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Arsenic speciation as well as toxic and nutrient elements in pantavat (overnight steeped rice)☆

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ABSTRACT

This study assessed the effect of soaking on the retention and removal of arsenic (As) along with other toxic elements and nutrients in three types of soaked rice or overnight steeped rice (*pantavat*), as this food dish was highlighted on the Australian MasterChef program in 2021 as a popular recipe. Results showed that brown rice contained twice as much As as basmati and kalijira rice. Cooking with As-free tap water using a rice cooker removed up to 30% of As from basmati rice. Around 21–29% removal of total As was observed in soaked basmati, brown, and kalijira rice. However, while 13% of inorganic As was removed from basmati and brown rice, no changes were noted in the kalijira rice. Regarding nutrient elements, both cooking and soaking rice caused significant enrichment of calcium (Ca) whereas potassium (K), molybdenum (Mo) and selenium (Se) were reduced substantially for the tested rice varieties. The nutrients like magnesium (Mg), iron (Fe), sulfur (S) and phosphorus (P) did not significantly change. The results indicated that soaking can minimize up to 30% As and soaked rice reduced few nutrients like K, Mo and Se. Data in this study highlights the retention and/or loss of toxic and beneficial nutrient elements in *pantavat* when As-free water is used to prepare this food.

1. Introduction

Pantavat (soaked rice or overnight steeped rice) is a common breakfast food that millions of inhabitants throughout Asia consume, especially in hot and humid climates. Consumption of *pantavat* can be observed in most families living in areas affected by arsenic (As) in rural regions of India and Bangladesh. Traditionally, the Bengali populace consume *pantavat* with fried hilsa fish and varieties of bhorta (various mixtures of mashed herbs, vegetables, or fish that are typically blended with mustard oil, garlic, onions, and red chili). Generally, cooked rice is soaked in water overnight and consumed at breakfast with mashed potato and/or cooked vegetables along with onion, fried chili and salt. *Pantavat* was first recognized as an important food exposure pathway of As for rural villagers in the Bengal Delta region in 2001 (Chowdhury et al., 2001). Cooked rice soaked overnight in As-contaminated water results in considerable amounts of As absorption by rice (Chowdhury et al., 2001). This poses a significant risk to the rural population since both rice and water usually contain toxic and carcinogenic inorganic As (arsenite and arsenate). Both are linked to various health problems including cancers, cardiovascular disease, and DNA damage (Banerjee et al., 2013; Biswas et al., 2020; Joardar et al., 2021; Karagas et al., 2019; Sobel et al., 2020).

Exposure to As from *pantavat* varies from region to region. Adults usually consume 250 g of *pantavat* every morning, according to a study conducted in a Kolsur village situated in West Bengal, India (Chowdhury et al., 2001). This study showed that the daily As burden from *pantavat* was 368 µg for an adult (268 µg from cooked rice and 100 µg from water added for *pantavat* preparation), whereas this value was 318 µg for children up to 11 years of age (Chowdhury et al., 2001). Another West Bengal based study showed that adults consume 307 µg of As from

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pantavat (174 μg from rice + 133 μg from water used for *pantavat* preparation) in Jalangi and 275 μg (175 μg from rice + 100 μg from water used for *pantavat* preparation) in Domkal in Murshidabad (Roychowdhury et al., 2003). For children, the As consumption originates from *pantavat* was 159 μg and 143 μg in Jalangi and Domkal, respectively (Roychowdhury et al., 2003). It was also reported that for adults exposure to As in Nadia, India, amounted to 187 μg (135 μg from rice and 52 μg from water used for preparing *pantavat* and other foods), while for children, the value was almost half (98.2 μg) (Roychowdhury et al., 2008). Except for these three articles, no information on As in *pantavat* is available in the literature. Since 2008, research on As in *pantavat* has been overlooked; this warrants further investigation, especially the fate and transformation of As when As free water is used for cooking and soaking. Hence, this is the first attempt to investigate As, other toxic and nutrient elements in *pantavat* and the impact of cooking and soaking rice with As free tap water.

However, the custom of eating *pantavat* was not confirmed in two rural communities of northwestern Bangladesh (Watanabe et al., 2004) although, there is no doubt that *pantavat* is an important food component in the Bengali population especially during *Pohela Boishak* (Bengali New Year). It has been shown that *pantavat* contains substantial amounts of As when As-containing water is used for preparation (Roychowdhury et al., 2003). To date, no single study has investigated total and inorganic As concentrations and other toxic elements along with retention and/or loss of beneficial nutrient elements in *pantavat*, especially when As-free water is used to prepare this food. Therefore, it is crucial to investigate the composition of toxic elements and beneficial nutrients in *pantavat*. This is particularly important given that this ethnic dish was highlighted to a very popular television show (Australian MasterChef TV program in 2021), after which many Asian migrants in Australia prepared and consumed this food and posted their experience on social media. *Pantavat* is not consumed globally and mainly consumed in Bengal delta regions of Bangladesh and India. Both regions are well recognized as worst As hot spots and millions of populace are exposed to As from two major exposure pathways (drinking water and rice). As half of the world population rely on rice-based diets and *pantavat* dish is made of cooked rice that is soaked in water overnight, so it is crucial to investigate the contents of As and other toxic and nutrient elements in *pantavat* due to the growing interest on ethnic cuisines in many cooking shows. There is a lack of studies on the retention and/or loss of toxic and essential elements in soaked rice. Thus, this is the first study we investigated the reduction/retention of toxic and essential elements from rice after cooking and then soaking.

Here, we quantified the amount of As (both total and speciated), other toxic and nutrient elements in *pantavat* when cooked and soaked with As-free water. To do this, three rice types commercially available in the Australian market were employed. Our gained results will advance our knowledge on As exposure from soaked rice where drinking water with As contamination is not widespread.

2. Experiment

All experiments were performed in triplicate. Three different type of rice (long grain Indian Basmati rice, medium grain Australian brown rice, and aromatic and short grain Bangladeshi Kalijira rice) were purchased from a local Australian market and analyzed for total As, inorganic As, other toxic elements including cadmium (Cd), chromium (Cr), manganese (Mn) and lead (Pb) and beneficial nutrient elements including calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), sulfur (S), iron (Fe), molybdenum (Mo), selenium (Se), copper (Cu) and zinc (Zn). Although Se, Cu and Zn are essential trace elements of the human body, excessive amounts of these elements in food could pose serious risk to human. The tap water used for cooking and preparing the *pantavat* contained most of the elements below the instrument detection limit (<0.5 μg/l) except Mn (1.3 μg/l), Cu (52 μg/l) and Zn (97 μg/l) and were below the guideline values set by the World Health Organization. Washed rice was cooked in a rice cooker using tap water until all the water was absorbed (1:2 ratio). A portion of the cooked rice (~100 g) was soaked with tap water (200 ml) and left overnight. The next morning, portions of uncooked, cooked, and soaked rice were freeze-dried using a freeze dryer. The freeze-dried samples were ground using a mortar and pestle. The powdered samples were analyzed for total As, speciated As (inorganic As – the sum of arsenite [AsIII] and arsenate [AsV]; dimethylarsinic acid [DMA]; and monomethylarsonic acid [MMA]) and other elements using our published methods (Rahman et al., 2021; Rahman and Naidu, 2020). The rice samples were digested using a microwave acid digestion system as per the procedure of Islam et al. (2017) followed by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer, NexION 350, USA) analysis. High-performance liquid chromatography (HPLC, Agilent 1200) coupled with ICP-MS (Agilent 7900) was used for As speciation analysis (Islam et al., 2017). Appropriate quality assurance and quality control were used during digestion, extraction and analysis and this was reported elsewhere (Rahman et al., 2021). We used IBM SPSS Statistics Data Editor version 28 for statistical analysis and OriginPro 2023b was used for plotting the graphs. We performed one-way analyses of variance (ANOVA) and Tukey multiple comparison tests were used in comparison to uncooked rice data with a significance level of 0.05.

3. Results and discussion

3.1. Total and speciated As in uncooked, cooked and soaked rice

Mean As concentrations in uncooked basmati, brown and kalijira rice were 91, 180 and 74 μg/kg, respectively (dry weight, dw) (Fig. 1). Brown rice had higher concentrations of As than basmati and kalijira rice. Generally, brown rice is known to have more As than white rice and it contains many more nutrients (Lee et al., 2019; Rahman et al., 2014; Saleh et al., 2019).

Previously it was reported that the mean As concentration in Indian and Pakistani basmati rice was 61 (range: 30–87) μg/kg, dw (Rahman et al., 2009). Thus, the mean concentration of As in basmati rice in this study was higher than that found in the previous study (Rahman et al., 2009). Arsenic concentrations in three kalijira rice samples from Matlab, Bangladesh were 100, 130 and 80 μg/kg, respectively (Sandhi et al., 2017), which were somewhat higher than those found here. Kalijira rice had almost two-fold less As than that found in the previous study (Rahman et al., 2014). Brown rice (medium grain) had considerably less

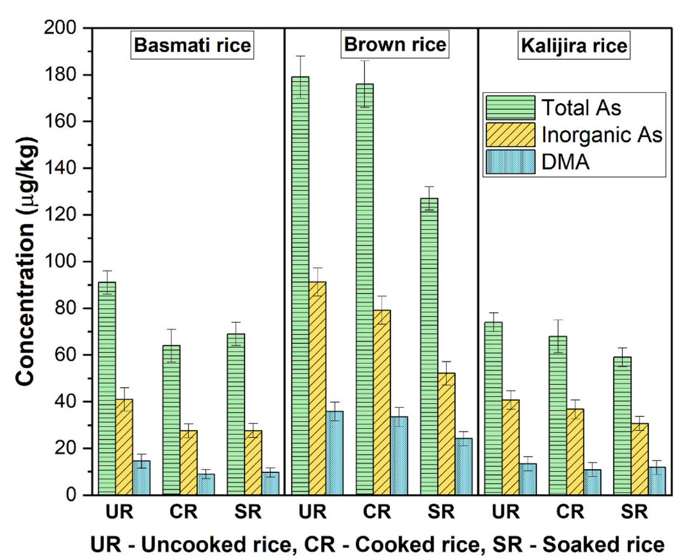

Fig. 1. Concentrations of total As, inorganic As and DMA and in uncooked, cooked and soaked rice.

As compared with the previous analysis (287 μg/kg), conducted in Australia (Rahman et al., 2014).

The average As concentrations in cooked basmati, brown and kalijira rice were 64, 176 and 68 μg/kg, respectively. Thus, the reduction in As in cooked rice was significant [30% for basmati ($F = 794$, $p < 0.001$), 2.2% for brown ($F = 1009$, $p < 0.001$) and 8% for kalijira rice ($F = 238$, $p < 0.001$)] as a result of cooking rice with tap water. One study noted that there was no removal of As from cooked rice when the rice was cooked with <3 μg/l of As-containing water (rice:water ratio was 1:1.5 or 2) without washing and with no water discarded (Sengupta et al., 2006). In this study, when rice was soaked with tap water, As concentrations were removed significantly ($p < 0.001$) by 23, 29 and 21% for basmati, brown and kalijira rice, respectively, compared with uncooked rice. However, when we compared the cooked and soaked rice, As removals were 27 and 13% in brown and kalijira rice, respectively, although no variation was observed for basmati rice. It should be noted that previous research has reported that low and moderate concentrations of As in cooking water significantly reduced the As contents in cooked rice (Chowdhury et al., 2020). For example, when rice from West Bengal in India was cooked with low-As containing water (<3 μg/l), As reduction was significant for both sun dried rice (34–89%) and parboiled rice (23–84%) (Chowdhury et al., 2020).

Rice is the staple food in many countries, and it is also a substantial benefactor to inorganic As exposure to many populations who rely on rice based diets. As adult in south Asian countries consume around 430 g of rice daily (Rahman et al., 2019; Rahman et al., 2009), rice consumption is a crucial source of inorganic As and other toxic elements to human. One of the most relevant findings of this study is that the total As was removed up to 30% in basmati rice when cooked with As-safe water (As<0.5 μg/l). Another finding is that soaking of rice can remove 21–29% As from rice.

Arsenic speciation of uncooked, cooked and soaked rice (Fig. 1) was conducted; on average 69% speciated As (sum of inorganic As and DMA) was extracted compared with total As. The percentages of inorganic As for uncooked basmati, brown and kalijira rice were 45, 51 and 55%, respectively, whereas DMA accounted for 16, 20 and 18%, respectively. Furthermore, MMA was not detected. Inorganic As in cooked rice was reduced significantly by approximately 13, 12% and 4% for basmati ($p < 0.001$), brown ($p < 0.001$) and kalijira rice ($p < 0.01$), respectively, compared with uncooked rice. No significant levels of inorganic As or DMA reduction were observed in cooked and soaked basmati and kalijira rice. Regarding the brown rice sample, the concentrations of inorganic As and DMA reduction in soaked rice were around 27 and 9%, respectively, compared with cooked rice. In the previous study, it was found a 1–17% reduction in inorganic As in 10 raw rice samples from Bangladesh when cooked in a rice cooker with a 1:2 rice-to-water ratio after being washed three times (Shahriar et al., 2022). Another study reported that cooking rice with deionized water (1:6 ratio) can remove approximately 35 and 45% of the total and inorganic As concentrations from long-grain and basmati rice, respectively (Raab et al., 2009). Similarly, cooking rice with excess deionized water (1:10 rice-to-water ratio) removed inorganic As contents by 40, 60, and 50% in long-grain polished, parboiled, and brown rice, respectively (Gray et al., 2016). Consequently, rice type and cooking practice influenced the removal of inorganic As from both cooked and soaked rice.

The maximum recommended level of inorganic As for milled, white or polished rice is 200 μg/kg for adults by the European Food Safety Authority (EFSA) (EFSA, 2021). However, the value is 100 μg/kg in rice used for the manufacture of foods for infant and young children (EFSA, 2021). The cocentrations of inorganic As in all rice tested in this study were below the maximum level (100 μg/kg) of EFSA. Although uncooked, cooked and soaked rice of all 3 tested samples showed lower inorganic As values set by the EFSA, the risk cannot be ignored for prolonged exposure.

3.2. Concentrations of other elemental (toxic and nutrients) composition in uncooked, cooked and soaked rice

Fig. 2 shows the concentrations of toxic and beneficial elements in uncooked, cooked, and soaked rice of the three selected rice types (basmati, brown and kalijira). For toxic elements, no differences were observed in the concentrations of Mn and Cd in uncooked, cooked and soaked rice (Fig. 2a). The concentrations of Cr and Pb were slightly enriched in both cooked and soaked rice compared with uncooked rice (Fig. 2a). The enrichment of Cr and Pb could be due to the lead pipes which carry these elements in tap water (Harvey et al., 2016). Cadmium pollution in rice has emerged as a major exposure pathway in many countries, including China, Bangladesh, India, Sri Lanka, and Iran where Cd is inadvertently added to the soil through the application of phosphatic fertilizer (Meharg et al., 2013; Shahriar et al., 2020; Shi et al., 2020).

Regarding beneficial nutrient elements, Ca was substantially enriched by 110, 94 and 53% in cooked rice, and 242, 146 and 146% in soaked rice for basmati, brown and kalijira, respectively, compared to uncooked rice (Fig. 2b). The Ca enrichment in both cooked and soaked rice was significant for basmati ($F = 424$, $p < 0.001$) and brown rice ($F = 316$, $p < 0.001$) compared to uncooked rice. Potassium significantly ($p < 0.001$) declined by 13, 8 and 9% in cooked rice whereas it was 47, 23 and 27% in soaked rice for basmati, brown and kalijira, respectively, compared with uncooked rice (Fig. 2b). The loss of K in soaked rice was almost triple for all examined rice samples compared with cooked rice. No significant difference was observed for Mg, Fe or P between cooked and soaked rice derived from the examined rice brands (Fig. 2b). S enrichment amounted to 4% only whereas Mo significantly ($p < 0.001$) decreased in both cooked rice (9–20%) and soaked rice (25–33%). A past study from Bangladesh showed that Mo in parboiled rice was significantly reduced by 20% compared to non-parboiled and rough rice (Rahman et al., 2019). A substantial reduction of Se (52–90%) was observed in both cooked and soaked rice ($p < 0.001$) compared with uncooked rice although no difference was observed between cooked and soaked rice (Fig. 2b). Cu enrichment in cooked rice was not significant; however, it reached 29% in soaked rice while Zn in both cooked and soaked rice was virtually unchanged (Fig. 2b).

It has been noted that past studies investigated soaking treatment of rice with water to remove toxic elements before cooking. For example, a study showed that soaking rice with water at 70 °C for 4 h reduced 37% of As in japonica rice and 33% in indica rice (Zhang et al., 2020). When rice was rinsed 3 times with water, 32%–66% reduction of Cd was observed in uncooked rice (Al-Saleh and Abduljabbar, 2017; Ziarati and Azizi, 2014). Washing followed by soaking for 3 h reduced 18% and 7% of As and Cd, respectively, from raw rice (Adibi et al., 2014). The removal of elements from raw rice usually depends on the washing times as well as soaking duration and temperature (Adibi et al., 2014).

Mineral elements play a crucial role in human metabolism and minimize malnutrition and its related diseases. In the present study, a significant reduction of the content of K, Mo, Se by cooking and soaking was observed, while the content of Mg, Fe, S and P was not significantly affected. The concentration of Cd and Mn in tested rice did not vary while Cr and Pb levels were somewhat supplemented. The enrichment of Ca and the presence of toxic Cr and Pb in rice could be sourced from water used for cooking and soaking, which warrants further investigation. As a result, this study discovers that cooking and soaking of rice with As-safe water could reduce the toxic elements in rice except Cr and Pb, however, also reduce some essential elements.

It is important to note that previous studies have shown that cooking may affect the elemental contents including inorganic As in cooked rice (Shahriar et al., 2022). Rice can be cooked using different cooking methods with varying rice to water ratio and this process can alter the elemental concentrations in rice (Shraim et al., 2022). Previously it was reported that rinsing and cooking may vary toxic elements such as As, Cd and Pb in cooked rice (Shahriar et al., 2022).

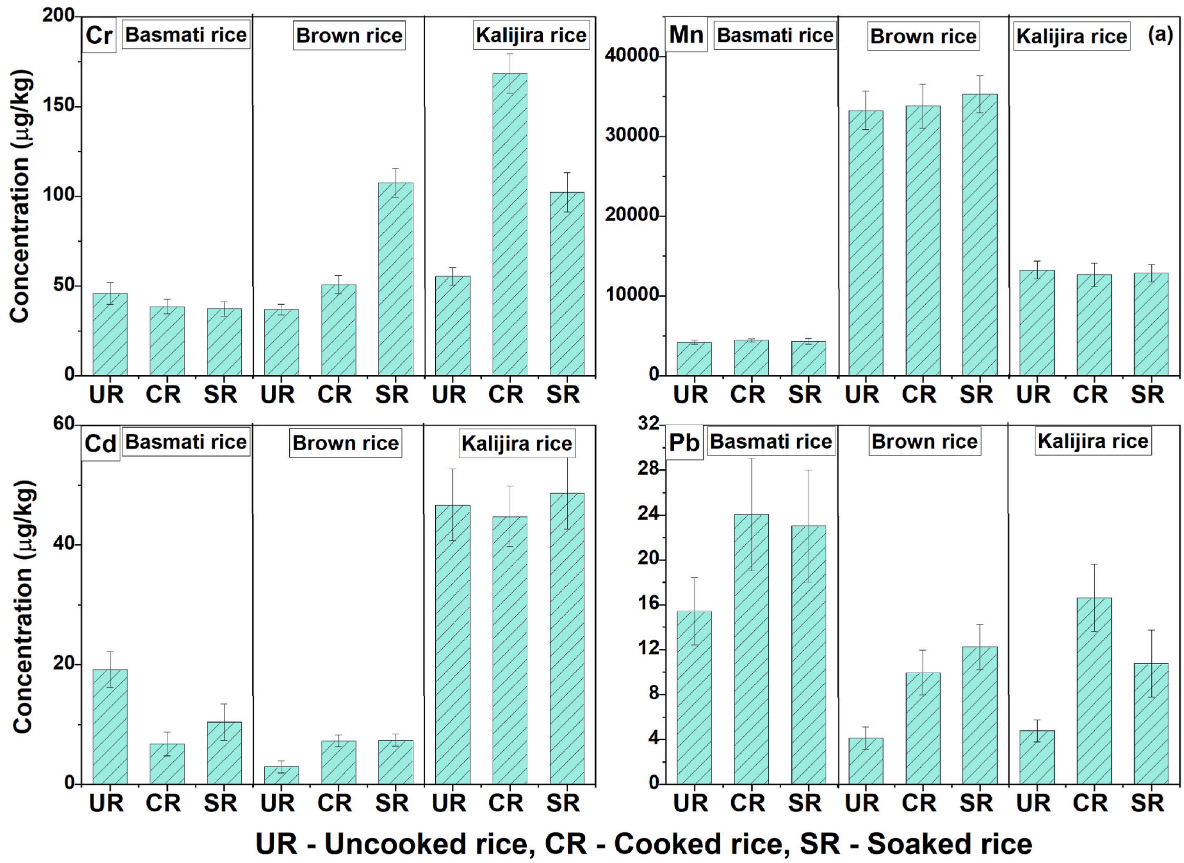

Fig. 2a. Concentrations of toxic elements (Cr, Mn, Cd and Pb) in uncooked, cooked and soaked rice.

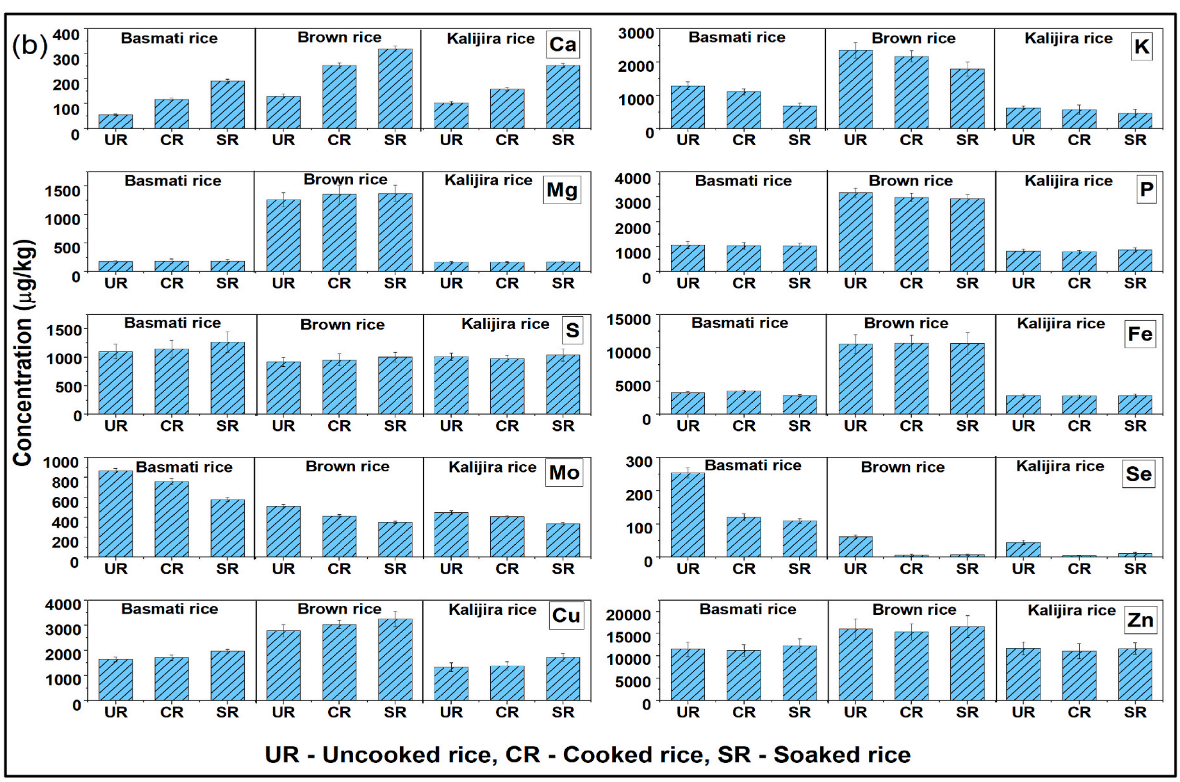

Fig. 2b. Concentrations of major nutrient elements (Ca, K, Mg, P and S) and trace nutrient elements (Fe, Mo, Se, Cu and Zn) in uncooked, cooked and soaked rice.

4. Conclusion

The results demonstrated that the concentrations of total As, inorganic As and DMA did not change in soaked rice when As-safe tap water was used for cooking and soaking. Beneficial nutrients such as Ca were enriched whereas K, Se and Mo declined in cooked and soaked rice. However, the scenario is completely different in As-endemic areas of Bangladesh and India where As-contaminated water is used and provides an important pathway for As exposure to inhabitants. Although Australian potable water is low in As, exposure to toxic inorganic As cannot be ignored by Asian migrants owing to the consumption of contaminated rice sold in Australian markets. We used rice cooker for cooking rice and hence future studies should investigate traditional cooking methods normally used in Bengal delta communities. This study provides new insights into the composition of As, toxic and nutrient elements in soaked rice. In areas such as Australia, where tap water is not contaminated with As, consuming soaked rice shall be safe. However, in regions with contaminated groundwater, such as Bangladesh and India, As exposure increases. In the future, a large-scale analysis should be conducted to explore (i) the effect of various As levels (low, moderate and elevated) for cooking rice types (variety, processing and grain size) using multiple cooking procedures; (ii) any changes in toxic inorganic As species in cooked and soaked rice; (iii) bioaccessibility of As in soaked rice; and (iv) consumers' exposure to As in *pantavat* and the risk involved.

Author contributions

MMR: Conceptualization, Methodology, Investigation, Validation, Visualization, Writing-Original Draft; **JR**: Writing-Reviewing & Editing; **RN**: Writing-Reviewing & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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