Reduction of Arsenic Content in Imported Polished Rice: Association of Cooking Method

Ranna Jafari-Moghadam\textsuperscript{1,2} and Parisa Ziarati *\textsuperscript{1,3}

\textsuperscript{1}Young Researchers & elite Club, Pharmaceutical Sciences Branch, Islamic Azad University, (IAUPS), Tehran, Iran
\textsuperscript{2}Pharmaceutical Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS)
\textsuperscript{3}Department of Medicinal Chemistry, Pharmacy Faculty, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran –Iran

ABSTRACT

Rice is the second food in high consumption among Iranian people. Arsenic (As) in rice is of special concern in countries where it is consumed daily. Rice plants absorb As from soil and water. Total arsenic and arsenic speciation was performed on rice that had undergone various varieties and forms of cooking. Basmati, long-grain, polished (white) imported popular rice brands as well as parboiled rice, were investigated. The effect of rinsing rice samples by different volume of water as well as the effect of soaking rinsed rice samples by NaCl and cooking methods on Arsenic contents were studied by ICP-MS. The minimum and maximum As contents in rinsing rice and cooked polished rice were 0.103 and 0.385 (mg/kg DW) in imported rice variety respectively. It was found here that cooking rice in a large volume of water and soaking rinsed rice samples by NaCl 2\% at least for 2 hours had the greatest effect (significantly affect p<0.003) with regards to lowering arsenic levels in cooked rice. Specifically, it preferentially reduced the inorganic arsenic content by 87\% of that in the raw rice, when combined with rinse washing and being soaked by salt.

Key words: Arsenic, Rice, cooking method, Salt, Rinse, Food safety.

INTRODUCTION

Arsenic (As) is a well-known toxic element that can be present in the environment and foodstuffs. Consequently it is a highly regulated element that is widely monitored. However, As speciation analysis rather than total As analysis is important because its biological toxicity is highly dependent on its chemical form. For example, if a seaweed sample is known to contain a high level of As, but mainly in the form of AsB, then there is no potential risk from its consumption as AsB is non-toxic. Among the five major species of arsenic, As(V), MMA, As(III) DMA and AsB, only the two inorganic forms (As(III) and As(V)) are toxic. They are also carcinogenic to humans, so need to be measured, especially in foodstuffs. Arsenic in rice is of special concern in countries where it is consumed daily. Rice plants absorb As from soil and water. Since rice requires a lot of water to grow compared to other plants, the potential for arsenic accumulation is increased. That FSA report identified a need to examine if the arsenic content from rice could be reduced by the manner in which the rice was cooked\textsuperscript{[1]}. A number of previous studies had suggested that rice cooking was important to the arsenic content of the cooked grain \textsuperscript{[2-7]}. A number of these studies focus on how cooking technique may reduce rice arsenic content, while others focus on how arsenic in cooking water affects arsenic content of cooked rice \textsuperscript{[4-7]}.  

Rice is unusual amongst terrestrial foods, particularly for staple crops, in that that it is grown anaerobically. Under anaerobic conditions arsenic in soil is converted readily to arsenite which is mobile, leading to arsenic in rice grain being around 10-fold higher than for other crops. This occurs in soils which have no or limited anthropogenic contamination. Rice grain arsenic levels elevated further when grown in soils subject to anthropogenic contamination such as: arsenical pesticide use, base and precious mining and smelting impacted soils, and contaminated water irrigated soils [1,8].

Rice, especially white rice, *Oryza sativa* L. is the staple food in the diet of various people including Asian countries [9, 10]. Rice is the second food in high consumption among Iranian people. Half of the world populations consume rice as their main food [11, 12]. It is the commonest crop grown in agricultural lands in the north of Iran [13]. Rice is the seed of the monocot plants (*Oryza sativa*), for example Asian rice or (*Oryza glaberrima*), for example African rice of the family, Graminaeae (grass family) [14]. Environmental contaminants are chemicals that are present in the environment in which the food is grown, harvested, transported, stored, packaged, processed, and consumed. The physical contact of the food with its environment results in its contamination. Several factors may influence contaminant accumulation such as spices, level and duration of contaminant exposure, topography, agricultural field conditions, amending soil with compost made from municipal sewage sludge and potential bioaccumulation [15]. In agricultural crops the main sources of heavy metal contamination are irrigation with contaminated water, metal based pesticides, industrial emissions, fertilizers and transportation, harvesting process, storage, and/or sale of crops [16-18]. As rice is one of the major agricultural products on the national and international market [19], during last decades many researchers have reported heavy metal concentrations, especially for As, Pb, Cd, Cr and the other elements in rice grains from various countries such as Iran, India, China, South and North Korea, Taiwan and Turkey [15, 20-24]. Daily consumption of rice in Asia countries ranges between 158-178 g/person-day [15, 25-26]. Iranian people eat an average of 40 kg of rice every year [27]. Although Iran is eleventh producer of rice at the world with an annual production 2600000 tons in 2010, during the last years the demand for rice has considerably been increased in comparison with its production, as a result, currently Iran is known as one of the large-scale importer of rice countries [28]. In this study rice was observed for its special individual consumption as a staple food in Iran [18] and probable hazards of its heavy metal contents on population health. Rice variety, treatment of rice and diversity of cooking may affect elemental content and intake of heavy metals [10, 15, 19, 20]. This research builds upon: Determination and comparing Arsenic contents in 5 popular imported brands in raw rice samples.

- Investigation the Effect of Cooking and heating processes in studied rice samples on inorganic and total Arsenic contents.
- Apply the best cooking methods in order to avoid the side effects of Arsenic in edible an consumed rice.
- Assess the associated health risk posed to the population through exposure to total Arsenic in imported rice.

**EXPERIMENTAL SECTION**

**Sampling Method**

480 samples of imported rice were purchased randomly from 5 popular brands of recognized rice market in 2015. Two varieties of Basmati (packed in 10 kg portions, 5 portions were mixed before use) and three polished (packed in 5 kg portions, 5 portions, mixed before use) were of Indian origin. Polished long-grain (5 times 5 kg portions, mixed before use) originated according to label from more than one brand. All samples were purchased at the same time from recognized Tehran-markets in 2015. All experiments were conducted with triplicate replication.

**Preparing Method**

The polished rice samples were crushed and sieved through a 30 mesh strainer to make rice flour. 1.00 g of the rice flour sample was weighed into a polypropylene centrifuge tube (50 mL). 15 mL of 0.15 M HNO3 was added and the sample was vortexed for 30 seconds. The tightly capped tube was left to stand overnight at room temperature; it was then placed in an oven at 90 °C for 2.5 hours where it was agitated for 30 seconds, every 30 minutes. After the extraction had finished, the sample was cooled in a refrigerator at 4 °C. The cooled rice flour suspension was centrifuged at 8000 rpm for 15 minutes and then the supernatant was passed through a 0.22 μm polyether sulphide (PES) filter. The filtrate was stored at 4 °C and analyzed within 24 hours to minimize any species inter-conversion. The sample was allowed to sit for 5 minutes with routine agitation. The water was decanted and then the process repeated again with another 500 ml of water and soaking in NaCl solution (2%) for 2 hours. The decanted water was then freeze dried. Dry weight determination was then made on both the raw and rinsed rice by oven drying at 800 °C.
until constant weight was reached. The quantity of freeze dried residue was recorded. Rinse washed rice was used in all subsequent cooking experiments.

The quantity of packet weight used in all boiling experiments was 100 g. Double distilled deionized water was used for the cooking water. All rice, including parboiled, was subject to 3:1 (low volume) water to rice (packet weight) cooking, where the water was cooked to dryness. All rice with the exception of par-boiled, were also subject to 6:1 (high volume) water and rice cooking, where the rice was cooked to eating texture. The residual water was drained off and then freeze dried. Cooked rice was then dried at 800 °C until constant weight was reached. Steamed rice Rinse washed rice (100 g packet weight) was soaked for 2 h in an acid washed 400 mL beaker with 200 mL of double distilled deionized water. On termination of soaking the water was decanted and freeze dried. The steamer was filled with 200 mL of double distilled deionized water and the soaked rice placed on arsenic and lint-free cotton-cloth. Steaming time was 2 times 15 min with stirring in between. Cooked rice was then dried at 800 °C until constant weight was reached.

Total arsenic determination

Digestion Total digestion of the samples was conducted by measuring out 0.5 g dry weight of sample into 50 ml polypropylene centrifuge tubes and adding 2.5 ml of Aristar nitric acid and 4 ml of hydrogen peroxide suprapur, followed by microwave digestion using a CEM Mars5 Microwave system [1]. On digestion the sample was diluted to 25 ml using double distilled deionized water with Rhodium (0.02 mL 10 mg Rh/L) as internal standard.

Analysis Arsenic content was measured using an Agilent 7500c ICP-MS with hydrogen as collision/reaction gas. Extraction Samples were extracted in 1% Aristar nitric acid and 1% (vol/vol) hydrogen peroxide suprapur using a CEM Mars5 microwave system [1]. The supernatant was used for determination of extractable arsenic and As-speciation. This oxidizes arsenite to arsenate, improving chromatographic resolution as arsenate elutes at some distance to MMA and DMA, where arsenite elutes adjacent to MMA and DMA. Analysis Arsenic species were separated on a Hamilton PRP X 100 anion exchange column using phosphate buffer. Internal standard Indium (0.01 mg/kg) in 1% (v/v) nitric acid was added during the analysis via a Tpiece as an internal standard. Standards Solutions (0.1mL) containing known amounts of DMA (10 to 100 μg/kg) was subjected to LC-ICP-MS under the same conditions as the supernatants. Peak areas from these measurements were used to construct a calibration curve. Single species standards DMA, MMA and As (V) were used for identification of species by retention time. Calibration standard solutions containing the five As species at different concentrations (1 ppb, 5 ppb, 10 ppb, 50 ppb, 100 ppb and 200 ppb) were prepared and analyzed. The chromatogram for the 10 ppb calibration standard (5 μL injection) shows the five As species are well separated in 4 minutes. The supernatants (0.1 mL) were used as they were and injected onto the column. Peak areas were used for quantification of As-species.

Total As determination

Every 10th sample was digested in duplicate and measured. Each analytical batch contained procedural blanks, spiked samples (for recovery estimate purposes) and CRM. Data were accepted if the spike recovery was between 80 and 120%. Replicate values for a given sample must have a relative standard deviation of < 20%. The reference material results for each batch should be within the certified range. The limit of detection is defined as three times the standard deviation of the signal from procedural blanks, corrected for sample weight and dilution.

Statistical Method

State differences on the basis of the states ( raw, rinsing, soaking, boiled-drained and cooked) of samples and different sates of cooking (Raw, Rinsed, Drained and cooked) were determined by student t-test. The changes were calculated by one way ANOVA and for analysis of the role of multiple factors univariate analysis was used by SPSS 17. Probability values of <0.05 were considered significant. Concentrations were expressed in terms of mg/Kg on a dry weight basis.

Risk Assessment

To evaluate the potential risk of rice consumption containing the heavy metals, Provisional Tolerable Daily Intake (PTDI) for a 60kg adult person was calculated by the following equation in which C is the Arsenic concentration in rice, Cons is the average consumption of rice in country (110g per capita per day) and BW is body weight of an Iranian adult person (60kg). The output was compared with the WHO/FAO and Iranian standard level.

PTDI = C × Cons / Bw
The Iran standard PTDI limits have been recommended for Cd, Pb and As 0.001, 0.0036 and 0.0021 mg/day/kg Bw, respectively [28-31].

RESULTS AND DISCUSSION

As shown, As(V), As(III) and DMA were found in all 480 rice samples. The inorganic As and DMA content varied significantly among the different brands of rice samples, likely reflecting the As concentration in the growing environment of the original plant. Although the concentration of toxic inorganic As varied among the samples, 56.8% of the rice samples contained lower limit than the upper level of 0.2 mg/kg recommended by Codex Alimentarius Commission.

### Table 1. AsT (total), As(arsenate and arsenite) and Aso (DMA and MMA) concentrations, in Bastami rice cooked in various ways. Data are the averages of 3 replicates. Numbers in italics are the standard deviation (SE) of the mean

<table>
<thead>
<tr>
<th>Rice Type &amp; State</th>
<th>Cooking Method</th>
<th>AsT Content ± SE (µg/kg)</th>
<th>Asi Content ± SE (µg/kg)</th>
<th>Aso Content ± SE (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished Bastami</td>
<td>Raw</td>
<td>198 ± 4</td>
<td>98 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td></td>
<td>Raw Washed</td>
<td>140 ± 2</td>
<td>85 ± 2</td>
<td>19 ± 1</td>
</tr>
<tr>
<td></td>
<td>Raw Washed 3:1 Water: Rice and Soak with NaCl 2%</td>
<td>127 ± 2</td>
<td>51 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td></td>
<td>Raw Washed 6:1 Water: Rice and Soak with NaCl 2%</td>
<td>122 ± 1</td>
<td>48 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td></td>
<td>Boiled Rice after washing and soaking in salt solution for 2 hours</td>
<td>112 ± 2</td>
<td>56 ± 2</td>
<td>18 ± 1</td>
</tr>
<tr>
<td></td>
<td>Cooked Rice (Separating Water from Rice During Cooking Method) after washing and soaking in salt solution for 2 hours</td>
<td>103 ± 1</td>
<td>52 ± 1</td>
<td>16 ± 2</td>
</tr>
</tbody>
</table>

### Table 2. AsT (total), As(arsenate and arsenite) and Aso (DMA and MMA) concentrations, in Polished long-grain rice cooked in various ways. Data are the averages of 3 replicates. Numbers in italics are the standard deviation (SE) of the mean

<table>
<thead>
<tr>
<th>Rice Type &amp; State</th>
<th>Cooking Method</th>
<th>AsT Content ± SE (µg/kg)</th>
<th>Asi Content ± SE (µg/kg)</th>
<th>Aso Content ± SE (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished long-grain</td>
<td>Raw</td>
<td>385 ± 3</td>
<td>195 ± 3</td>
<td>91 ± 2</td>
</tr>
<tr>
<td></td>
<td>Raw Washed</td>
<td>340 ± 3</td>
<td>180 ± 2</td>
<td>86 ± 1</td>
</tr>
<tr>
<td></td>
<td>Raw Washed 3:1 Water: Rice and Soak with NaCl 2%</td>
<td>237 ± 4</td>
<td>123 ± 1</td>
<td>77 ± 2</td>
</tr>
<tr>
<td></td>
<td>Raw Washed 6:1 Water: Rice and Soak with NaCl 2%</td>
<td>201 ± 1</td>
<td>114 ± 2</td>
<td>75 ± 1</td>
</tr>
<tr>
<td></td>
<td>Boiled Rice after washing and soaking in salt solution for 2 hours</td>
<td>232 ± 3</td>
<td>156 ± 2</td>
<td>88 ± 1</td>
</tr>
<tr>
<td></td>
<td>Cooked Rice (Separating Water from Rice During Cooking Method) after washing and soaking in salt solution for 2 hours</td>
<td>203 ± 2</td>
<td>152 ± 1</td>
<td>66 ± 1</td>
</tr>
</tbody>
</table>

All rice types tested for high volume cooking (6:1 water to rice ration), that is all the non-parboiled types tested, considerably reduced both total and inorganic arsenic content. There was no reduction in organic arsenic content on high volume cooking. Total arsenic content was reduced to 62.4% of raw rice content following rinsing and high volume cooking, ranging from 55% in whole grain basmati to 68.3% in polished Khaterah brand long-grain. This reduction was on average 59.5% for inorganic arsenic content, ranging from 50.6% for polished long-grain to 63.8% for polished basmati. Even though the rinse washing was not significantly effective for both types of the long-grain rice, high volume cooking water and soaking in NaCl solution (2%) for 2 hours, reduced inorganic arsenic contents to those of basmati rice where rinsing was more effective. This suggests that high volume cooking and soaking in NaCl solution (2%) for 2 hours, by itself is enough to reduce total and inorganic arsenic content, though rinse washing is normally recommended as part of the preparation of rice.

There was variation in the effectiveness of rinse washing in removing total/inorganic arsenic from raw rice (Table 1 & 2). Washing and soaking in NaCl solution (2%) for 2 hours, removed more total arsenic for both the polished (to 87% of raw rice content) and wholegrain (to 85% of raw rice content) basmati, while for all other rice percentage arsenic remaining ranged only from 36-49% of raw rice content, including parboiled. It appears that rinse washing
is more effective for basmati rice than for other types of rice, though more samples would need to be analyzed to confirm this. Virtually all the arsenic lost was inorganic while (90% on average of raw rice concentration).

The results of arsenic contents in 480 samples of raw, rinsing, soaking by NaCl 2%, boiling – drained and cooking rice samples are shown in figure 2. All concentrations are expressed as mg/kg DW. Results show that the mean content of Arsenic in the most samples from samples is much higher than maximum levels set by national standard and FAO/WHO. ANOVA analysis showed that there was a significant difference in As contents in Rinsing and raw and drained rice samples (p < 0.01 and p < 0.05 respectively). The minimum and maximum As contents in all studied samples of rice were 0.103 for Cooked Rice (Separating Water from Rice During Cooking Method) after washing and soaking in salt solution for 2 hours and 0.385 (mg/kg DW) for raw polished long-grain imported rice variety respectively.

The results revealed that all raw, rinsing, soaking, boiling - drained and cooked rice and polished rice samples (82.1%) had total arsenic content above maximum level 0.2 mg/kg and in all samples except 35% of samples (in rinsing and soaking with NaCl states), the concentrations of As were over than maximum level which is recommended by FAO/WHO Expert Committee on Food Additives and national standard [31,32].

The most comparable to the present study, though more limited in cooking treatments and rice types, was a high water rice (6:1) investigation conducted by Mihucz et al. [2-4]. Two Hungarian and one Chinese rice types, for none of which it was recorded if the rice was wholegrain or polished, were used in that study. They found a 42-63% reduction in total arsenic in cooked rice, with the cooking liquor containing most of the removed arsenic from the rice (26-49%), while the quantity removed by rinse washing was less (8-17%). It was found that raw rice contained both arsenate and arsenite, and it was primarily arsenite that was removed from the rice on rinsing and boiling. Arsenite (As3+) is uncharged at physiological pHs and hence more mobile than arsenate (As5+) or DMA (As5+), both of which are anionic. The DMA findings in that study [2-3] confirm our results. In another comparable study, three West Bengali samples, where rice polishing state was not recorded, were rinse washed and then cooked in a large water volume [3]. Total arsenic, not speciation was determined. The rinse washing step was more exhaustive, involving 5-6 rinses until the rinse water discard was clear, the traditional Indian preparation, rather than double rinse wash step used in the experiments reported here. The rinse wash step removed 28% of the arsenic compared to raw rice. Combined rinse washing and large volume (6:1 water : rice) reduced arsenic up to 58% of raw rice content. This is compared to an average of 87% removal of As by NaCl (2%) reported in the current study. The increased efficiency of removal for the Indian rice study of that study may be due to more exhaustive rinse washing, or due to the intrinsic nature of the rice used in that study. 14 Other studies have been conducted on the effects of cooking rice on arsenic content, but have focused on the impact of arsenic contaminated cooking water on rice arsenic burdens [4-8]. The low level in basmati rice is confirmed in other studies, such as Kuramata et al., in 2013 [33] and Food and Drug Administration (2013)[34], which also shows lower levels for jasmine rice.

**CONCLUSION**

Rice absorbs arsenic from the ground and water and this absorption is dependent on the rice type itself and content in the soil rather than on whether the rice is produced organically or not. The more arsenic there is in the ground where the rice is growing, the more arsenic there will be in the rice grain. It was found here that cooking rice in a large volume of water (6:1 water : rice) and soaking rinsed rice samples by NaCl 2% at least for 2 hours had the greatest effect with regards to lowering arsenic levels in cooked rice. Specifically, it preferentially reduced the inorganic arsenic content by 87% of that in the raw rice, when combined with rinse washing. It is recommended that to reduce total and inorganic arsenic content of rice that rice is rinse washed and cooked in a 6:1 water to rice ration and then soak by salt at least for 2 hours. Exhaustive rinse washing, as practiced in Iran and some other countries, may reduce arsenic content even further when combined with large cooking water volume.

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