

Inorganic arsenic removal in rice bran by percolating cooking water

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1	Inorganic arsenic removal in rice bran by percolating cooking water
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13	
14	Abstract
15	Rice bran, a by-product of milling rice, is highly nutritious but contains very high
16	levels of the non-threshold carcinogen inorganic arsenic (i-As), at concentrations
17	around 1 mg/kg. This i-As content needs to be reduced to make rice bran a useful
18	food ingredient. Evaluated here is a novel approach to minimizing rice bran i-As
19	content which is also suitable for its stabilization namely, cooking bran in percolating
20	arsenic-free boiling water. Up to 96% of i-As removal was observed for a range of
21	rice bran products, with i-As removal related to the volume of cooking water used.
22	This process reduced the copper, potassium, and phosphorus content, but had little
23	effect on other trace- and macro- nutrient elements in the rice bran. There was little
24	change in organic composition, as assayed by NIR, except for a decrease in the
25	soluble sugar and an increase, due to biomass loss, in dietary fiber.

- 26
- 27 Keywords: inorganic arsenic, rice bran, cooking water, nutrient elements, and rice
- 28 bran composition.

29 1. Introduction

30 Rice bran has high concentration in micro- and macro nutrient elements, vitamins and 31 soluble fiber, and is considered a good source of hypoallergenic protein (Zhang, 32 Zhang, Wang, & Guo, 2012). It is becoming a popular ingredient in health-promoting 33 value-added products, it is marketed as a superfood, and has been considered as a 34 health food supplement for malnourished children in international aid programs 35 among other applications (Nagendra Prasad MN, Kr, & Khatokar M, 2011; Qureshi, 36 Sami, & Khan, 2002; Sun et al., 2008; Zhang et al., 2012). However, the realization 37 that rice bran also contains high levels of the carcinogen inorganic arsenic (i-As) 38 (Andrew A. Meharg et al., 2008; Sun et al., 2008), has stalled the development of the 39 utilization of this otherwise very valuable product.

40

41 Rice accumulates much higher levels of i-As than other cereals and foodstuff, in 42 general, due to being cultivated in flooded soils (A. A. Meharg & Zhao, 2012; Sun et 43 al., 2008; Williams et al., 2007). In rice grain most of the i-As is accumulated in the 44 outer bran layers, the pericarp and the aleurone, having i-As concentrations as high as 45 <1 mg/kg (Andrew A. Meharg et al., 2008; Sun et al., 2008). The European Union has 46 formulated regulations on the maximum levels of i-As in rice in order to reduce 47 exposure, and the most restrictive one has been established at 0.1 mg/kg for rice 48 destined for the production of food for infants and young children, the level of which 49 has also been recently proposed as a maximum limit in infant rice cereals by the U.S. 50 Food and Drug Administration (EC, 2015; FDA, 2016). The UN WHO has also set an 51 advisory maximum level of i-As in polished rice grain at 0.2 mg/kg (WHO, 2014), 52 which is also the EU standard. Fortification with rice bran has become popular in 53 health/organic/whole-meal foodstuffs, and such rice bran fortified foods, such as

- 54 baby/toddler foods, tend to be elevated in i-As, leading them to have i-As 55 concentrations above EU standards, for example (Signes-Pastor et al., 2016).
- 56

Previous studies have shown that i-As in rice is quite soluble in cooking water, and 57 58 that the larger the volume of cooking water used the greater the i-As removal (Raab, 59 Baskaran, Feldmann, & Meharg, 2009). The method of cooking rice might enable i-60 As mitigation, especially when low i-As cooking water is available (Carey, Jiujin, 61 Gomes Farias, & Meharg, 2015). Carey et al. (2015) developed this observation to 62 pioneer a novel approach to rice cooking to maximize i-As removal. Their findings 63 showed that if rice was percolated with clean, i.e. i-As free, cooking water, up to 85% 64 of i-As could be removed from rice grains while cooking. The percolated cooking 65 water was either recycled (as steam then condensed to form percolating water) or 66 discarded. In the study reported here the efficacy of such percolating cooking 67 technologies in removing i-As from rice bran was trialed. Key to this study was that 68 this cooking of whole rice bran had minimal impact on the beneficial nutritional 69 qualities of bran such as fiber, protein and mineral nutrient content.

70

71 **2. Material and Methods**

72 2.1. Rice bran cooking

Commercial rice bran samples (16) were purchased, including pure rice bran (n = 14)
and rice bran water-soluble (n = 2) products. An off-the-shelf coffee percolator by
Andrew James, with no adaptation, was used to cook rice bran, as per Carey *et al.*(2015). This type of coffee-maker provides a continual stream of percolating, near
boiling, water through a filter unit. Here, the water reservoir was filled with 1.5 L of
deionized water, which took 15 min. to fully discharge through the filter unit. In the

metal-mesh filter unit 20 g of bran was placed. The bran samples were then cooked in
1-415 min. cycles with the reservoir re-filled at the end of each cycle, water-to-rice
bran ratios of which were 75:1, 150:1 and 300:1, respectively.

82

83 **2.2. Sample preparation and chemical analysis**

84 The raw and cooked rice bran samples were freeze-dried using a Christ Alpha 1-4 LD 85 Plus, and then powdered using a Retch PM100. The powder was used for X-ray 86 fluorescence (XRF) and near infrared (NIR) spectroscopy analyses. For arsenic 87 speciation powdered sample, 0.1 g, was accurately weighed into 50 ml polypropylene 88 centrifuge tubes and 10 ml of 1% concentrated nitric acid was added and left 89 overnight. Then samples were microwave digested in a CEM MARS 6 instrument for 90 30 min. at 95°C using a 3 stage slow heating program: to 55°C in 5 min. held for 10 91 min., to 75°C in 5 min., held for 10 min. to 95°C in 5 min., held for 30 min. The 92 digestate was centrifuged with a Sorvall Legend RT at 4,500 g and a 1 ml aliquot was 93 transferred to a 2 ml polypropylene vial and 10 \Box l of analytical grade hydrogen 94 peroxide was added to convert any arsenite to arsenate to facilitate subsequent 95 chromatographic species separation by ion chromatography with mass spectrometric 96 detection (IC-ICP-MS). All samples were analyzed in 2 batches including 3 blanks 97 and 3 replicate samples of the certified reference material (CRM) NIST 1568b rice 98 flour per batch. For total element analysis by inductively coupled plasma - mass 99 spectrometric (ICP-MS) 2 ml of concentrated nitric acid and 2 ml of hydrogen 100 peroxide were added into 50 ml polypropylene centrifuge tubes containing 0.1 g of 101 powdered sample and left to stand overnight. The samples were microwave digested. 102 The temperature was raised to 95°C in 5 min. and held for 10 min. and then to 135°C 103 in 5 min. and held for 10 min. Finally the digest was taken up to 180°C in 5 min. and

104 maintained for 30 min. Samples were cooled to room temperature and then an internal 105 standard (30 µl of 10 mg/kg rhodium) was added to the digestate and accurately 106 diluted to 30 ml with deionized distilled water. Several blanks and samples of NIST 107 1568b rice flour CRM were included per batch of total element analysis. 108 109 A Thermo Scientific IC5000 ion chromatography (IC) system, with a Thermo AS7, 110 2x250 mm column and a Thermo AG7, 2x50 mm guard column interfaced with a 111 Thermo ICAP Q ICP-MS in collision cell mode was used to quantify arsenic 112 speciation. A linear gradient mobile phase was carried out over 15 minutes starting at 113 100% mobile phase of 20 mM ammonium carbonate and finishing at 100% mobile 114 phase of 200 mM ammonium carbonate. The resulting chromatogram was compared 115 with that for authentic standards; dimethylarsinic acid (DMA), i-As, 116 monomethylarsonic acid (MMA), tetratmethylarsonium and arsenobetaine. DMA 117 concentration series were used to calibrate the arsenic present under each 118 chromatographic peak. 119 120 Total elements were also measured using the Thermo ICAP Q but in direct solution 121 acquisition mode. All elements reported were present both in calibration standards 122 and in CRM NIST 1568b with only elements with good CRM recoveries reported. 123 Additional elements were also analyzed by bench-top XRF (Rigaku CG), including 124 samples of NIST 1568b rice flour CRM in each batch of samples. Only elements 125 present in the CRM and with good analytical recoveries were presented. Rice bran 126 composition was also analyzed with a Thermo near infrared (NIR) spectroscopy. Each 127 rice bran samples was analyzed in triplicate and the mean value was used to calculate 128 the percentage of compositional variation of individual samples.

129

130 **2.3. Statistical analyses**

131 The median and range concentration of the main arsenic species in commercial rice 132 bran samples were determined. Likewise, total elements concentration (Ca, Cu, Fe, 133 Mn, P and S) and the percentage of the rice bran organic composition variation (fat, 134 fiber, protein, starch and sugar) according to the cooking percolating water-to-rice 135 bran ratio was also analyzed. The analysis of variance (ANOVA) and the Tukey's 136 range test were used to determine any significant differences in the main arsenic 137 species and total elements concentration between groups according to the volume of 138 percolating cooking water. All statistical analyses and plots were performed using the 139 R Statistical Software (R Core Team, 2014). The limit of detection (LOD) was 140 calculated as the mean of the blank concentrations plus three times the standard 141 deviation of the blank concentrations multiplied by the dilution factor. The ½ LOD 142 value was assigned for statistical analyses of the data when samples were below the 143 LOD.

144

145 **3. Results**

146 The mean \pm SE concentration and recovery of rice CRM flour NIST-1568b for

147 arsenic species were: 0.099 ± 0.001 mg/kg and $107 \pm 2\%$ for i-As, 0.184 ± 0.007

148 mg/kg and $102 \pm 4\%$ for DMA, and 0.010 ± 0.001 mg/kg and $89 \pm 3\%$ for MMA,

- based on N = 6. The arsenic species in the rice CRM had i-As, DMA and MMA
- 150 certified at $0.092 \pm 0.010 \text{ mg/kg}$, $0.182 \pm 0.012 \text{ mg/kg}$, and $0.0116 \pm 0.0035 \text{ mg/kg}$,
- 151 respectively. The limit of detection (LOD) for arsenic speciation, calculated from
- 152 DMA calibration, was 0.002 mg/kg. All samples presented were above the LOD for
- 153 DMA and i-As, however, almost half of the rice bran samples analyzed had MMA

154 content below the LOD, and in this case ½ LOD was used in statistical analysis of the
155 data.

156

157 The predominant arsenic species in the commercial rice bran samples analyzed was i-158 As, followed by DMA and MMA (Table 1). The median and range percentage of i-As 159 in the entire commercial raw/uncooked rice bran dataset were 95.4% and 93.4% -160 97.7 %, respectively. The commercial rice bran water-soluble samples, obtained with 161 the carbohydrases treatment (Qureshi et al., 2002), had 1.6-fold higher median i-As 162 (0.916 mg/kg) than that found in uncooked pure rice bran (0.561 mg/kg). The DMA 163 concentration in uncooked rice bran was about an order of magnitude lower than that 164 of i-As, with a median of 0.025 mg/kg and a range from 0.013 to 0.055 mg/kg for the 165 entire commercial uncooked rice bran dataset. Only ~half of the commercial raw rice 166 bran dataset had traces of MMA higher than the LOD, with a median of 0.003 mg/kg 167 ranging from <LOD to 0.006 mg/kg.

168

169 The i-As concentration in cooked rice bran was significantly lower compared to that 170 in the uncooked rice bran (p < 0.001) (**Table 2**). This study shows that greater i-As 171 removal from cooked rice bran can be achieved with greater water-to-rice bran ratio, 172 but only up to a certain extent (**Table 2** and **Figure 1**). The i-As concentration in 173 cooked rice bran with 150:1 and 300:1 water-to-rice bran ratios did not differ 174 statistically (Table 2). A median percentage of 68% and 76% of i-As could be 175 removed at the highest water-to-rice bran ratios (150:1 and 300:1, respectively), and 176 even higher than 90% in some individual samples (Figure 1). The DMA 177 concentration in cooked rice bran was significantly lower compared with that in the 178 uncooked rice bran (p < 0.001), however, the volume of cooking water did not affect

179 statistically the DMA concentration in the cooked rice bran (**Table 2**). A median

180 percentage of 52%, 62% and 65% of DMA could be removed at 75:1, 150:1 and

181 300:1 water-to-rice bran ratios, respectively (Figure 1). The cooking process did not

182 affect the MMA concentration in the rice bran. The MMA traces found in the

183 uncooked samples were still found in the cooked rice bran regardless of the volume of

the cooking water tested.

185

186 When a range of trace- and macro- elements were analyzed between uncooked and cooked rice bran with different volumes of cooking water, only copper (p = 0.002), 187 188 potassium (p < 0.001), and phosphorus (p < 0.001) were significantly different, while 189 calcium, iron, manganese, sulfur and zinc were non-significant (Table 2). The loss of 190 copper, potassium and phosphorus during the cooking process was 37%, 54% and 191 16%, respectively, regardless of cooking water volume tested, which did not 192 statistically affect the concentration of these elements in the cooked rice bran (Table 193 2).

194

195 When the compositional variation in rice bran due to the cooking process with 196 different volumes of water was explored only the fiber and the sugar content seemed 197 to differ from the original content in the uncooked rice bran, while fat, protein and 198 starch appeared to be stable throughout the cooking process regardless of the volume 199 of cooking water (Figure 2). The fiber content in cooked rice bran had a median 200 percentage increment of 14%, 35%, and 40% compared to that in uncooked rice bran 201 when 75:1, 150:1 and 300:1 water-to-rice bran ratios were performed, respectively, 202 increment of which is probably due to the overall rice bran biomass decrease caused 203 during the cooking process. On the contrary, the relationship between fiber and sugar

had a negative correlation coefficient of -0.63, with a median percentage reduction of
sugar content of 35%, 57%, and 82% according to the level of percolating cooking
water volume.

207

208 4. Discussion

209 Rice bran has become a popular ingredient in "health-products" due to its positive 210 nutritional aspects. However, rice bran contains high concentrations of i-As, up to 1.1 211 mg/kg in this study here, which needs to be reduced to make rice bran suitable for the 212 human consumption. Using a continuous flow of arsenic-free near boiling water 213 percolated through pure rice bran enables an i-As removal from rice bran of up to 214 96%, a higher percentage than that previously reported for whole-grain and polished 215 rice samples where a maximum removal value of 85% was obtained for individual 216 rice samples (Carey et al., 2015). This may be related to the larger cooking water-to-217 rice bran ratio used in this study (*i.e.* 300:1) compared to that previously tested with 218 rice (i.e. 12:1). A moderation of i-As removal efficiency from rice bran was described 219 for the higher volumes of cooking water, reaching a plateau at a cooking water-to-rice 220 bran ratio of 150:1. The i-As removal approach described here provides a novel 221 solution to significantly reduce the i-As concentration in pure rice bran below the UN 222 WHO advisory level and the maximum EU i-As limit for non-parboiled milled rice 223 (0.200 mg/kg). A patented methodology to remove arsenic in rice bran protein has 224 been previously developed in China; however, the patent differs from the approach 225 detailed in this study focused on i-As removal from whole rice bran instead of from 226 the subcomponent rice bran protein. In addition, the patented approach is for an 227 industrial setting, and combines a static cooking chemical extraction with sodium 228 hydroxide at pH 11.5 and a centrifugation step (China Faming Zhuanli Shenqing,

229 2013). Conversely, the approach described here can be applied from a

home/homestead to an industrial setting, and only uses pure water in a continuousnovel percolation cooking technique.

232



241

242 The removal approach reduced the copper, potassium and phosphorus content in the 243 cooked rice bran; however, the concentrations of these elements were still very high 244 compared to that found in rice (Carey et al., 2015), and if necessary, they could be 245 refortified after cooking process. The i-As removal approach described here also 246 reduced the soluble sugar content in favor of an increment of insoluble dietary fiber in 247 treated rice bran, possibly due to the decrease in biomass. This could help in creating 248 healthier food products due to the cooked brans lower sugar and higher fiber content 249 (The Lancet, 2016; Wang, Suo, de Wit, Boom, & Schutyser, 2016). Neither vitamins 250 nor other bioactive compounds removed due to rice bran processing with percolating 251 near boiling water were assessed here, and thus further studies are required to address 252 this, especially for those water-soluble and thermo sensitive, *i.e* B-vitamins group and 253 phenolic compounds, which rice bran contains in notable amounts (Kim & Lim, 2016;

254	Patil et al., 2016; Tuncel, Yilmaz, Kocabiyik, & Uygur, 2014). Again, if key vitamins
255	are remove, these could be refortified if necessary.

256

257	The approach	studied here	demonstrates	that the	continual	percolating	of near 1	boiling
						F		0

- 258 cooking water flow principle is an efficient i-As whole rice bran removal method. The
- 259 high volumes of water used here could be greatly reduced if the cooking water was
- 260 recycled through distillation by using the previously validated for i-As removal from
- rice grain (Carey et al., 2015).
- 262

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- 333 334

Commercial RB	Ν	i-As (mg/kg d.w.)	DMA (mg/kg d.w.)	i-As %
RB	14	0.561 (0.376 - 0.818)	0.025 (0.013 - 0.032)	95.5 (93.4 - 97.7)
RB_WS	2	0.916 (0.753 - 1.079)	0.041 (0.028 - 0.055)	95.6 (95.0 - 96.3)
RB_1	3	0.668 (0.664 - 0.818)	0.016 (0.013 - 0.018)	97.6 (96.5 - 97.7)
RB_2	4	0.570 (0.535 - 0.626)	0.025 (0.024 - 0.025)	95.6 (95.4 - 95.9
RB_3	2	0.533 (0.504 - 0.562)	0.027 (0.024 - 0.030)	94.5 (94.3 - 94.7
RB_4	1	0.583 (0.583 - 0.583)	0.027 (0.027 - 0.027)	94.8 (94.8 - 94.8
RB_5	1	0.561 (0.561 - 0.561)	0.030 (0.030 - 0.030)	94.1 (94.1 - 94.1
RB_6	2	0.521 (0.484 - 0.559)	0.030 (0.029 - 0.032)	93.5 (93.4 -93.6)
RB_7	1	0.376 (0.376 - 0.376)	0.017 (0.017 - 0.017)	95.6 (95.6 - 95.6
RB_8_WS	1	1.079 (1.079 - 1.079)	0.055 (0.055 - 0.055)	95.0 (95.0 - 95.0
RB_9_WS	1	0.753 (0.753 - 0.753)	0.028 (0.028 - 0.028)	96.3 (96.3 - 96.3)

Table 1: Inorganic arsenic and DMA concentration in commercial rice bran, and

percentage of inorganic arsenic (median (min - max)). *RB = Pure rice bran and RB

337 WS = Rice bran water-soluble.

Water:Rice ratio Ν i-As (mg/kg) DMA (mg/kg) Ca (mg/kg) Cu (mg/kg) K (mg/kg) 10.60 (9.350 -15.300 (14,000 -Uncooked $0.601 (0.535 - 0.818)^{a}$ $0.025 (0.013 - 0.030)^{a}$ 515.0 (402.0 - 769.0) 7 13.90)^a $19.700)^{a}$ 6.980 (6.380 -8,430 (8,030 -75:1 0.374 (0.277 - 0.526)^b $0.012 (0.006 - 0.020)^{b}$ 379.0 (299.0 - 787.0) 7 $10.00)^{b}$ $12.000)^{b}$ 6.860 (5.090 -6,720 (4,040 -0.212 (0.032 - 0.376)^c $0.007 (0.003 - 0.014)^{b}$ 489.0 (228.0 - 661.0) 150:1 7 $11.10)^{b}$ $10.800)^{b}$ 6.195 (5.850 -4.805 (1.890 -300:1 $0.167 (0.028 - 0.260)^{\circ}$ $0.006 (0.003 - 0.011)^{b}$ 445.5 (389.0 - 567.0) 4 9.890)^b $10.500)^{b}$ *p*-value < 0.001 < 0.001 0.543 0.002 < 0.001 Water:Rice ratio Ν Fe (mg/kg) Mn (mg/kg) P (mg/kg) S (mg/kg) Zn (mg/kg) 18,679 (17,131 -Uncooked 84.90 (79.90 - 131.0) 276.1 (214.7 - 417.1) 1,530 (1,340 - 1,830) 60.60 (46.00 - 73.50) 7 20,703)^a 16.238 (14.649 -75:1 71.50 (62.30 - 91.40) 291.2 (231.4 - 408.6) 1,390 (1,250 - 1,840) 52.90 (38.60 - 56.00) 7 17,376)^{ab} 14.776 (11.064 -150:1 75.50 (53.40 - 149.0) 303.5 (233.5 - 427.1) 1,450 (1,090 - 2,120) 54.30 (35.90 - 84.50) 7 17,237)^b 14,494 (11,151 -300:1 67.25 (51.00 - 118.0) 382.8 (319.4 - 424.8) 1,235 (1,010 - 2,090) 48.50 (34.50 - 77.40) 4 $16,536)^{b}$ *p*-value 0.504 0.362 < 0.001 0.635 0.601

Table 2: Arsenic speciation (i-As and DMA), and total calcium, copper, potassium, iron, manganese, phosphorus, sulfur, and zinc in raw and

cooked rice bran according to the cooking time (median (min - max)).



Figure 1: Inorganic arsenic and DMA concentration in rice bran, and removal percentage according to water-to-rice bran ratio.

Figure 2: Percentage of compositional variation according to the water-to-rice bran ratio. Each point at 75:1 and 150:1 ratios shows the median percentage obtained from 7 rice bran samples, respectively. Each point at 300:1 ratio shows the median percentage obtained from 4 rice bran samples.

