



## **Effect of Cooking on Arsenic Reduction in Two Rainfed Rice Varieties of Bangladesh and Their Health Risk Assessment**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author MS designed the study, carried out ICP-MS analysis and wrote the draft of the manuscript. Author MT contributed to the design of the work and prepared of the manuscript. Authors MR, MHAM and MKA contributed to the design of the work and collected the samples. Authors MRI, YM and TA contributed to the preparation of the manuscript. Author SM involved in planning and supervised the work. All authors read and approved the final manuscript.*

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## ABSTRACT

To investigate the effects of cooking on reducing the health risk, the concentrations of total arsenic in raw and cooked rice were measured. Raw rice of two rainfed rice varieties was purchased from market of Dhaka, Bangladesh in 2015. Minikit is a long slender grained popular rice variety in Bangladesh while Kataribhogh is a fine grained aromatic rice which is famous for its aroma and texture. One hundred grams of raw rice was washed with Milli-Q water three times. Then, raw rice was cooked with Milli-Q water: grain ratio of 5:1 in an iron pan covered with lid and the excess water after cooking was decanted. Cooked rice was freeze-dried and ground for arsenic analysis. A microwave digestion was used to digest the rice samples for analysis. Samples were analyzed using inductively coupled plasma mass spectrometer (ICP-MS). The arsenic concentrations in raw rice of Minikit and Kataribhogh in this study were 0.123–0.153  $\mu\text{g g}^{-1}$  and 0.611–0.783  $\mu\text{g g}^{-1}$ , respectively. On the other hand, the total arsenic contents in cooked rice of Minikit and Kataribhogh were  $0.115 \pm 0.004 \mu\text{g g}^{-1}$  and  $0.386 \pm 0.004 \mu\text{g g}^{-1}$ , respectively. There was 17% and 46% reduction of arsenic due to washing and cooking of Minikit and Kataribhogh rice, respectively. Based on the concentration of arsenic in raw and cooked rice, the carcinogenic risk of arsenic via rice ingestion was estimated. The estimated risk of arsenic with cooked rice was  $1.2 \times 10^{-5}$  and  $4.1 \times 10^{-5}$  while that of raw rice was  $1.5 \times 10^{-5}$  and  $7.7 \times 10^{-5}$  for Minikit and Kataribhogh rice, respectively. The results of this study revealed that health risk assessment on the basis of arsenic content of raw rice would be overestimated than the actual health risk.

*Keywords: Arsenic; rainfed rice; cooking; risk assessment.*

## 1. INTRODUCTION

Arsenic (As) is a toxic and carcinogenic element to humans (Group I in the IARC classification of carcinogens) widely distributed in the environment, particularly in water, and the earth's crust. Shallow tube-well water in different locations of Bangladesh is heavily contaminated with As. Irrigating rice with As-contaminated water lead to decrease in rice yield [1-3] but increase in As-concentration in grains [3-5]. Among foods consumed in Bangladesh, rice is an important dietary source of arsenic for Bangladesh population, and the caloric intake of rice is about 70% of the total [6]. Islam et al. [7] reported that rice contributed about 62% of the total As intake by the household of Dhaka city. In Bangladesh, approximately 35 and 57 million people are exposed to arsenic contaminated water whose arsenic concentration exceeds the guideline values of 50 and 10  $\mu\text{g L}^{-1}$ , respectively [8]. A large amount of people in Bangladesh are affected by arsenic contaminated water and foods, which leads to adverse health outcomes such as skin cancer, dermatitis, mild pigmentation keratosis of the skin, lung cancer, hepatic dysfunction and diabetes [9]. There has been a number of health risk assessment based on the total As content in raw rice [10,11]. A number of previous studies had suggested that the total As content in raw rice and cooked rice differ widely depending on rice variety [12,13], washing before cooking [13,14], quality of

cooking water and volume of water added for cooking [15,16]. Therefore, the actual health risk assessment from rice will be obtained if it is done on the basis of As in cooked rice. Until now, cooking experiments from Bangladesh rice were limited to irrigated rice varieties [6,12]. Irrigated rice comprises about 60% of the total rice in the country while the rest is coming from rainfed rice. Rainfed rice varieties are palatable and preferred by the consumers compared to irrigated rice. In this study, Minikit, a long slender grained rice and Kataribhogh, a fine grained aromatic rice was selected to assess the effect of cooking on reducing the level of total As in cooked rice and health risk assessment was calculated due to consumption of these cooked rice.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

Nitric acid and hydrogen peroxide were obtained from Kanto Chemical Co., Japan. All chemicals were analytical grade reagents, and Milli-Q (Elix UV5 and Milli-Q Adv.A10, Millipore, USA) water was used for each solution preparation. Argon gas was supplied by Tomoe Shokai Co., Ltd., Japan. Arsenic standard solution and tuning solution (1  $\mu\text{g L}^{-1}$  each of lithium (Li), yttrium(Y), cerium (Ce), thallium (Tl), magnesium (Mg), and cobalt (Co) in 2 wt % nitric acid) were purchased from Agilent Technologies, USA. Internal calibration standard solution containing 1.0 mg

L<sup>-1</sup> each of beryllium (Be) and tellurium (Te), and 0.5 mg L<sup>-1</sup> each of indium (In), Y, Co, and titanium (Ti) was purchased from SPEX CertiPrep, USA.

## 2.2 Rice Cooking Procedure

Raw rice of two rainfed rice varieties was purchased from market of Dhaka, Bangladesh. Minikit is a long slender grained popular rice variety in Bangladesh and Kataribhogh is a fine grained aromatic rice which is famous for its aroma and texture. One hundred grams of raw rice was washed with Milli-Q water three times. Then, raw rice was cooked with Milli-Q water: grain ratio of 5:1 in an iron pan covered with lid and the excess water after cooking was decanted. Cooked rice of Minikit and Kataribhogh had 74% and 70% moisture. Cooked rice was freeze-dried and ground for As analysis. Cooking and analytical procedures are schematically summarized in Fig. 1.

## 2.3 Sample Preparation

A microwave digestion was used to digest the rice samples (raw and cooked rice) for analysis. The polytetrafluoroethylene (PTFE) digestion vessels and polypropylene containers were cleaned, soaked in 5% nitric acid for 24 h, then rinsed with Milli-Q water and dried. For arsenic analysis, 0.5 g of rice samples was treated with 5 mL 69% nitric acid and 2 mL 30% hydrogen peroxide in closed digestion vessel. After mixing for 20 min, the vessels containing samples were placed in a microwave digestion system (Berghof-MWS2, Berghof speedwave, Germany). The following microwave program was applied: 10 min at 180°C with 800 W, 10 min at 190°C with 900 W, and as a last step 10 min at 100°C with 400 W. After digestion, acid solutions with samples were transferred into a Teflon graduated cylinder and total volume was made up to 50 mL with Milli-Q water. The digested acid solutions were then filtered by using syringe filter (DISMIC-25HP PTFE, pore size = 0.45 µm; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and stored in 50 mL polypropylene tubes (Nalgene, NY, USA).

## 2.4 Instrumental Analysis

Samples were analyzed using inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700 series, USA). The internal calibration standard solution was added into each sample. Working standards (0, 10, 20, 50, and 100 µg L<sup>-1</sup>) were prepared by dilution of the

arsenic standard solution, then the concentrations of arsenic were determined by an internal standard method. The arsenic concentration in raw and cooked rice were based on the dry weight of rice. All test batches were evaluated using an internal quality approach and validated if they satisfied the defined Internal Quality Controls (IQCs). For each batch experiment, one blank, one certified reference material (CRM) and several samples were analyzed in duplicate to eliminate any batch-specific error. Finally, the arsenic concentration was quantified by calibration based on internal standards.

## 2.5 Quality Assurance and Quality Control

Arsenic standard solution was used to prepare a calibration curve. The calibration curves with R<sup>2</sup>>0.999 were accepted for concentration calculation. Before starting the sequence, relative standard deviation (RSD < 5%) was checked by using the tuning solution. The quality of quantitative analysis for arsenic in the rice was checked by using a certified reference material (NMIJ CRM 7402-a, Cod fish tissue) purchased from the National Institute of Advanced Industrial Science and Technology (AIST) and yielded a good accuracy of the analysis. The comparison is made with the certified values, which in both cases confirmed that the sample preparation and instrumentation conditions provided good levels of accuracy and precision.

## 2.6 Risk Assessment

Estimated daily intake (EDI) for the arsenic was calculated by multiplying the respective average concentration in rice sample by the average weight of rice consumption per day dividing by the average body weight of Bangladeshi. EDI were calculated by the following equation:

$$EDI = \frac{FIR \times C}{BW} \quad (1)$$

where FIR is the rice ingestion rate (420 g day<sup>-1</sup> for adult), C is the arsenic concentration in rice (µg g<sup>-1</sup>), and BW is the average body weight (59 kg for adult). In this study, the carcinogenic health risk related with the consumption of rice was measured based on the target cancer risk (TR) or the risk of cancer over a lifetime. For carcinogens, a risk was estimated as the incremental probability of an individual to develop cancer over a lifetime exposure to that potential carcinogen (i.e., incremental or excess individual

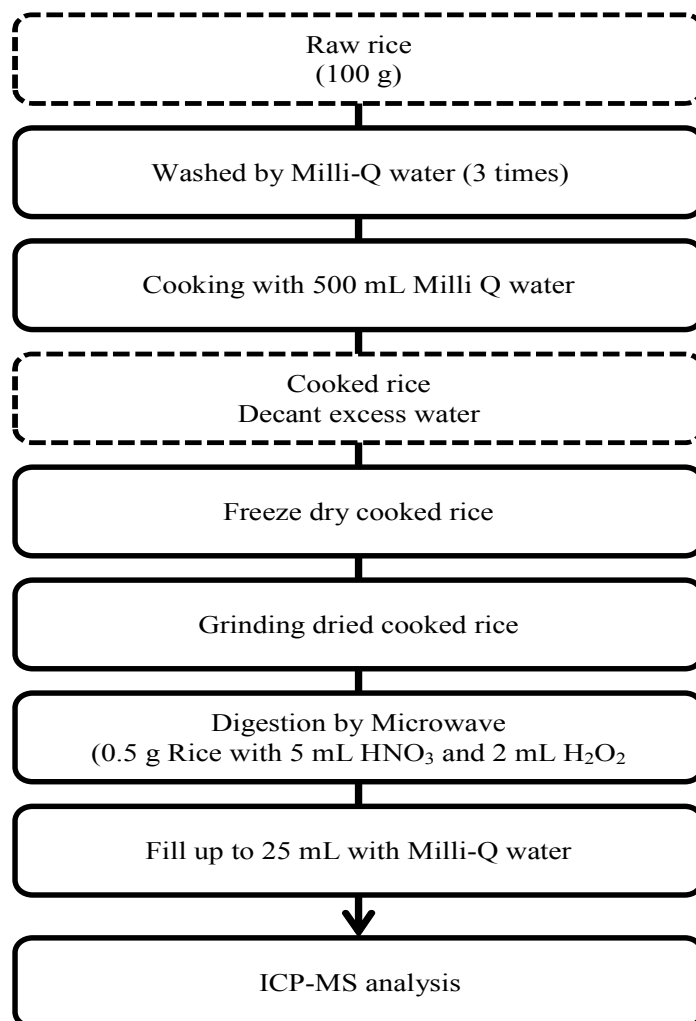


Fig. 1. Analysis procedures of arsenic in rice during cooking

lifetime cancer risk) [17]. Acceptable risk levels for carcinogens range from  $10^{-4}$  (risk of developing cancer over a human life time is 1 in 10,000) to  $10^{-6}$  (risk of developing cancer over a human lifetime is 1 in 1,000,000). The equation used for estimating the target cancer risk (lifetime cancer risk) is as follows [17].

$$TR = \frac{EF \times ED \times EDI \times CSF_o}{TA} \times 0.001 \quad (2)$$

where TR represents the target cancer risk or the risk of cancer over a lifetime, EF is the exposure frequency ( $365 \text{ day year}^{-1}$ ), ED is the exposure duration (60 year for Bangladesh) equivalent to the average lifetime, TA is the averaging exposure time ( $365 \text{ day year}^{-1} \times ED$ ), and CSF<sub>o</sub> is the oral carcinogenic slope factor from the

Integrated Risk Information System database ( $1.5 \text{ mg}^{-1} \text{ kg day}$  for As) [18].

### 3. RESULTS AND DISCUSSION

#### 3.1 Arsenic Concentration in Rice

The As concentrations in raw rice of Minikit and Kataribhogh in this study were  $0.123\text{--}0.153 \mu\text{g g}^{-1}$  ( $0.139 \pm 0.012 \mu\text{g g}^{-1}$ ) and  $0.611\text{--}0.783 \mu\text{g g}^{-1}$  ( $0.721 \pm 0.077 \mu\text{g g}^{-1}$ ), respectively. The arsenic concentration in rice varied widely depending on levels of As in irrigation water, soil, season and rice varieties (Table 1). The grain arsenic content of collected rice varieties is quite high compared to the values available for rainfed rice in the literature indicating that the rice might

have grown in highly arsenic contaminated soils of Bangladesh.

The total As contents in cooked rice of Minikit and Kataribhogh were  $0.115 \pm 0.004 \mu\text{g g}^{-1}$  and  $0.386 \pm 0.004 \mu\text{g g}^{-1}$ , respectively. There was 17% and 46% reduction of As due to washing and cooking of Minikit and Kataribhogh rice, respectively (Table 2). Kataribhogh, a fine grained aromatic rice when cooked showed higher removal of As than the Minikit, a long slender rice. Jitaru et al. [23] reported that there was 49% loss of As due to cooking from an aromatic white Basmati. During washing the raw rice, outer side of the rice could be sheared off by rubbing where the concentration of As is higher in rice grains. Besides, during cooking the inorganic As from raw rice might have dissolved in cooking water which was decanted after cooking. Rahman et al. [12] carried out a cooking study on BRR1 dhan28 and BRR1 hybrid dhan1 and reported that As in the cooked rice of BRR1 dhan28 and BRR1 hybrid dhan1 reduced by 30% and 16%, respectively. Sengupta et al. [14] compared the three major rice-cooking procedures followed globally and confirmed that total As content in cooked rice depends strongly on the method of cooking rice. They reported up to 57% reduction of total As from rice by washing until clear (5 or 6 times) and high-volume (water: rice of 6:1) cooking followed by discarding excess water. Mihucz et al. [22] demonstrated that total As content in two types (one brown and one white) of Hungarian rice and one type of Chinese white rice were reduced to a mean of 54% (ranging from 42% to 61%) of that in raw rice by washing rice with deionized water three times and high-volume cooking (water: rice ratio 6:1) followed by decanting of excess water. These results indicate that decanting of excess

water after high-volume cooking effectively decreases total As in rice. However, As removals from raw rice upon cooking varied widely depending on cooking procedures and rice types as described in Table 2, which ranged from 3–49%.

### 3.2 Risk Assessment

Based on the concentration of arsenic in raw and cooked rice, the carcinogenic risk of arsenic via rice ingestion was estimated. The estimated risk of arsenic with cooked rice was  $1.2 \times 10^{-5}$  and  $4.1 \times 10^{-5}$  while that raw rice was  $1.5 \times 10^{-5}$  and  $7.7 \times 10^{-5}$  for Minikit and Kataribhogh rice, respectively. Compared with acceptable risk levels for carcinogens ( $10^{-4}$  to  $10^{-6}$ ), estimated risks were comparable, which implied that the arsenic exposure with rice consumption could be significant. The results in this study revealed that the cooking could lower 17% and 46% of the arsenic exposure and risk due to consumption Minikit and Kataribhogh rice. There are a number of methods for decreasing As in rice grains namely use of arsenic free surface water and water from deep tube wells, use of alternate wetting and drying technique, cultivation of rice genotypes taking up less As from soil, and use of silicon fertilizers. All the methods have limitations for their use in the real situation. However, still if the rice grains have elevated arsenic levels, the last chance to reduce the arsenic levels in rice is by proper washing, cooking with arsenic free water and decanting excess water after cooking. This is easy to practice and easy to be disseminated among the farm families. Therefore, the impact of this method to reduce human arsenic intake via rice consumption in Bangladesh could be significant.

**Table 1. Arsenic concentrations in raw rice of Bangladesh**

Growing condition	Number of samples	Range of arsenic concentration ( $\mu\text{g g}^{-1}$ )	Mean As concentration ( $\mu\text{g g}^{-1}$ )	Reference
Irrigated	78	0.108–0.311	0.183	Duxbury et al. [19]
Rainfed	72	0.072–1.70	0.117	
Irrigated	13	0.058–1.835	0.496	Meharg and Rahman [20]
Irrigated	10	0.04–0.270	0.13	Das et al. [21]
Irrigated	35	0.05–2.05	-	Islam et al. [1]
Irrigated	133	0.040–0.910	-	Williams et al. [4]
Rainfed	189	<0.040–0.920	-	
Irrigated and rainfed	965	0.003–0.680	0.126	Islam et al. [11]
Irrigated	23	0.047–0.506	0.1523	Jahiruddin et al.[5]
Rainfed	48	0.047–0.535	0.140	
Rainfed	Minikit	0.123–0.153	0.139	This study
Rainfed	Kataribhogh	0.611–0.783	0.721	

**Table 2. Arsenic concentration in cooked rice and removal rates by cooking**

Rice variety	Cooking technique	Raw rice as conc. ( $\mu\text{g g}^{-1}$ )	Cooked rice As conc. ( $\mu\text{g g}^{-1}$ )	As removal (%)	Reference
BRR1 dhan28	Washing - 3 times	$0.57 \pm 0.04$	$0.39 \pm 0.04$	32	Rahman et al. [12]
BRR1 hybrid dhan1	Water: rice ratio - 5:1 Excess water – Decanted	$0.69 \pm 0.21$	$0.44 \pm 0.03$	36	
Zhenshan 97	Washing - 3 times Water: rice ratio - 3:1 Excess water – Decanted	$0.171 \pm 0.007$	$0.103 \pm 0.004$	40	Mihucz et al. [22]
Basmati	Washing- 2 times Water: rice ratio - 6:1 Excess water- Decanted	$0.162 \pm 0.003$	$0.103 \pm 0.005$	36	Raab et al. [15]
White Basmati	Washing - 6 times Water: rice ratio - 3:1 Excess water - Evaporated to dry	$0.129 \pm 0.009$	$0.064 \pm 0.017$	49	Jitaru et al. [23]
Koshihikari	Washing - 3 times Water: rice ratio - 2:1 Excess water - Evaporated to dry	$0.501 \pm 0.010$	$0.487 \pm 0.009$	3	Naito et al. [13]
Minikit	Washing - 3 times	$0.139 \pm 0.012$	$0.115 \pm 0.004$	17	This study
Kataribhogh	Water: rice ratio - 5:1 Excess water - Decanted	$0.721 \pm 0.007$	$0.386 \pm 0.004$	46	

#### 4. CONCLUSION

Cooking of Minikit and Kataribhogh raw rice with Milli-Q water: grain ratio (5:1) and decanting the excess cooked water reduced the total As by 17% and 46%, respectively compared to the As concentration in raw rice. The carcinogenic risk with consumption of cooked rice of Minikit was  $1.2 \times 10^{-5}$  while for Kataribhogh rice was  $4.1 \times 10^{-5}$ . The results in this study revealed that the cooking rice lowers the arsenic exposure and risk of cancer for humans compared to raw rice.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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