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## Improved rice cooking approach to maximise arsenic removal while preserving nutrient elements

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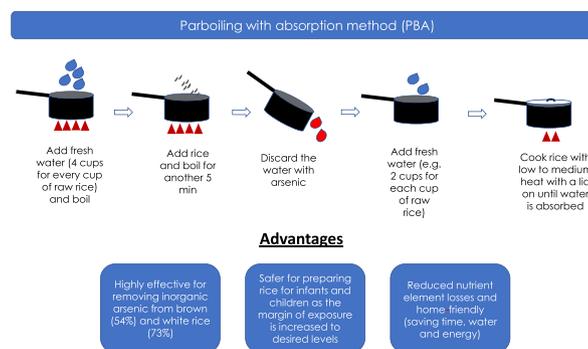
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### HIGHLIGHTS

- Parboiled and absorbed (PBA) method removed 54% & 73% iAs from brown & white rice.
- Washing and pre-soaking prior to absorption cooking reduced iAs in white rice only.
- PBA raised MOE by 3.7 and 2.2 times for white and brown rice, respectively.
- Brown rice retained more nutrients than white rice under absorption methods.
- Absorption methods preserved micro-nutrient such as Zn in brown & white rice.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Inorganic arsenic (iAs) is a group 1 carcinogen, and consumption of rice can be a significant pathway of iAs exposure in the food chain. Although there are regulations in place to control iAs for marketed rice in some countries, additional measures are explored to remove arsenic from rice. Due to the surface-bound and soluble nature of iAs, previous studies have shown that it can be removed to a significant extent using different cooking methods. Towards this goal we modified and tested the absorption method in combination with four home-friendly cooking treatments (UA = unwashed and absorbed, WA = washed and absorbed, PSA = pre-soaked and absorbed, and PBA = parboiled and absorbed) using both brown and white rice (3 types each). The nutrient elements were measured using ICP-MS and arsenic speciation was carried out using LC-ICP-MS. Overall, our results show that PBA was the optimum approach assessed, removing 54% and 73% of inorganic arsenic (iAs) for brown and white rice respectively, raising the margin of exposure (MOE) by 3.7 for white rice and 2.2 times for brown rice, thus allowing the consumption of rice more safely for infants, children and adults. Other cooking treatments were effective in reducing the iAs concentration from white rice only. Here we also report changes in selected nutrient elements (P, K, Mg, Zn and Mn) which are relatively abundant in rice. In general, the treatments retained more nutrients in brown rice than white rice. No significant loss of Zn was observed from both rice types and the loss of other nutrients was similar or less than in comparison to reported losses from rice cooked in excess water in the literature. We conclude that PBA is a promising technique and further research is needed by including different regional rice types and water quality levels.

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### 1. Introduction

Rice is consumed by nearly half of the global population, providing 30–70% of energy (Ranum et al., 2014) along with various micro and

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macronutrients. However, rice can be a significant source of inorganic arsenic (iAs, comprised of As<sup>III</sup> and As<sup>V</sup>), a poison and Group 1 carcinogen, according to the IARC (International Agency for Research on Cancer, 2012), when grown in an arsenic-contaminated environment (e.g. soil or water). Apart from iAs, rice also contains lower concentrations of less toxic organic arsenic (oAs) compounds such as dimethylarsenic acid (DMA), and traces of monomethylarsonic acid (MMA) (Bakhat et al., 2017; Islam et al., 2017).

Rice is known to accumulate around ten times as much iAs as other cereals (Williams et al., 2007) due to increased concentrations of plant-available iAs in lowland rice cultivation systems (Upadhyay et al., 2019). In rice grains, iAs is concentrated in the outer bran layer surrounding the endosperm (Meharg et al., 2008; Sun et al., 2008), and therefore brown rice, (unmilled or unpolished rice that retains its bran) contains more iAs than white rice. Though the bulk of iAs is removed in this process, it also removes 75–90% of nutrients which are mainly concentrated in the bran (Steiger et al., 2014).

The iAs exposure risk to a given population is estimated using the margin of exposure (MOE) which is a function of average daily consumption (ADC) of rice, average concentration (AC) of iAs in the rice consumed, and body weight (bw) (Guillod-Magnin et al., 2018; Menon et al., 2020; Rintala et al., 2014). Due to high food consumption rate relative to their body weight, children are up to three times more vulnerable to arsenic exposure than adults (EFSA, 2014). National (e.g. USA, China, Australia) and regional (EU) regulations place maximum limits on iAs in marketed rice (raw or uncooked) to reduce these risks. For instance, based on European Commission regulations (European Commission, 2015), the maximum permissible iAs concentrations are 0.25 and 0.20 mg kg<sup>-1</sup> for brown and white rice, respectively. However, for rice destined for infant food production and consumption, it is 0.10 mg kg<sup>-1</sup>. No such regulations exist in many Asian countries where the problem is severe, including India and Bangladesh. Although regulated, a recent study found that nearly half of the rice brands sold in the UK are potentially unsafe for infants and young children (Menon et al., 2020). However, the above finding is based on raw or uncooked rice analysis, and the iAs concentrations is likely to change depending on the cooking method warranting further rigorous re-evaluation of risks through the consumption of cooked rice.

Due to its solubility and the surface-bound nature of iAs in rice grains (Lombi et al., 2009; Moore et al., 2010), pre-cooking (washing/rinsing, pre-soaking etc.) and cooking methods have the potential to reduce iAs. Though there are many investigations on this topic, only a few of them reported iAs data. For instance, washing or rinsing rice in cold water was found to reduce iAs in white rice by 10–40% (Atiaga et al., 2020; Gray et al., 2015; Naito et al., 2015; Raab et al., 2009). Others have reported ~5% reduction in total As (tAs, which the sum of iAs and oAs) upon washing 3–5 times (Mandal et al., 2019; Sharafi et al., 2019). Similarly, Zhang et al. (2020) extensively investigated the effect of soaking with varying rice to water ratio (1:2–1:5), temperature (30–70 °C) and duration of soaking (2–48 h) for *indica* and *japaonia* rice types and up to 40% reduction in tAs could be obtained by high temperature 60–70 °C.

A range of studies investigated the effects of cooking rice in excess water (e.g. rice to water ratio of 1:6–12 by weight) which is widely popular in some countries such as India (Sengupta et al., 2006). For example, a recent study (Atiaga et al., 2020) used both white and brown rice and cooked with excess water (1:6 rice to water ratio); this treatment reduced iAs 60% (mean), with variation (range: 29–90%) observed between the rice types. Similarly, Gray et al. (2015) reported that rice cooked with excess water cooking (1:6–10 rice to water ratio) reduced iAs by 40% from long white rice and 50% from brown rice. Carey et al. (2015) used percolating cooking water to remove arsenic and found that iAs removal effectiveness is linearly related to the amount of water used in cooking and found that highest water ratio (1:12 rice to water ratio) removed 53 ± 5% from white rice and 61 ± 3% from

brown rice. Most studies given above often included washing treatment in their studies.

However, the absorption method is a popular rice cooking approach in Western countries (Sengupta et al., 2006) and some Asian countries in which rice is cooked in a covered pot or rice cooker with a low water to rice ratio (e.g. 1:2–3 rice to water by volume) under low to medium heat until all the water is absorbed. This same principle is used when using a rice cooker or pressure cooker. As expected, this method is not as effective as cooking in excess water in removing iAs (Atiaga et al., 2020; Liao et al., 2019; Naito et al., 2015) though it is likely to preserve nutrients in rice as no water is discarded. For this reason it is the recommended method for cooking fortified rice to prevent loss of added nutrients. Also, this method is often quicker than alternatives as it uses less water in comparison to cooking in excess water, thus saving energy and time.

There are only a few studies that investigated the micronutrient concentrations along with As in cooked rice. In their study, Gray et al. (2015) reported that rinsing brown rice does not significantly reduce Fe, Mn or vitamin (folate, niacin and thiamine) contents whereas the same treatment for white rice removed 90% of Fe and 80% of the vitamins. When brown and white rice was cooked with excess water, the loss of Fe was not significant for brown rice whereas both moderate and high volume excess water cooking (1:6–10 rice to water ratio) removed as much as 70% Fe and on average 50% reduction of vitamins from white rice. Mn was not lost from brown rice by rinsing or cooking whereas both treatments caused substantial loss of Mn from white rice. Two recent investigations (Mwale et al., 2018; Sharafi et al., 2019) reported nutrient losses along with total arsenic (tAs) reduction using a range of different cooking methods. Mwale et al. (2018) reported 4.5, 30, 44% reduction in total arsenic in 1:3, 1:6 and 1:10 rice to water ratios, respectively, and all nutrients except Se, Fe, and Cu displayed significant losses at 1:6 rice to water ratio. Sharafi et al. (2019) tested washing five times, washing + soaking, washing + a Persian cooking method called 'Kateh' (somewhat similar to absorption method with 1:2 water ratio by volume) and washing and 'rinse cooked' on the concentrations of As and other toxic trace elements along with beneficial nutrient elements. The rinse cooking method was found to be the best suited for tAs removal (up to 52.6%) compared to the control group (only washing). They reported up to ~34% reduction for Fe and ~26% for Zn and these were from 'Kateh' and 'rinse cooked' methods.

Much attention has been paid to the removal of arsenic through cooking methods, but we require more data on nutrient losses along with arsenic and its species (iAs and oAs) in order to develop/recommended an optimum method suitable for various rice types. This is particularly important when the rice consumers do not know the concentration of iAs in the marketed rice. Both cooking with excess water and absorption methods have their own advantages and disadvantages. A major problem with cooking with excess water is the loss of important nutrients, even though it is highly effective in reducing iAs. Additionally, it may require more energy, time and water in comparison to the absorption technique; we note that in many arsenic affected areas, people often have a limited quantity of clean water available. The absorption method, on the other hand, has not been sufficiently and thoroughly explored in the past and therefore, in this study we focus on modifying the absorption method to adapt this approach for use as a home-friendly method. To this end, we used pre-cooking treatments such as washing, pre-soaking and brief parboiling when combined with an absorption method for iAs and nutrient element concentrations in the cooked rice. The effect of temperature on dissolution of arsenic from rice has already been demonstrated by a previous study (Zhang et al., 2020), therefore we hypothesise that brief parboiling would amplify the dissolution of surface-bound iAs (and nutrients) in comparison to washing or pre-soaking with cold water. Furthermore, for the most efficient iAs removal method, we have used the most recent approach to risk assessment using MOE (Margin of Exposure). MOE assessment provides a robust, internationally-recognised method

used by researchers, regulators and policy makers to assess the long-term exposure risk to carcinogens.

## 2. Methods

### 2.1. Cooking of rice samples

We selected three brown rice types namely Thai Brown rice (B1), short grain brown rice (B2) brown basmati (B3) along with three white rice types which were everyday white long-grained rice (W1), white pudding rice (W2) and sushi rice (W3). These were chosen as they were with relatively high concentrations of iAs from a batch of 55 commercially available rice brands which we have analysed and reported previously (Menon et al., 2020) from the UK. These rice types represent the two dominant rice types (white or brown) sold through major supermarket chains in the UK. The mean iAs concentration of the above uncooked (raw or R) samples were B1 = 0.235 ( $\pm 0.032$ ); B2 = 0.326 ( $\pm 0.014$ ); B3 = 0.195 ( $\pm 0.031$ ); W1 = 0.161 ( $\pm 0.016$ ); W2 = 0.234 ( $\pm 0.047$ ) and W3 = 0.129 ( $\pm 0.010$ ) mg kg<sup>-1</sup>.

The experimental design included four cooking treatments as detailed below. Each treatment was performed with three replicates for each of the six rice types, providing a total 72 (=4 × 6 × 3) subsamples.

1. Unwashed and absorbed (UA) in which rice was cooked without washing and cooked directly in deionised water using the absorption method.
2. Washed and absorbed (WA) in which rice samples were washed for 5 min in an orbital shaker (100 rev min<sup>-1</sup>) simulating energetic washing whilst stirring. The water was discarded after shaking before it was cooked using the absorption method.
3. Pre-soaked and absorbed (PSA) method involved soaking rice samples for 30 min, then these were cooked using the absorption method. The water was discarded after shaking before it was cooked using the absorption method.
4. Parboiled and absorbed (PBA), in which water was boiled first, and then the rice was added to cook for 5 min; the water was then discarded, and the rice was then cooked using the absorption method in fresh deionised water.

For each sample, we used 10 mL or ~8 g rice. Deionised water (40 mL); corresponding to a rice to water ratio of 1:4 by volume or 1:5 by weight) was used for the initial washing, pre-soaking and parboiling (i.e. in 2, 3 & 4 below) and discarded. After this, rice samples were cooked in fresh deionised water using the absorption method (rice to water 1:2 by volume or 1:2.5 by weight) in a clean 100 ml beaker for all treatments. Note that in treatment 1, rice was cooked with the absorption method using deionised water with the above ratio.

The cooking was performed using a laboratory-grade hotplate (JENWAY 1000), starting with medium power until boiling and then held at minimum power until all water was evaporated (~10–20 min, depending on the rice type). The beaker was kept loosely covered with a glass lid to prevent building up of pressure and bubbling up. Note that we carried out preliminary cooking experiments to optimise the rice - water ratio, and the cooking time required for each rice type. After each cooking, we made sure the rice was cooked (i.e. without a hard white centre in the grain after cooking and with soft texture). Based on the weights recorded, we found that, on average, the weight of rice increased by a factor of 3.10 (UA) to 3.75 (PBA) after final cooking.

After cooking, all beakers containing samples were then cooled to around 60 °C, transferred to a ventilated pre-heated oven (at 50 °C) and dried for two days; the final moisture content of samples was found to be 9–10% (w/w), similar to raw rice moisture contents. Due to high water content in the cooked rice, we used this approach as it allowed us to dry all samples at once and it is often quicker than other methods such as freeze-drying. Dried sub-samples were placed in a stainless steel RETSCH Mixer Mill Grinding Jar with a stainless steel grinding ball and milled using an MM200 Mixer Mill set at 250 rev min<sup>-1</sup> for 3 min.

### 2.2. Elemental analysis

The samples were first analysed using ICP-MS (Thermo-Fisher Scientific iCAP-Q and iCAP-TQ; Thermo Fisher Scientific, Bremen, Germany) to obtain elemental composition. For this analysis, approximately 0.2 g (dry weight) of rice flour from each sample was microwave-digested in 6 mL HNO<sub>3</sub> (Primar grade, Fisher Scientific, UK) in perfluoroalkoxy (PFA) vessels (Multiwave; Anton Paar GmbH, St. Albans, UK). The digested samples were diluted to 20 mL, and then 1-in-10 with Milli-Q water (18.2 MΩ cm), before conducting elemental analysis by inductively coupled plasma mass spectrometry or ICP-MS at the School of Biosciences, University of Nottingham. The instrument was run employing a collision-cell (Q cell) using He with kinetic energy discrimination (He-cell) to remove polyatomic interferences. Samples were introduced from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). Internal standards were introduced to the sample stream on a separate line via the ASXpress unit and included Ge (10 µg L<sup>-1</sup>), Rh (10 µg L<sup>-1</sup>) and Ir (5 µg L<sup>-1</sup>) in 2% HNO<sub>3</sub>. External multi-element calibration standards (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA) included Ag, Al, As, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Ti, Tl, U, V and Zn, in the range 0–100 µg L<sup>-1</sup> (0, 20, 40, 100 µg L<sup>-1</sup>). A multi-element (1000 mg L<sup>-1</sup>) calibration solution (Qmx Laboratories Ltd., Thaxted, UK) was used to create Ca, Mg, Na and K standards in the range 0–30 mg L<sup>-1</sup>. Phosphorus, boron and sulphur calibrations utilised in-house standard solutions (KH<sub>2</sub>PO<sub>4</sub>, H<sub>3</sub>BO<sub>3</sub> and K<sub>2</sub>SO<sub>4</sub>). Peak dwell times were 10 ms with 300 scans per sample. Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilising external cross-calibration between pulse-counting and analogue detector modes when required.

### 2.3. Arsenic speciation

All samples were analysed for arsenic species (As<sup>III</sup>, As<sup>V</sup>, DMA and MMA) using LC-ICP-MS at the School of Biosciences, University of Nottingham, UK, using the methods described in previous publications (Huang et al., 2010; Menon et al., 2020; Phiri et al., 2019). In this method, approximately 1.5 g of each of powdered rice sample was suspended in 15 mL 2% nitric acid (Primar Plus grade, Fisher Scientific, U.K.) in polypropylene 'DigiTubes' (SCP Science, Quebec, Canada), digested using a Teflon-coated graphite block digester (Model A3, Analysco Ltd., U.K.) at 95 °C for 1.5 h. After cooling the suspensions, their volumes were made up to 50 mL with ultrapure water (18.2 MΩ cm). An aliquot (c. 6 mL) was syringe-filtered (<5 µm) for arsenic speciation using a coupled LC-ICP-MS (HPLC 5000 series, Thermo Scientific) with a PRP-X100 anion exchange column (PS-DVB/Trimethyl ammonium exchanger; 5 µm particle size; 4.6 mm ID; 250 mm length). The eluent was treated with 20 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> (analytical grade) at pH = 5.6, pumped at 1.5 mL min<sup>-1</sup> in isocratic mode. We used 5.0 µg L<sup>-1</sup> arsenite (As<sup>III</sup>) and arsenate (As<sup>V</sup>) (Spex Certiprep, Stanmore, U.K.), and 5.0 µg L<sup>-1</sup> dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) (purity >98%; Sigma/Merck, Darmstadt, Germany) as standards. The concentrations of individual species to obtain the sum of inorganic (As<sup>III</sup> and As<sup>V</sup>) and organic (DMA and MMA) species. We used certified rice reference material (NIST1568b), and determined recovery values for As<sup>III</sup>, As<sup>V</sup>, and DMA of 121.0%, 99.9% and 90.6%, respectively. Recovery of essential nutrients present in rice (P, K, Mg, Mn, Zn) ranged from 88 to 120% in comparison to the rice standard.

### 2.4. Calculation of margin of exposure (MOE)

The procedure to calculate the Margin of Exposure (MOE) can be found in previous publications (Guilod-Magnin et al., 2018; Jallad, 2019; Menon et al., 2020; Rintala et al., 2014); a brief summary of

these steps is provided below. The minimum value of MOE that is required to avoid adverse iAs exposure is 1, although higher values are desirable.

$$MOE = \frac{BMDL_{0.1}}{EDI} \quad (1)$$

The MOE will depend on the BMDL values used (Benchmark dose lower confidence limit; the subscript indicates the dose needed for a 0.1% increase in the incidence of cancers) and its range is from 0.0003 to 0.008 mg kg<sup>-1</sup> bw d<sup>-1</sup> (EFSA, 2014). In this study we used the lowest reported iAs value of 0.0003 mg kg<sup>-1</sup> bw d<sup>-1</sup>.

The denominator, EDI (Estimated Dietary Intake) is calculated as:

$$EDI = \frac{AC \times ADC}{bw} \quad (2)$$

where AC is the average concentration of iAs in rice (mg kg<sup>-1</sup>), ADC is the average daily consumption rate of rice (kg d<sup>-1</sup>), and bw represents the average body weight of the local population (kg). Also, rearranging the equation allows us to calculate the maximum allowed rate of Average Daily Consumption (ADC<sub>max</sub>) to achieve an MOE of 1, as shown below:

$$ADC_{max} = \frac{BMDL_{0.1} \times bw}{MoE \times AC} \quad (3)$$

Substituting values of 0.0003 mg kg<sup>-1</sup> bw d<sup>-1</sup> for BMDL<sub>0.1</sub> and MOE of 1, the above equation can be simplified as:

$$ADC_{max} = \frac{0.0003 \times bw}{AC} \quad (4)$$

Using the iAs concentrations both from raw (R) rice and cooked rice (e.g. PBA) we calculated ADC<sub>max</sub> for three different age groups for brown and white rice. In Table 1, we provide an example scenario using a typical portion of rice and population characteristics (1-year-old infant, 7-year-old children and adults, male and female) for the UK (Office for National Statistics, 2010; The UK Government Service, n.d.).

## 2.5. Statistical analysis

We used GraphPad Prism (Version 8.4.2, San Diego, California USA, www.graphpad.com) for statistical analyses and plotting the graphs. We carefully examined all rice analytical data individually first and then pooled all brown (B1–B3) and white types (W1–W3) for the statistical analyses. We also analysed the data using one-way ANOVA as the data were normally distributed and Dunnett's multiple comparison tests in comparison to raw rice data (R). While comparing treatments, the following notations were used in figures to indicate the level of significance: "\*" for  $P \leq 0.05$ , "\*\*" for  $P \leq 0.01$ , "\*\*\*" for  $P \leq 0.001$  and "\*\*\*\*" for  $P \leq 0.0001$ . The error bars in graphs represent standard error of the mean; please note the differences in the Y-axis scale between graphs.

## 3. Results and discussion

### 3.1. Arsenic species

The cooking treatments used significantly influenced the concentration of iAs as shown in Fig. 1a when we pooled and analysed data from all brown (F (4, 37) = 8.038;  $P < 0.0001$ ) and white rice (F (4, 40) = 25.93;  $P < 0.0001$ ). The newly developed PBA was the most effective in reducing the iAs among all treatments with a 54% and 73% decrease in iAs in brown and white rice respectively, in comparison to raw rice (R) (Fig. 1c). In brown rice, UA caused a 9% decrease in iAs, which was not significantly different from R; the same treatment, however, led to a significant reduction of iAs in white rice of 32%. Both WA and PSA treatments reduced iAs to a similar extent in both rice types (~18% in

**Table 1**

Determination of maximum rate of Average Daily Consumption (ADC<sub>max</sub>) for different age groups in the UK to achieve a minimum target MOE of 1 for raw and cooked (PBA) white and brown rice. It can be seen that ADC<sub>max</sub> is increased by a factor of 3.7 and 2.2 times for white and brown rice under PBA in comparison to raw rice, allowing the population to consume more rice safely.

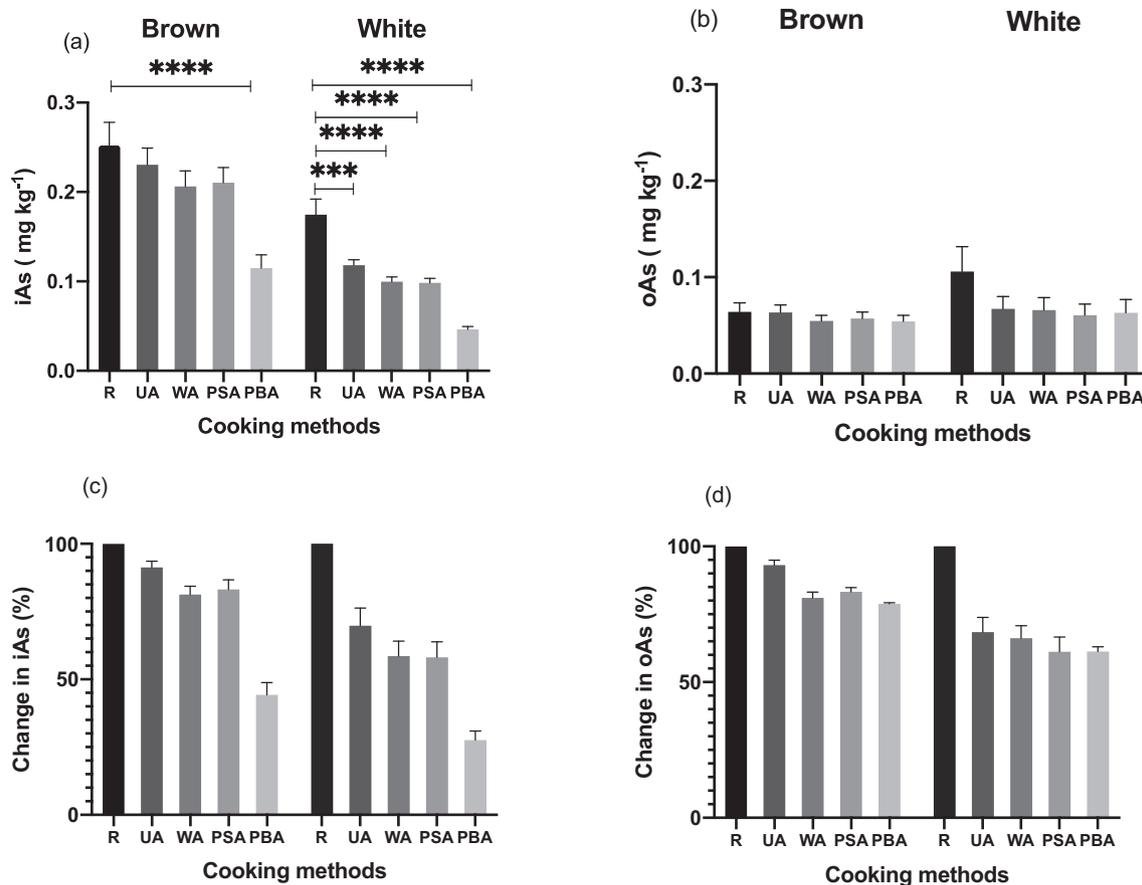
Target Population	bw (kg)	Rice type + cooking method	AC iAs (mg kg <sup>-1</sup> )	Target MOE	ADC <sub>max</sub> (kg d <sup>-1</sup> )
Adult male	83.0	White raw	0.175	1.00	0.142
Adult female	70.0				0.120
Children (7 y)	23.0				0.039
Children (1 y)	9.0				0.015
Adult male	83.0	White-PBA	0.047	1.00	0.530
Adult female	70.0				0.447
Children (7 y)	23.0				0.147
Children (1 y)	9.0				0.057
Adult male	83.0	Brown-raw	0.252	1.00	0.099
Adult female	70.0				0.083
Children (7 y)	23.0				0.027
Children (1 y)	9.0				0.011
Adult male	83.0	Brown PBA	0.115	1.00	0.217
Adult female	70.0				0.183
Children (7 y)	23.0				0.060
Children (1 y)	9.0				0.023

bw = body weight (constant); AC = average concentration of iAs of white or brown rice types; MOE = margin of exposure; ADC<sub>max</sub> = maximum allowed rate of Average Daily Consumption.

brown and ~44% in white); however, the reduction was only statistically significant for white rice, as shown in Fig. 1a. It appears that iAs bound to the bran layer is resilient to removal that does not include a raised temperature treatment. Further research is required to understand whether bran is acting as a physical or chemical barrier or a combination of both.

The concentration of the less harmful organic As (oAs; sum of monomethylarsonic acid or MMA, and dimethylarsinic acid or DMA) was mostly unaffected by the cooking methods studied (Fig. 1b & d) and not statistically different from raw rice (R). This is similar to previous research work (Atiaga et al., 2020; Carey et al., 2010; Raab et al., 2009) and it is due to the fact that DMA is found in the inner endosperm as opposed to iAs which is mostly surface-distributed and therefore reduced by washing and cooking. It is worth noting that while cooking the rice, the lid was not completely closed to avoid frothing and this may have caused a small reduction in arsenic concentration in UA in comparison to R.

Though the above analysis was based on pooled data (average of all brown vs all white rice), in Fig. 2, we show the distribution of iAs and oAs in individual rice types (B1–B3; W1–W3) along with changes in their concentrations in response to cooking methods. The iAs fraction was dominant in most rice types except for sample W3 which had more oAs than iAs (leading to slightly higher SEs in Fig. 1b when the data were pooled). We performed ANOVA with multiple comparisons for iAs and oAs for each rice type. The cooking treatments significantly influenced iAs in B1 (F (4, 9) = 44.62;  $P < 0.0001$ ), B2 (F (4,9) = 73.02;  $P < 0.0001$ ), B3 (F (4,10) = 12.10;  $P = 0.0008$ ). Similarly, ANOVA results showed significant influence of cooking on iAs concentrations for W1 (F (4,10) = 41.92;  $P < 0.0001$ ), W2 (F (4,10) = 28.03;  $P < 0.0001$ ) and W3 (F(4,10) = 79.30;  $P < 0.0001$ ). The results of multiple comparisons are shown as grey asterisks in Fig. 2 plots for iAs only owing to its importance and to avoid clutter in the graphs. In contrast to iAs data, oAs concentrations were affected by cooking in B1 only (F (4, 9) = 5.161;  $P = 0.0193$ ) and it was also true for W1 (F (4, 10) = 15.65;  $P = 0.0003$ ) and W3 (F (4, 10) = 54.34;  $P < 0.0001$ ). Multiple comparison data showed that oAs concentrations were statistically different from R for both WA ( $P = 0.0171$ ) and PBA ( $P = 0.0298$ ) in B1, whereas PBA was the only method which was statistically different from R for B2 ( $P = 0.0476$ ) and B3 ( $P = 0.0453$ ). The oAs concentrations in all cooking methods were statistically different from R for W1 ( $P$



**Fig. 1.** (a–d). Comparison of average inorganic As (a) and organic As concentrations (a, b) and in per cent (c, d) in brown and white rice under different cooking treatments (R = raw rice; UA = unwashed and absorbed; WA = washed and absorbed; PSA = pre-soaked and absorbed and PBA = parboiled and absorbed). Each bar represents the average of three rice samples (brown or white) with three replicates. The error bars indicate standard error of means (SEM). Note that in c & d, R was assumed to be 100% (hence no error bars) and all other treatments were normalised to R.

values for UA, WA, PSA and PBA were 0.0021, 0.0011, 0.0006 and <0.0001 respectively) and W2 ( $P < 0.0001$  for UA, WA, PSA and PBA), whereas cooking method did not significantly alter oAs in W2.

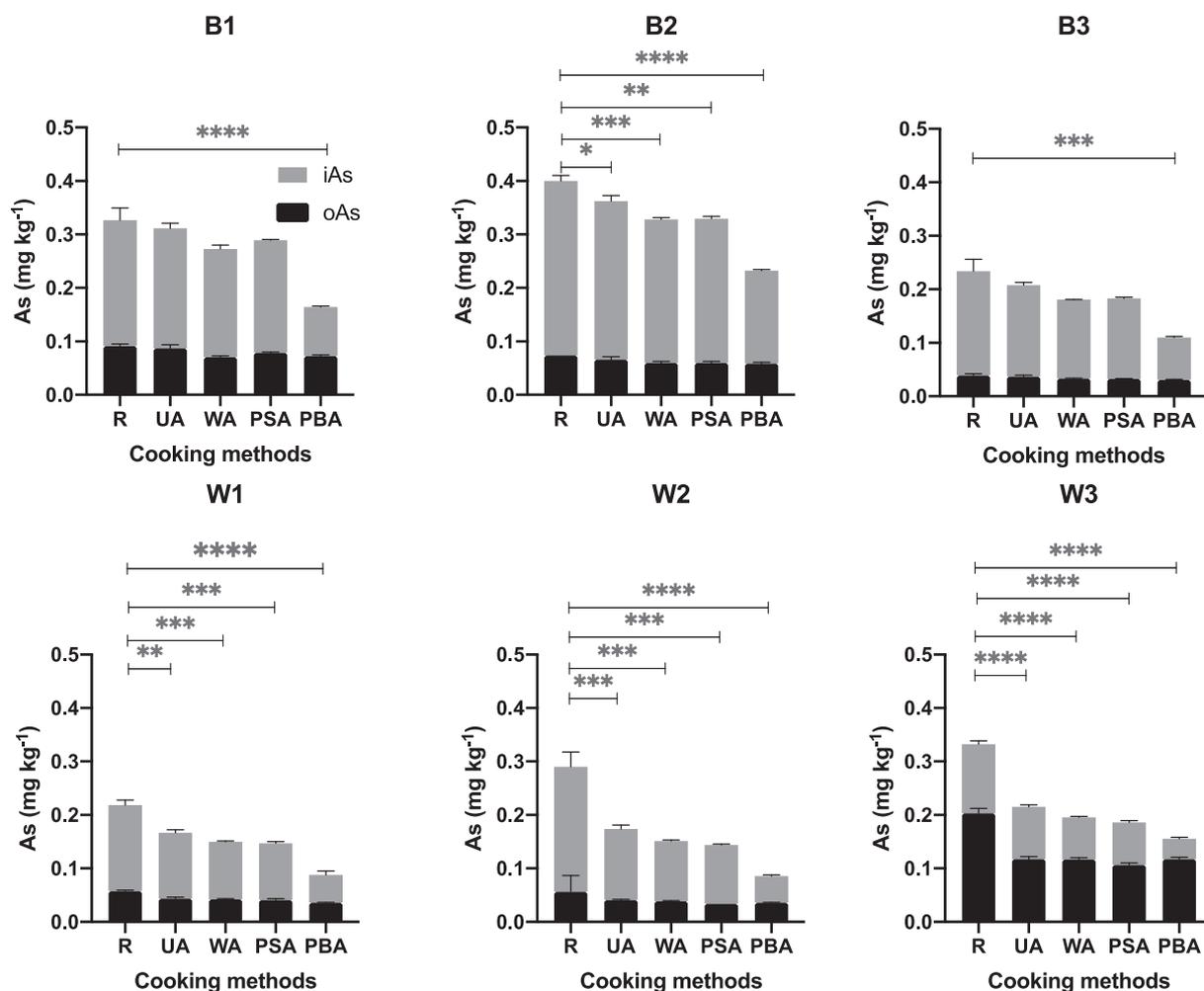
Previous studies (Atiaga et al., 2020; Gray et al., 2015; Naito et al., 2015; Raab et al., 2009) have shown that cold rinsing/washing can reduce iAs in white rice by 10–40% and our results also showed a comparable and significant reduction in iAs in both WA (18%) and PSA (44%) treatments. Similar to our results, Gray et al. (2015) also reported that iAs concentrations in brown rice were not significantly changed by rinsing.

Earlier studies reported different rates of iAs removal from both white and brown rice. For instance, a 1:6 rice to water ratio reduced iAs by ~80% in brown and ~60% in white rice (Atiaga et al., 2020) when it was combined with rinsing treatments. Another study reported a reduction of iAs 49% in white rice and 44% in brown rice (Raab et al., 2009) which was somewhat similar to the findings by Gray et al. (2015) where they reported a reduction of iAs by 40% from long white rice and 50% from brown rice in cooking in excess water (1:6–10 rice to water ratio) treatments. Using percolating water methods, Carey et al. (2015) reported iAs reduction up to  $53 \pm 5\%$  from white rice and  $61 \pm 3\%$  from brown rice. The efficiency of PBA to remove iAs from white (73%) and brown (54%) rice was found to be better for white rice than the results from other studies, but in the case of brown rice, iAs removal was comparable to that reported by Carey et al. (2015) and better than by some others (Raab et al., 2009; Gray et al., 2015). We acknowledge that the rice samples used in these investigations are not the same, which could influence the results. As demonstrated in Fig. 2, different rice types respond to cooking differently. Future experiments may compare PBA along with excess water cooking methods using different rice types.

### 3.2. Mineral nutrients

Though PBA was very effective in removing iAs to a great extent, it is also important to consider the nutrient losses. Note that each rice sample had a different nutrient composition and the results shown here represent averages of 9 replicates (i.e. 3 per each rice sample) per treatment for both white and brown rice. In Fig. 3 (a–e) & Suppl. Fig. 1, we show nutrients which are relatively abundant ( $>5 \text{ mg kg}^{-1}$ ) in rice as well as affected by cooking (P, K, Mg, Zn and Mn). The concentrations of these nutrients were higher in brown rice than white rice. The cooking methods did not significantly influence P and K concentrations in brown rice in our experiment. However, white rice P ( $F(4,40) = 13.02$ ;  $P < 0.0001$ ) and K ( $F(4,40) = 33.49$ ;  $P < 0.0001$ ) concentrations were changed significantly during the cooking and for both nutrients significant reductions from R were observed in WA (P loss = 27%; K loss = 30%), PSA (P loss = 15%, K loss = 22%) and PBA (P loss = 22%; K loss = 48%) as shown in Fig. 3a & b (white rice bars). In contrast, the cooking methods significantly influence Mg concentrations in both brown ( $F(4,40) = 9.991$ ;  $P < 0.0001$ ) and white rice ( $F(4,40) = 9.512$ ;  $P < 0.0001$ ) and all treatments were significantly different from R, as shown in Fig. 3c. On average, ca. 7–8% Mg loss occurred across the cooking methods except for UA in brown rice whereas for white rice WA, PSA and PBA caused 40, 18 and 22% reduction in Mg, respectively.

Zn concentrations did not significantly change (Fig. 3d) as a function of these cooking methods, whereas Mn concentrations were significantly influenced by cooking treatments in brown ( $F(4,39)$ ; 4.829  $P = 0.0028$ ) and white ( $F(4,39)$ ; 2.627;  $P = 0.0490$ ) rice; we found only WA (loss = 7%) and PBA (loss = 5%) significantly influenced Mn



**Fig. 2.** Distribution of inorganic and organic arsenic (iAs and oAs) in 6 rice samples (average of three replicates) under different cooking treatments (R = raw rice; UA = unwashed and absorbed; WA = washed and absorbed; PSA = pre-soaked and absorbed and PBA = parboiled and absorbed). The error bars indicate standard error of means (SEM). Please note that grey asterisks are for iAs (for oAs, please refer to Section 3).

concentrations in brown rice whereas it was only WA (loss = 21%) for white rice as shown in Fig. 3e. The concentrations of Se were very small ( $0.05 \pm 0.03$  and  $0.03 \pm 0.01$  mg kg<sup>-1</sup> in raw, brown and raw white samples respectively) and did not change significantly in response to cooking treatments for both rice types and hence are not shown here.

Only a few studies (Gray et al., 2015; Mwale et al., 2018; Sharafi et al., 2019) have examined changes in micronutrients along with arsenic with cooking. Gray et al. (2015) reported loss of Fe and Mn along with vitamins such as folate, niacin and thiamin when exposed to rinsing, absorption or being cooked with moderate and high volume of water. Though we did not report Fe in this paper due poor quality data, their results showed that the absorption method did not change Fe concentrations whereas as much as 75% of Fe is lost when cooked with excess water (a rice to water ratio of 1:10) from polished/white or parboiled rice whereas it did not change for brown rice. Their findings show that up to 93% of Mn was lost when cooked with excess water whereas rinsing only removed up to 35% Mn; brown rice Mn concentration was not affected by rinsing or cooking method (Gray et al., 2015). This was a much higher loss than the values reported in this study for WA and PBA for white rice.

Mwale et al. (2018) found that cooking in excess water (1:6–10 rice to water ratio) could result in loss of K (50–58.9%), Mg (22.4–23.8%), Mn (16.5–20.8%), Zn (7.7–14.2%) and Fe (8.2–24.4%) in comparison to raw rice. We found that PBA produced a similar reduction in K and Mg (50 and 24%) only in white rice whereas the loss of these from brown rice

was minimal (3 and 7%; see Fig. 3). A similar trend was also found for P. Most importantly, the new PBA method did not cause a significant reduction in Zn. Zn in rice grains is not distributed on the surface of the grain (Lombi et al., 2009) and less likely to be lost by our PBA cooking treatment, whereas boiling in excess water produced a 7.7–14.2% loss (Mwale et al., 2018). Zn deficiency is widespread in regions where rice is the main staple (Dipti et al., 2012) and PBA could form part of the solution to tackle Zn deficiency in the population. We also observed a loss of just ~5% Mn with PBA from both rice types, compared to greater loss (16.5–20.8%) when cooking rice with excess water (Mwale et al., 2018).

In a recent study, Sharafi et al. (2019) reported changes in toxic and essential elements under different cooking methods including rinsing (5 times), pre-soaking (1, 5 and 12 h soaking) as well as other methods similar to washing and absorption method called 'Kateh method' and 'washing and rinse cooking' (somewhat similar to PBA in our study). Though there are differences in the methodology, they found that Kateh and rinse cooking produced a reduction of approximately 25 and 45% in total arsenic, along with ca. 10–30% reduction in Fe and Zn. However, they did not report iAs in that investigation, rather tAs. To summarise, in general, absorption methods did not affect nutrients such as P and K in brown rice whereas white rice was shown to respond to the various treatments used. Both Mg and Mn concentrations were reduced by absorption methods in both rice types whereas Zn was largely preserved in both white and brown rice. PBA, in particular, is

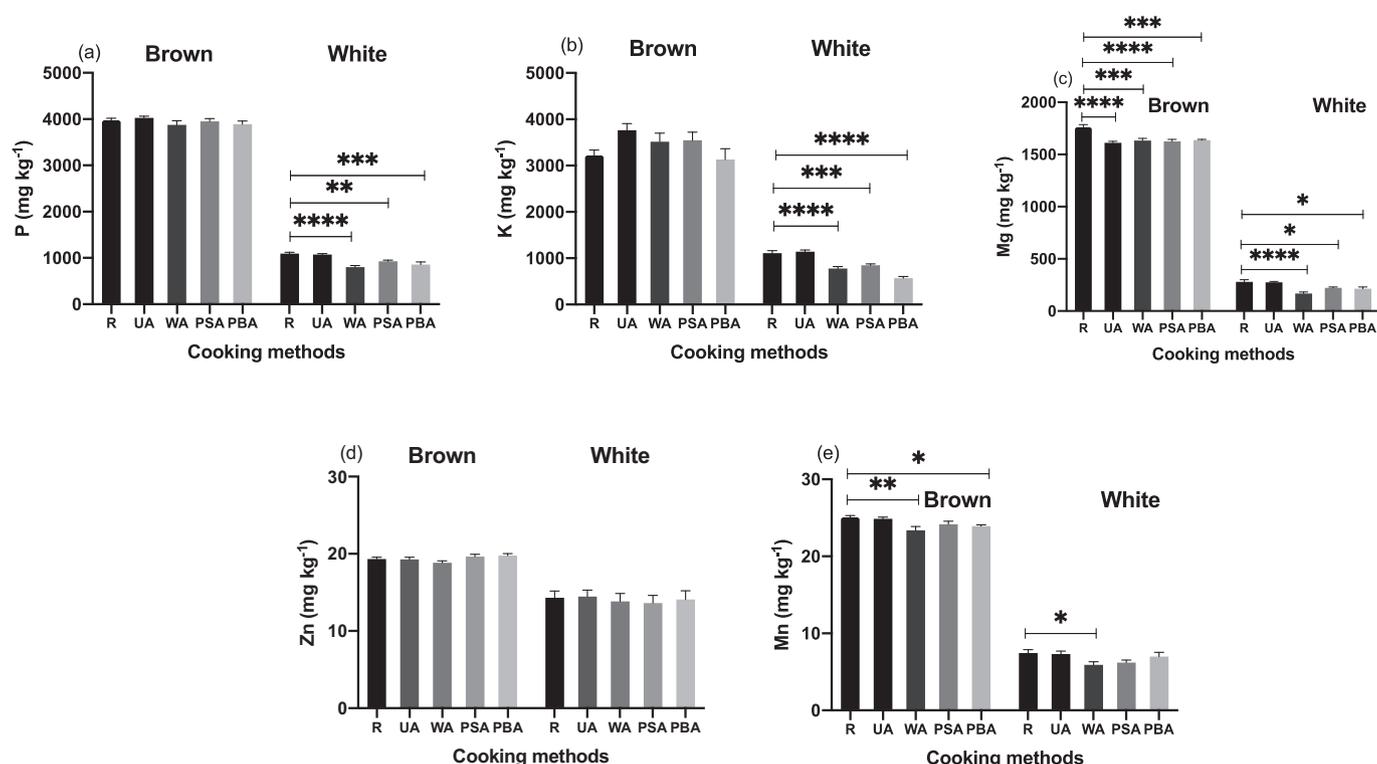


Fig. 3. (a–e). Changes in P, K, Mg, Zn and Mn of brown and white rice under different cooking treatments (R = raw rice; UA = unwashed and absorbed; WA = washed and absorbed; PSA = pre-soaked and absorbed and PBA = parboiled and absorbed). Each bar represents the average of three rice samples (brown or white) with three replicates. The error bars indicate standard error of means (SEM).

shown to impact K and Mg in white rice, and produced a comparable loss to the rice samples cooked in excess water in a previous study (Mwale et al., 2018) whereas for Mn losses were much smaller in comparison to excess water cooking methods (Gray et al., 2015; Mwale et al., 2018) and PBA did not produce significant losses in Zn in contrast to previous studies that used excess water cooking methods (Mwale et al., 2018; Sharafi et al., 2019).

### 3.3. Margin of exposure

Based on the analytical data on iAs, we determined the ADC<sub>max</sub> with a minimum target MOE of 1. In Table 1, we compared ADC<sub>max</sub> of PBA with raw (R) white and brown rice. The PBA approach increased the ADC<sub>max</sub> by 3.7 times for white rice and 2.2 times for brown rice in comparison to raw (R) rice for all age groups, allowing them to consume more rice safely under PBA. In other words, if we keep all values constant (i.e. bw, AC and ADC) then PBA increases the MOE by 3.7 and 2.2 for white and brown rice, respectively.

Furthermore, we evaluated whether cooking methods could reduce iAs exposure in the European population in Table 2. Across Europe, estimated 95th percentile dietary exposure (EDI) among infants, toddlers and children ranged from 0.00036 to 0.00209 mg kg<sup>-1</sup> bw d<sup>-1</sup> whereas for adults it was 0.00014 to 0.00064 mg kg<sup>-1</sup> bw d<sup>-1</sup> (EFSA, 2014).

Based on these values, corresponding MOE for infants, toddlers and children groups is from 0.83 to 0.14 and for adults it is from 2.14 to 0.47, clearly highlighting the risk to high rice consumers. If we use PBA for white rice then it can potentially be raised to 3.08–0.53 for infants, toddlers and children and 7.93–1.73 for adults (Table 2). For brown rice, it will be 1.83–0.32 for infants, toddlers and children and 4.71–1.03 for adults. The data suggested that infants, toddlers and children are at risk of exposure and therefore, ADC<sub>max</sub> in Table 1 could be used for these groups.

It may be noted that we assumed a minimum 1 for MOE, however, higher values are desirable in all cases. This can be achieved by reducing the daily consumption rate of rice (e.g. reducing the portion size or substituting rice through diet diversification) or selecting rice types which are low in iAs. In this study we used rice types which are somewhat close to the maximum permissible iAs concentration in raw rice (see Section 2.1) stipulated by the European Union. However, currently marketed rice varieties do not provide a clear warning about whether the rice is safe for children or not (Menon et al., 2020), therefore cooking methods such as PBA and other methods (e.g. cooking with excess water) can help to reduce these risks considerably.

Another important point to note here is that MOE will depend on the BMDL used. Here we used the lowest value reported (BMDL<sub>0.1</sub> = 0.0003 mg kg<sup>-1</sup> bw d<sup>-1</sup>) and therefore provide the safest possible

Table 2  
Mean dietary exposure and MOE assessment in European population.

Target population	Range of 95th per centile EDI <sup>a</sup> (mg kg <sup>-1</sup> bw d <sup>-1</sup> )	MOE (mg kg <sup>-1</sup> bw d <sup>-1</sup> )	MOE if PBA was used for white rice (=MOE × 3.7)	MOE if PBA was used for brown rice (=MOE × 2.2)
Infants, toddlers and children	0.00036–0.00209	0.83–0.14	3.08–0.53	1.83–0.31
Adults	0.00014–0.00064	2.14–0.46	7.92–1.73	4.71–1.03

<sup>a</sup> Based on EFSA (2014).

rates of consumption. Although the BMDL range extends up to 0.008 mg kg<sup>-1</sup> bw d<sup>-1</sup> in the UK, 0.003 mg kg<sup>-1</sup> bw d<sup>-1</sup> was previously used in assessing iAs risks by the Food Standards Agency (FSA, 2016) which was based on BMDL<sub>0.5</sub>. Higher values of BMDL, i.e. 0.003 and 0.008 mg kg<sup>-1</sup> bw d<sup>-1</sup> would raise the MOE by 10 and 26.7 times, and therefore, it is safer to calculate MOE using the lowest BMDL (i.e. BMDL<sub>0.1</sub> = 0.0003 mg kg<sup>-1</sup> bw d<sup>-1</sup>) for assessing the risk from carcinogens. In other words, MOE using BMDL<sub>0.1</sub> = 0.0003 mg kg<sup>-1</sup> bw d<sup>-1</sup> is the most conservative estimate of the risk and probably most suitable for assessing the risks to vulnerable populations (infants, toddlers and children) as shown in some recent publications (Guillod-Magnin et al., 2018; Menon et al., 2020; Rintala et al., 2014).

Absorption methods may not be the most widely used method in some Asian countries such as India, where cooking rice with excess water is the normal practice (Sengupta et al., 2006) and in particular, arsenic affected states of India, access to arsenic-free water can be a problem. Washing, soaking and cooking rice with arsenic contaminated water is generally shown to increase the arsenic in the cooked rice (Chowdhury et al., 2020; Clemente et al., 2021; Roy Chowdhury et al., 2018; Signes-Pastor et al., 2012). Further experimentation is required with spiked arsenic water to fully evaluate whether methods such as PBA could be useful in these regions. Therefore, PBA is recommended if households have access to water which is safe according to WHO recommendation for iAs (i.e. 0.01 ppm).

For wider applicability of PBA, inclusion of parboiled rice in future experiments. We did not use parboiled rice as this is less popular in the UK. However, Gray et al. (2015) reported a significant loss of iAs, mineral nutrients and vitamins from parboiled rice when cooked with excess water. To support millions who are living with arsenic in their environment, we need to develop or adapt these rice cooking methods based on the cooking preferences of the affected population.

#### 4. Conclusions

In this study, we compared and evaluated the efficiency of iAs removal from three brown and white rice types using absorption methods. The cooking treatments were unwashed and absorbed (UA), washed and absorbed (WA), pre-soaked and absorbed (PSA), and parboiled and absorbed (PBA). Of these treatments, PBA was found to be most efficient, removing 54% and 73% of inorganic arsenic (iAs) for brown and white rice respectively, whereas the other treatments significantly reduced iAs in white rice only. PBA is not only practical to perform domestically, but also was found to be the only method suited to all rice varieties in order to obtain a desirable MOE for all population groups. It increased MOE by factor of 3.7 for white rice and 2.2 for brown rice, allowing us to consume more rice safely. This study showed that absorption methods could be further explored not only due to potential savings of water, energy use and cooking time but also due to its efficiency in removing iAs. In general, brown rice nutrients were higher and better retained under cooking than white rice nutrients. In particular, a crucial micronutrient Zn in rice, was not lost in any of the cooking treatments studied. The loss of K and Mg from white rice in PBA was similar to when rice is cooked with excess water whereas Mn losses were much smaller compared to excess water cooking methods reported in the literature. Further research is required to adapt methods such as PBA for its wider applicability by incorporating regional rice types and water quality levels.

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#### CRedit authorship contribution statement

MM = Funding acquisition, conceptualisation, investigation, methodology, project administration, supervision, formal analysis, visualisation, and writing (original draft). WD & XC = Experimentation and writing (review and editing). JH = Experimentation, formal analysis,

writing (review and editing); ER = Funding acquisition, writing (review and 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.

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