packed and processed in Kabwohe- Sheema District of Uganda; Kibimba = a rice brand, Grown and Packaged in Kibimba, Bugiri district, along Kampala-Malaba Highway in Uganda; York Taste = Punjabi Indian Rice brand, with long grains; Ordinary = a rice brand, Grown in the swampy areas of Kayunga District of Uganda. All comparisons were done using an unpaired t-test between each raw and the respectively treated rice brand, the p-values were all unsignificant (see table 1).
Figure 3

Shows comparison of arsenic levels between raw and washed rice brands (panel A); arsenic levels in unboiled tap water, boiled tap water and water used in soaking of the various rice brands (panel B). Bars represent mean ± SEM of two separate measurements of arsenic levels. Ppm = parts per million; SWT Ravi = rice brand, Milled processed and packaged in Pakistan; SWT1 = Long grain white rice brand, milled processed and packaged in Uganda; Zhong Yi = rice brand Grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda; Turkey = An Indian Basmati Rice brand imported to Uganda; Numa = a rice brand, Packed and processed in Kabwohe- Sheema District of Uganda; Kibimba = a rice brand, Grown and Packaged in Kibimba, Bugiri district, along Kampala-Malaba Highway in Uganda; York Taste = Punjabi Indian Rice brand, with long grains; Ordinary = a rice brand, Grown in the swampy areas of Kayunga District of Uganda. All comparisons were done using an unpaired t-test between each raw and the respectively treated rice brand, the p-values were all unsignificant (see table 1).