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Arsenic levels in rice brands sold in Kampala: an experimental study to show the modifying effect of boiling, soaking and washing

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ABSTRACT

Arsenic is an important environmental pollutant with potential cancer-causing effects. It contributes to acute and chronic toxicity depending on the doses or duration of exposure. In this study, we estimated the concentration of total arsenic in different rice brands sold in Uganda, where rice is a staple food. We conducted an experimental study. Different rice brands were obtained from supermarkets and grocery shops, and assessed for arsenic using atomic absorption spectrometry. The concentrations of arsenic in rice were estimated after boiling, washing, or overnight soaking in plenty of water to see if these methods reduce arsenic levels efficiently in food. The concentrations of arsenic in the different rice brands were compared using an unpaired t-test after setting a p-value of ≤0.05 as significant. The G-rice brand had the lowest arsenic levels of 1.4±0.000 ppm and the C-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±0.000 ppm, leading to increased retention of the heavy metal in the rice. Rice brands sold in Kampala city seem to have higher than acceptable arsenic levels. Increased vigilance in terms of routine monitoring for the levels of arsenic in rice and water used during food preparation by the Ugandan food or water safety regulatory authorities is highly recommended.

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1. Introduction

Arsenic is a major toxic heavy metal alongside others including Cadmium (Cd), Mercury (Hg), and Lead (Pb), which cause toxicity by polluting water, human food and animal feeds.

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Arsenic exists both in organic and inorganic forms, with the latter being more toxic than the former (1,2). The heavy metal is naturally found to be bivalent, either as Ars- III (arsenite) or as Ars-V (arsenate) (3). Rice production and consumption has been increasing steadily across the globe providing nourishment to over 50% of the world population (4).

Rice is cultivated in two major species: Oryza glaberrima and Oryza sativa, with the latter being the most widely cultivated across the globe. Oryza glaberrima was originally indigenously grown in Africa, but it is almost getting replaced with O. sativa (4). Ninety percent (90%) of the world's rice comes from Asia, and the rest is contributed by other countries such as Brazil, USA, Egypt, Italy, Madagascar and Nigeria (5,6). There has been an increasing trend of rice production and consumption in Africa, with only 54% of consumption satisfied locally, but the rest is from imported rice (4,5). The rice production chain goes through the following sequence: starting from the farm, paddy rice is harvested or produced, which may be stored temporarily; the paddy rice is then milled by cleaning, husking, and polishing; finally, the rice if released to the rice traders (both wholesale and retailers) who avail it to the consumers (4). In this production sequence, rice contamination with arsenic is most highly expected during farming due to environmental exposures (7-11). The storage and processing or milling stages are mostly associated with increased loss of micronutrients from the rice, which can be mitigated through fortification with vitamins or mineral supplements (6,12).

The majority of environmental arsenic pollution of geological derivation from underlying rocks, but industrial pollution also plays a huge role in this regard (13). Arsenic from industrial pollution of the environment comes as additives to pesticides (or insecticides), herbicides, cosmetics and herbal remedies (14,15), which subsequently pollute water bodies (1,16). The ensuing use of polluted water in the irrigation of crops consequently introduces arsenic into the food chain, which can potentially cause poisoning of human beings and their animals (17,18). Because plants have the capacity to absorb and hoard arsenic from various forms of soil or settings, there is always a likelihood of finding either some trace amounts or substantial amounts of arsenic in the reaped and industrially processed foodstuffs (19). Likewise, food crops such as rice are said to assimilate and hoard 10 times as much arsenic as compared to other comparable crops (3,20). In a bid to increase rice production, some genetically modified rice varieties have been introduced in Uganda, though with relatively unknown safety standards. It is still not well known how the genetically modified rice varieties compare to the indigenous varieties in terms of their ability to accumulate arsenic and comparative studies are needed to tease out this important safety measure of such a potential toxin to both human beings and animals (21). Moreover, simple methods such as polymerase chain reaction (PCR) for detecting the genetically modified rice varieties have been developed elsewhere, but they do not indicate the ability of the plant to accumulate heavy metals such as arsenic (22).

Accordingly, the supervisory bodies including 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 50 μg/L, to prevent toxicity in humans and animals (1,23).

Arsenic harmfulness in the human population can occur mainly via direct consumption of significantly high doses of the heavy metal as a poison or indirectly through chronic/continued ingesting of small amounts via food and water over a protracted period (17,24).

This culminates into either acute or chronic toxicity amongst the human victims (1). Additionally, some

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people get exposed to arsenic toxicity through the inhalation of arsenic exhausts from industrial pollution or via direct skin contact with arsenic compounds (24). Acute arsenic poisonousness displays signs including extreme salivation, vomiting, excessive watery diarrhea (bloody diarrhea), and rapid dehydration with subsequent cardiovascular collapse (2). Chronic arsenic toxicity, on the contrary, 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). The major mechanism of arsenic toxicity is by causing oxidative damage through the production of reactive oxygen species causing 'oxidative stresses to both proteins and DNA, which is an important step in mutagenesis or cancer (25). Arsenic poisoning has been associated with hepatotoxicity via several mechanisms including apoptotic-caspase activation, TNF-alpha inflammatory activation, and mitogen-activated protein kinase – Extracellularly regulated kinase (MAPK-ERK) activation in the hepatocytes (26). The method of food preparation can help in avoiding exposure to excessive amounts of heavy metals, for example, cooking rice after rinsing has been reported to be the most effective way of reducing arsenic levels and other heavy metals in rice (27). However, the successful reduction of heavy metals like arsenic in rice during cooking or boiling comes at the expense of losing essential trace elements like iron, selenium, and zinc, which are essential for maintaining normal body metabolism (28). A number of other essential nutrients lost are due to the thermal effects of food preparation including vitamins such as carotenoids and several other antioxidants (29).

For purposes of averting arsenic toxicity, it is consequently vital to know or have data regarding the amount of this heavy metal or its residues dumped in the environment, in the food chain, and in water resources. We, therefore, aimed at determining the arsenic concentration in the various rice brands that are sold in grocery shops in Kampala city, because rice is one of the main foods in town areas of Uganda. Additionally, we also endeavored to determine if there is a modifying effect of boiling, soaking, or washing on the arsenic levels in the rice.

2. Materials and Methods

2.1. Study setting

We conducted an experimental study in the laboratory to estimate or quantify the Arsenic levels in rice. The different rice brands were consecutive/conveniently sampled from grocery stores around Kampala city and transported to the Nutrition Unit in the Department of Medical Physiology Laboratory for boiling, soaking, or washing. Subsequently, the prepared rice samples were taken for measuring their arsenic concentrations using an Agilent Atomic Absorption Spectrometer in the Department of Chemistry at Makerere University.

2.2. Sampling procedure

The different eight rice samples were collected casually in triplicates from some supermarkets and grocery shops around Kampala and taken to the Department of Medical Physiology Laboratory for processing.

2.2.1. Sample storage

The sampled rice brands were protected from adulteration and undesired loss of their metal content during the study. After exposing the rice to boiling, overnight soaking, or washing conditions, 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 sterilized laboratory sample bags at room temperature (around 25°C) until preparation for further analysis.

2.2.2. Sample preparation before analysis

Five hundred grams (500 g) 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 min until when ready. Fifteen milliliters (15 mL) of the water in which each sample is soaked or washed and boiled were stored in germ-free falcon containers for further analysis. The 500 g of the prepared samples were heated in an oven until dry. The rice samples were homogenized to a fine powder using a mincer, while the liquid samples were analysed for arsenic directly.

2.3.Analyzing for arsenic

All samples were prepared for analysis using a modified 'wet ashing' procedure as described by Adeloju, 1989 (30). Briefly, the analysis was done in two experiments or duplicates of samples. For all the solid rice samples, 1.25 g of the dried sample (dried in the oven for 24 h at 103°C) was weighed and transferred to the destruction tube; 25 mL of 65% nitric acid (HNO_3) was then added with three boiling chips and a funnel placed on top of the demolition tube. The tube was then heated to 100°C and maintained for 1 h, heat was increased to 125°C, 150°C, 175°C and, 200°C maintaining the temperatures for 15 min at each change. The remaining volume was concentrated to 5 mL and left to cool. After cooling, 1 mL of 30% hydrogen peroxide (H_2O_2) was added and destructed for 10 min. This destruction was repeated once. Then after cooling again, 3 mL of 30% H_2O_2 was added and destructed for 10 min. Twenty-five (25 mL) of deionized water was then added, mixed and heated till boiling. The solution was cooled and transferred to a 250 mL volumetric flask, filled up to the mark, mixed and left to settle for 15 h. The absorbance of the supernatant was then measured by Atomic Absorption Spectrometry (AAS) using an Agilent 240 AA spectrometer series machine (Agilent Technologies, Santa Clara, California, USA) following standard procedures (30).

2.4. Quality control

All chemicals 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 skilled laboratory technicians. Validation of analytical methods was done by assessing analytical figures of merit as recommended by the international conference on harmonization (ICH 1994 (31)) using a limit of detection (LOD of 0.001 ppm for arsenic in water/solution). The study approval reference number is SBS-2022-138.

2.5. Data management and analysis

All the data obtained was cleaned before summarizing in Excel spreadsheets. This was followed by data analysis for the variations in the arsenic content between the different brands of rice by performing an unpaired t-test and plotting the relevant graphical presentation of the results using Graph Pad Prism (version 8) software. For statistical significance, p-values of ≤ 0.05 were considered.

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3. Results

Following the initial assays for arsenic levels in the raw rice, the mean ± SEM concentration of arsenic in each rice brand was as follows: A (rice brand, Milled processed and packaged in Pakistan) had 1.675±0.0132 ppm, B (Long grain white rice brand, milled processed and packaged in Uganda) had 1.735±0.0132 ppm, C (rice brand Grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda) had 2.195±0.0132 ppm, D (An Indian Basmati Rice brand imported to Uganda) had 1.605±0.0132 ppm, E (a rice brand, Packed and processed in Kabwohe- Sheema District of Uganda) had 1.145±0.0702 ppm, F (a rice brand, Grown and Packaged in Kibimba, Bugiri district, along Kampala-Malaba Highway in Uganda) had 1.565±0.0702, G (Punjabi Indian Rice brand, with long grains) had 0.935±0.070 ppm and H (a rice brand, Grown in the swampy areas of Kayunga District of Uganda) had 1.335±0.0702 ppm (see Fig. 1A). The un-boiled tap water had an arsenic concentration of 3.356 ppm (N.B. comparison of the arsenic levels between the different rice brands was not found to be significant and the p-values are summarized in Table 1).

Following boiling of the rice brands, the mean ± SEM arsenic concentration of each brand was as follows: A had 1.123±0.004 ppm, B had 0.991±0.0008 ppm, C had 1.516±0.004 ppm, D had 1.616±0.0041 ppm, E had 1.116±0.009 ppm, F had 0.521±0.002 ppm, G had 2.358±0.018 ppm and H had 2.143±0.013 ppm (see Fig. 1B) (N.B. comparison of the arsenic levels between the different rice brands was not found to be significant and the p-values are summarized in Table 1).

Following overnight soaking of the rice brands, the mean ±SEM- arsenic concentration for each brand was as follows: SWT Ravi had 2.213±0 ppm, SWT1 had 2.062±0 ppm, Zhong Yi had 2.132±0 ppm, Turkey had 1.782±0 ppm, Numa had 2.518±0.008 ppm, Kibimba had 3.169±0.009 ppm, York Taste had 2.598±0.008 ppm and ordinary rice had 1.737±0.008 ppm (see Fig. 2A).

Then, after overnight soaking and boiling of the rice brands, the mean ±SEM – arsenic concentration for each brand was as follows: A had 2.238±0085 ppm, B had 1.757±0.0084 ppm, C had 2.158±0.0061 ppm, D had 2.208±0.0061 ppm, E had 2.658±0.006 ppm, F had 1.937±0.006 ppm, G had 2.278±0.011 ppm and H had 2.603±0.002 ppm (see Fig. 2B) (N.B. comparison of the arsenic levels between the different rice brands was not found to be significant and the p-values are summarized in Table 1).

In addition, after washing the rice brands, the mean ±SEM- arsenic concentration for each brand was as follows: A had 3.154±0.005 ppm, B had 5.516±0.007 ppm, C had 5.036±0.0002 ppm, D had 4.786±0.024 ppm, E had 2.353±0.0008 ppm, F had 1.342±0.003 ppm, G had 2.358±0.0012 ppm and H had 3.044±0 ppm (see Fig. 3A) (N.B. comparison of the arsenic levels between the different rice brands was not found to be significant and the p-values are summarized in Table 1).

Then lastly, the water used in all the above assays had the following mean ± SEM arsenic concentration for each sample involved: un-boiled water had 3.475±0.001 ppm, boiled tap water had 3.275±0.001 ppm, water for A had 3.029±0.001 ppm, water for B had 2.538±0.003 ppm, water for C had 3.614±0.0002 ppm, water for D had 3.479±0.001 ppm, water for E had 3.814±0.0002 ppm, water for F had 3.144±0.003 ppm, water for G 3.915±0.0002 ppm and water for H had 3.314±0.0002 ppm (see Fig. 3B).

Table 1. A summary of the comparison p-values following an un-paired t-test statistical analysis of arsenic levels in the respective rice sample treatment groups

Rice samples compared	p-value	Rice samples compared	p-value
Raw A vs Washed A	0.3333	Raw E vs washed E	0.3333
Raw B vs washed B	>0.999	Raw F vs washed E	0.3333
Raw C vs washed C	0.3333	Raw G vs washed E	0.3333
Raw D vs washed D	0.3333	Raw H vs washed E	0.3333
Raw E vs washed E	0.3333	Raw F vs washed F	0.3333
Raw F vs washed F	>0.999	Raw G vs washed F	0.3333
Raw G vs washed G	0.3333	Raw H vs washed F	0.3333
Raw H vs washed H	0.3333	Raw G vs washed G	0.3333
Raw B vs washed B	0.3333	Raw H vs washed G	0.3333
Raw C vs washed C	0.3333	Raw H vs washed H	0.3333
Raw D vs washed D	0.6667	Raw A vs overnight soaked A	0.3333
Raw E vs washed B	0.3333	Raw B vs overnight soaked B	0.3333
Raw F vs washed B	>0.999	Raw C vs overnight soaked C	0.3333
Raw G vs washed B	0.3333	Raw D vs overnight soaked D	0.3333
Raw H vs washed B	0.3333	Raw E vs overnight soaked E	0.3333
Raw C vs washed C	0.3333	Raw F vs overnight soaked F	0.3333
Raw D vs washed C	0.3333	Raw G vs overnight soaked G	0.3333
Raw E vs washed C	0.3333	Raw H vs overnight soaked H	0.3333
Raw F vs washed C	0.3333	Raw A vs boiled A	0.3333
Raw G vs washed C	0.3333	Raw B vs boiled B	0.3333
Raw H vs washed C	0.3333	Raw C vs boiled C	0.3333
Raw D vs washed D	0.3333	Raw D vs boiled D	0.3333
Raw E vs washed D	0.3333	Raw F vs boiled F	0.3333
Raw F vs washed D	0.3333	Raw E vs boiled E	>0.999
Raw G vs washed D	0.3333	Raw G vs boiled G	0.3333
Raw H vs washed D	0.3333	Raw H vs boiled H	0.3333

N.B: A = rice brand, milled processed and packaged in Pakistan; B = Long grain white rice brand, milled processed and packaged in Uganda; C = rice brand grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda; D = An Indian Basmati rice brand imported to Uganda; E = a rice brand, packed and processed in Kabwohe- Sheema district of Uganda; F = a rice brand, grown and packaged in Kibimba, Bugiri district, along Kampala-Malaba highway in Uganda; G = Punjabi Indian rice brand, with long grains; H = a rice brand, grown in the swampy areas of Kayunga district of Uganda. Vs=versus

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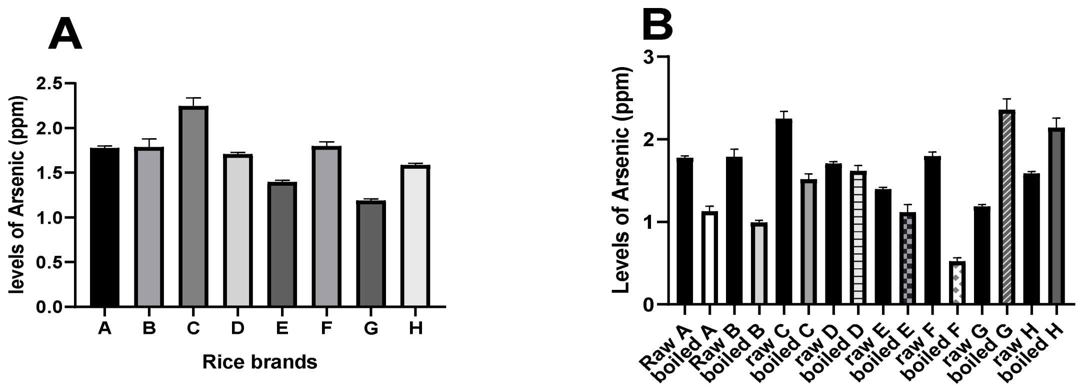

Figure 1. Levels of arsenic in raw rice brands **(panel A)**; a comparison of arsenic levels between raw and boiled rice brands **(panel B).** Bars represent mean ± SEM of two separate measurements of arsenic levels. ppm = parts per million; A = rice brand, milled processed and packaged in Pakistan; B = Long grain white rice brand, milled processed and packaged in Uganda; C = rice brand grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda; D = An Indian basmati rice brand imported to Uganda; E = a rice brand, packed and processed in Kabwohe- Sheema district of Uganda; F = a rice brand, grown and packaged in Kibimba, Bugiri district, along Kampala-Malaba highway in Uganda; G= Punjabi Indian rice brand, with long grains; H = 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**).

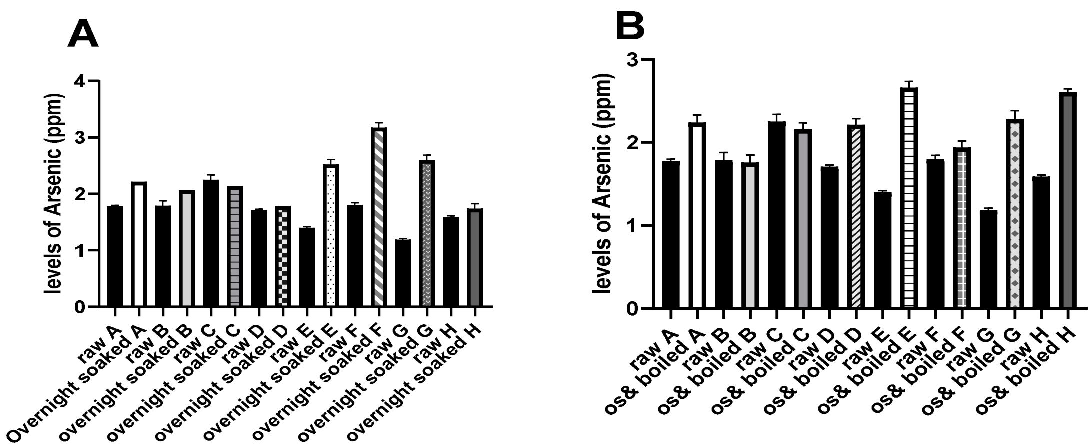

Figure 2. Comparison of arsenic levels between raw and overnight-soaked rice brands **(panel A)**; a comparison of arsenic levels between raw and overnight-soaked & boiled rice brands **(panel B).** Bars represent mean ± SEM of two separate measurements of arsenic levels. ppm = parts per million; os = overnight-soaked; A = rice brand, milled processed and packaged in Pakistan; B = Long grain white rice brand, milled processed and packaged in Uganda; C = rice brand grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda; D = An Indian basmati rice brand imported to Uganda; E = a rice brand, Packed and processed in Kabwohe- Sheema District of Uganda; F = a rice brand, Grown and Packaged in Kibimba, Bugiri district, along Kampala-Malaba highway in Uganda; G = Punjabi Indian rice brand, with long grains; H = 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**).

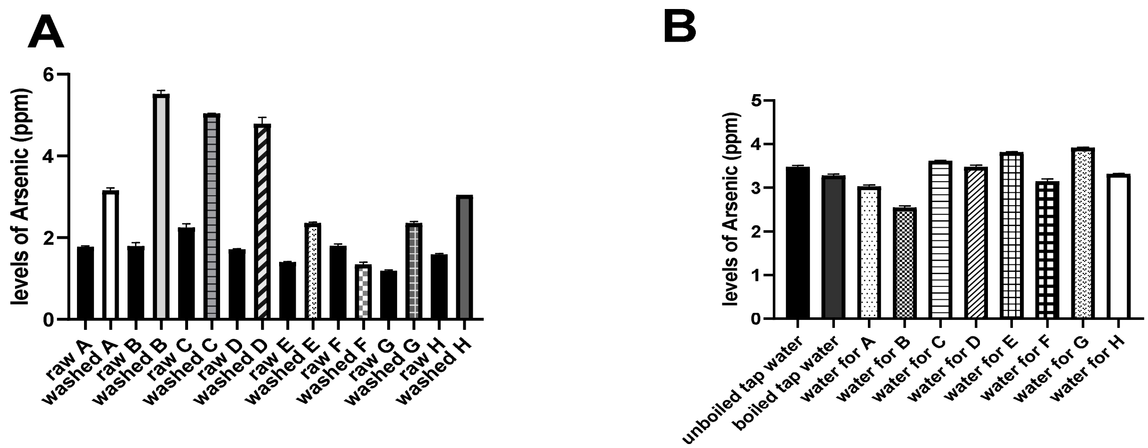

Figure 3. Comparison of arsenic levels between raw and washed rice brands (**panel A**); arsenic levels in un-boiled tap water, boiled tap water and water used in soaking of the various rice brands (**panel B**). Bars represent mean ± SEM of two separate measurements of arsenic levels. ppm = parts per million; A = rice brand, milled processed and packaged in Pakistan; B = Long grain white rice brand, milled processed and packaged in Uganda; C = rice brand grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda; D = An Indian basmati rice brand imported to Uganda; E = a rice brand, packed and processed in Kabwohe- Sheema district of Uganda; F = a rice brand, grown and packaged in Kibimba, Bugiri district, along Kampala-Malaba highway in Uganda; G = Punjabi Indian rice brand, with long grains; H = 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**).

4. Discussion

Rice is a major food eaten by many Ugandans, and we documented the arsenic levels in different rice brands sold in Kampala city, as well as examining any modifying effect of boiling, overnight soaking, and washing of the rice when preparing a rice meal.

From the results, we detected that the local rice brands such as C (grown in the Lwera wetland near Masaka city) and F (grown in the Bugiri district in Eastern Uganda) had slightly higher levels of arsenic than all the other brands in the market. This might imply that the soils and water resources in which these specific rice brands are grown, already have a substantially high concentration of arsenic. It could also be a pointer to the presence of mining activity in the neighborhood or proximity of the agricultural sites, which leads to the contamination of the soil and water with exceptionally higher levels of arsenic than what would be expected if there was no mining taking place.

Certainly, the intensified mining of sand in the Lwera marshland that has been previously highlighted (32, 33), might be contributing to the increased release of arsenic into the water used in the cultivation of rice in that area. The results postulated that all the rice brands included in this study have arsenic levels ranging from an average of 1.4 parts per million (ppm) (for G = Punjabi Indian Rice brand, with long grains) to 2.4 ppm (for C = rice brand grown and packaged in Lwera -Lukaya, Kalungu district near Masaka city of Uganda) (Fig. 1, panel A), which exceeds the acceptable levels of 0.05 ppm set by the American FDA and WHO as the maximum limit of arsenic to be tolerated in food or water (1,23,34). Our rice arsenic levels are certainly much higher than those got from another study done in Nigeria, which reported much lower levels of an average of 0.0304 ppm (35) in Zamfara state rice. This implies that continued consumption of these rice brands daily and for a long time might expose the general population to the toxic effects of arsenic in the body (1). In case there is no government oversight or intervention to test and constantly monitor the amount of arsenic in the foods sold to the general public, we can predict here that the high arsenic levels in the rice brands sold in Kampala city might even surge further years. In comparison to a similar analysis done in Iran by Abdi Leili et al., to show the levels of potentially toxic elements in maize and soya beans, our results still remain higher, showing a cause for concern (36). Moreover, some attempts have been made to show an association between chronic dietary intake of arsenic and hypertension, although, the findings are still inconclusive (37). Higher health complications have been found in Asian children exposed to chronic dietary intake or arsenic via wheat and rice (38).

We examined the modifying effect of boiling, overnight soaking, and washing of the rice during food preparation as possible methods of lowering arsenic that might be contained in the raw rice brands. We intended to imitate the general method used in preparing a rice meal locally by boiling, soaking, and washing the rice in tap water as trusted by the majority of the population in Kampala for cooking food. Surprisingly, the tap water contained a higher concentration of arsenic than that found in the raw rice brands as shown in Fig. 3 (panel B) and Fig. 1 (panel A). This clearly shows that whatever we did in attempting to decrease the arsenic concentration of the raw rice brands by boiling, soaking or washing did not yield the anticipated effect, instead triggered increased retention of arsenic as indicated in Fig. 1 (panel B), Fig. 2, and Fig. 3 (panel A). This indicates that the water sources used in cooking or food preparation in Uganda's capital city Kampala might be adulterating the food (such as rice) with higher levels of arsenic than what is initially found in the raw rice. Food preparation methods such as soaking and washing have been previously suggested to help in decreasing the arsenic levels in rice before it is cooked (39-41). Our study discoveries seem to challenge this assertion mainly because we used tap water, which already had a higher concentration of arsenic than what was found in the raw rice brands examined. Conversely, our findings seem to support a report published by Ujjal Mandal et al., which showed that boiling rice using arsenic-rich underground water in West Bengal – India, caused increased retention of arsenic in the food eaten by the local population of that region (42). Hence, there is a need for routine monitoring of the arsenic concentration in the different rice brands sold to the general population, as well as the levels of arsenic in the water used for domestic purposes in Uganda.

In this study, we measured 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 endeavored to imitate 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 puzzling effect of the additional arsenic already found in the tap water used in food preparation by the general population.

5. Conclusion

Our study illustrates that the rice brands sold in Kampala city have unusually higher levels of arsenic than the acceptable limit set by the WHO or the American FDA. Our efforts to study the modifying effect of boiling, soaking, and washing as means of decreasing the arsenic levels in the rice were unsuccessful because the tap water used had almost twice as much arsenic as that of the raw rice brands. Increased vigilance in terms of routine monitoring for the levels of arsenic in rice and water used during food preparation by the Ugandan food or water safety regulatory authorities is highly recommended.

Conflict of interest

All authors confirm that there is no conflict of interest to declare.

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