Developing a Method to Remove Inorganic Arsenic from Rice with Natural Substances

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SUMMARY
Many developing nations struggle with arsenic (As) exposure due to the over-pumping of groundwater wells, which results in higher concentrations of As in agricultural systems. In Bangladesh, there has been an ongoing crisis of As-related health issues, such as heart disease and various cancers, primarily due to the consumption of As-contaminated water and rice. Prior research has mainly focused on removing As from water, but there has been little research on removing As from rice, even though rice contains dangerous amounts of As and Bangladeshis have a heavily rice-based diet. We focus on developing a method to remove inorganic As from rice using natural substances. We developed a method utilizing activated charcoal and bentonite clay to lower levels of As in rice using these natural substances. We adapted a procedure to use the Quick Sensafe Rapid Arsenic Test Kit to measure As before and after treatment while simulating cooking conditions. Activated charcoal was the most effective, as it was able to remove 75% of As when used alone. Therefore, this method could be utilized by countries like Bangladesh to lower As intake and incidences of As-related health issues.

INTRODUCTION
Bangladesh is facing a major public problem of arsenic (As) contamination due to their As-polluted tube wells and groundwater systems (1). Unfortunately, this As contamination has spread to their food resources, as As has accumulated at significant concentrations in locally grown rice (2). Since rice is grown under flooded conditions, toxic inorganic As from polluted groundwater builds up in large amounts around the root and is absorbed in high concentrations into the grain which is then consumed (2). Rice plays a key role in Bangladesh As exposure (3). Bangladeshis have a heavily rice-based diet, and many individuals are inadvertently consuming high levels of As. In West Bengal and Bangladesh, rice is the main source of As exposure after water and on average, Bangladeshis consume 450 to 600 grams of rice each day (4). Consumption of inorganic As is a cause of numerous health issues, including skin lesions, heart disease, and skin, bladder, and lung cancers (5). Therefore, we looked at the As in rice that people consume and developed and tested methods for removing As from rice.

In a study conducted by the Health Effects of Arsenic Longitudinal Study (HEALS), participants from Bangladesh were tracked for over a decade, and those exposed to the highest concentrations of As displayed an increase of nearly 70% in all-cause mortality (6). HEALS also reported that even low-level exposures to As increased mortality rates, showing that any amount of As intake could potentially be deadly. There have also been recent studies which correlate high As exposure with higher levels of illnesses and death. In Matlab, Bangladesh participants were monitored for thirteen years, and the study concluded that higher consumption of As increases mortality risks among young adults and that As poisoning is prevalent in Bangladeshi society today (7).

Current methods to lower inorganic As concentration involve washing the rice multiple times, discarding the contaminated water, and then boiling the rice with extra water to lessen the reabsorption of As back into the grain (8). However, this method is not completely effective. One study showed that only up to 57% of total As levels could be removed with low-As water washing and boiling with extra water, and 28% could be removed with low-As water washing alone (9). Consumption of the remaining inorganic As is potentially dangerous (10). Thus, complete As removal from rice is an ongoing issue.

Inorganic As levels in rice vary based on the type of rice and where it is grown. Brown rice absorbs more inorganic As because As accumulates in the aleurone layer of the rice grain, which gives brown rice its color (10). Also, As concentration increases significantly as the size of the rice grain increases. Therefore, large-grain rice is more contaminated. That is why we used large-grain brown rice from India that contained significant levels of inorganic As.

The goal of this study was to develop a method to remove as much inorganic As (arsenite, AsIII and arsenate, AsV) from rice as possible using the natural substances activated charcoal and bentonite clay. Activated charcoal and bentonite clay have similar properties, including their ability to adsorb heavy metals due to their negatively-charged, porous surfaces. Particularly, activated charcoal has a large surface area that allows for the physical adsorption of dissolved substances, such as inorganic As, from liquids (11). The use of natural substances like activated charcoal and bentonite clay to lower inorganic As levels from solution is effective, but their abilities to lower inorganic As in solids, such as rice, have not been tested. We hypothesized that inorganic As levels in rice would be lowered after the addition of these substances.
We showed that activated charcoal and bentonite clay can remove As from rice and potentially help lower levels of As intake. Our research could benefit those living in Bangladesh, as it could help remove inorganic As from their diets and lower occurrences of As-related health issues.

RESULTS

The effectiveness of water wash methods, bentonite clay, and activated charcoal were examined for their efficiency in removing As from rice. For all tests, the rice was cooked for twenty minutes, dried in an incubator until no moisture remained, and homogenized with a coffee grinder. The powdered rice was then added to water and heated to extract the As. The resulting substance was then cooled and As levels were measured using the Sensafe Quick Arsenic field test kit. This test kit utilizes a patented, modified Gutzeit method for the reaction, which creates arsine gas and displays results on a mercuric bromide strip. These kits are reliable, fast, inexpensive, and produce results comparable to higher end machines (12). The Sensafe kit provided an Easy Read color chart that is used to match the color formed on the mercuric bromide strip with the amount of inorganic As in the initial sample. This As test kit was created for water, soil, and wood analysis, however, which is why we adapted this procedure to have the ability to measure As in rice.

We used two water wash methods: As water wash and clean water wash. The As water wash method, which used As-free water, lowered the 40 ppb to 10 ppb of inorganic As (Figure 1).

The usage of bentonite clay to remove inorganic As from rice is effective but requires a method to prevent the bentonite clay powder from mixing with the rice. The usage of a coffee filter with 10 grams of bentonite clay powder lowered the concentration of As from 40 ppb to 20 ppb (Figure 1). The usage of a five-gram activated charcoal brick lowered the original 40 ppb to 10 ppb (Figure 1). In addition to using activated charcoal on its own, we combined this method with the clean water wash method to attempt to remove even more inorganic As. The results of this were favorable, as the two methods combined are able to remove the most inorganic As, lowering the concentration of As to 5 ppb (Figure 1).

DISCUSSION

Our results support the hypothesis that natural substances, activated charcoal and bentonite clay, are able to remove inorganic As from rice. The various methods differ in the removal of As from the rice. Results appeared consistent throughout all three trials because the kit used for these experiments is not very sensitive (Figure 1). The combination of the clean water wash and activated charcoal method removed the most As, followed by the activated charcoal method tied with the clean water wash method, then the bentonite clay method, and finally the As water wash method which removed the least.

By using the As water wash method, 25% of inorganic As can be removed (Figure 1). Previous research found that washing rice with low-As water can remove up to 28% of natural substances. We showed that activated charcoal and bentonite clay can remove As from rice and potentially help lower levels of As intake. Our research could benefit those living in Bangladesh, as it could help remove inorganic As from their diets and lower occurrences of As-related health issues.
inorganic As (9). This supports the validity of our measurement procedure because the results closely align.

The method that resulted in the second-lowest level of inorganic As removal of 50% is bentonite clay (Figure 1). Bentonite clay is not the most effective at removing inorganic As, as only half could be removed when using the coffee filter method. This could be due to the usage of the coffee filter pouch that could have prevented exposure of inorganic As to the bentonite clay’s surface. Unfortunately, this coffee filter method is necessary to prevent the combination of the clay powder with the rice. Zahra et al. reports that bentonite clay is able to remove up to 95% of inorganic As from wastewater (13), but our study’s results show that only 50% of inorganic As can be removed from rice (Figure 1). However, this result of 50% removal still supports our hypothesis that bentonite clay can remove inorganic As.

Two methods resulted in removal of 75% of the inorganic As: the clean water wash and activated charcoal treatment (Figure 1). The clean water wash method is effective and results in a removal of 75% of inorganic As. However, this method can only be utilized by those with access to clean water, a potential limitation to those in Bangladesh with contaminated tube wells and groundwater systems (1). Activated charcoal is also effective and results in a removal of 75% of inorganic As. These results can be supported with previous research that reports that activated charcoal is able to adsorb AsV by 2.5% of its weight and AsIII by 1.2% of its weight from solution (14).

The clean water wash method combined with the activated charcoal method works the best to remove the most inorganic As. The combination of these methods is tested because both show the most potential for high As removal. This combination results in a removal of 87.5% of inorganic As, which is the greatest As removal percentage observed in our study (Figure 1). For those with access to clean water, this method would work. Future studies should investigate whether the combination of the As water wash method with the activated charcoal method is effective for people in Bangladesh. However, the activated charcoal method alone is still effective, as it is able to remove 75% of inorganic As without the clean water wash. Also, activated charcoal is very convenient due to its ability to remain activated for 1-2 months and its simplicity to use. In conclusion, activated charcoal would be the most applicable and effective for Bangladeshi.

One possible limitation is that the maximum amount of inorganic As that could be extracted from the plain Indian brown rice using the kit was 40 ppb. This is most likely not all of the As found in the rice. However, by keeping the procedure the same for testing the other methods, this gave us a baseline value to which the experimental results could be compared. Additionally, the test strips provided in the kit only had colors in 10 ppb increments to measure As levels, so there could have been possible errors in the precision of the measurements, as we were unable to measure removal to the nearest percent. To limit error, we had at least three trials per method and ensured that the colors on the test strip appeared consistent.

The findings resulting from our study could potentially help those in Bangladesh lower their As intake, therefore possibly lowering the occurrences of As-related health issues and death (6). In the future, we should test more methods, such as boiling with extra water, and test more types of rice, obtaining rice from Bangladesh or testing rice from the United States. Also, we should vary the amounts of adsorbent substance used in each experiment in order to determine if that could possibly have an effect on removal capabilities. Additionally, we could look at more ways to make these methods more accessible to those in Bangladesh by possibly creating activated charcoal out of easily obtainable resources such as coconut shells. As of now, the results are favorable because the initial goal to develop a method to remove inorganic As from rice using natural substances is met.

**MATERIALS AND METHODS**

**Measurement of Arsenic Levels**

We used the Sensafe Quick Arsenic field test kit (Industrial Test Systems Part No. 481396) to measure As levels. This test kit utilizes a patented reaction, a modified Gutzeit method, which creates arsine gas and displays results on a mercuric bromide strip. In this method, the inorganic As compounds in the sample are converted to arsine (AsH$_3$) gas, which is a product of the reaction of zinc and tartaric acid. Ferrous and nickel salts are added to speed up this reaction. The arsine gas reacts with the mercuric bromide on the test strip to form mixed mercury halogens like AsH$_2$HgBr to create a yellow or brown color. The test kit provided an Easy Read color chart with low sensitivity that was used to match the color with the amount of inorganic As in the initial sample. This As test kit was created for water, soil, and wood analysis, however, which is why we adapted this procedure to have the ability to measure As in rice.

**Preparation & Cooking**

To simulate cooking conditions, 25 grams of large-grain brown rice from India was cooked in 60 mL of water for 20 minutes and then dried in an incubator at 28°C to return it back into its dried form that could be measured for As with the Sensafe Quick Arsenic field test kit. Since the field test kit that we use detects levels of inorganic As in water, the rice had to be homogenized. First, a coffee grinder was used to grind the rice into a powder, and then 10 grams of this rice powder was placed into 100 ml of water. This mixture was heated on a consistent medium-high setting for five minutes to further extract As. After cooling, the mixture was ready to be tested using the test kit.

First, using the aforementioned procedure, we took multiple baseline samples of the As concentration in the rice alone as a control. After finalizing the control measurement procedure, we continued on testing the other methods. The current water wash method was tested by swirling the rice in
water for ten seconds and discarding and changing the water three times before cooking. Water washing was conducted before the rice was cooked, and then the rest of the procedure was followed. In an attempt to develop a new method to remove As that utilized activated charcoal or bentonite clay, we cooked the rice with these natural substances by either placing it in alone or putting it inside of a permeable coffee filter pouch. Specifically, we utilized five grams of activated charcoal and ten grams of bentonite clay powder within a permeable pouch throughout the entire cooking duration. Activated charcoal was in a solid brick form and placed in direct contact with the rice. The bentonite clay was in powdered form and placed inside of a coffee filter pouch. For all tests, the rice was cooked for twenty minutes, dried in an incubator until no moisture remained, and homogenized with a coffee grinder. Three trials of each method were conducted, and results appeared consistent.

Data Analysis
To analyze the data, we compared the results of the experimental groups to the results of the control group. Figure 1 was prepared by finding percent decrease compared to the control group. Each discrete color result of the test kit indicates an As concentration within a 10 ppb range.

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