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Inorganic arsenic removal in rice bran by percolating cooking water

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Abstract

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

Keywords: inorganic arsenic, rice bran, cooking water, nutrient elements, and rice bran composition.

1. Introduction

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

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

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

2. Material and Methods

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- 4 15 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.

2.2. Sample preparation and chemical analysis

The raw and cooked rice bran samples were freeze-dried using a Christ Alpha 1-4 LD Plus, and then powdered using a Retch PM100. The powder was used for X-ray fluorescence (XRF) and near infrared (NIR) spectroscopy analyses. For arsenic speciation powdered sample, 0.1 g, was accurately weighed into 50 ml polypropylene centrifuge tubes and 10 ml of 1% concentrated nitric acid was added and left overnight. Then samples were microwave digested in a CEM MARS 6 instrument for 30 min. at 95°C using a 3 stage slow heating program: to 55°C in 5 min. held for 10 min., to 75°C in 5 min., held for 10 min. to 95°C in 5 min., held for 30 min. The digestate was centrifuged with a Sorvall Legend RT at 4,500 g and a 1 ml aliquot was transferred to a 2 ml polypropylene vial and 10 µl of analytical grade hydrogen peroxide was added to convert any arsenite to arsenate to facilitate subsequent chromatographic species separation by ion chromatography with mass spectrometric detection (IC-ICP-MS). All samples were analyzed in 2 batches including 3 blanks and 3 replicate samples of the certified reference material (CRM) NIST 1568b rice flour per batch. For total element analysis by inductively coupled plasma - mass spectrometric (ICP-MS) 2 ml of concentrated nitric acid and 2 ml of hydrogen peroxide were added into 50 ml polypropylene centrifuge tubes containing 0.1 g of powdered sample and left to stand overnight. The samples were microwave digested. The temperature was raised to 95°C in 5 min. and held for 10 min. and then to 135°C in 5 min. and held for 10 min. Finally the digest was taken up to 180°C in 5 min. and maintained for 30 min. Samples were cooled to room temperature and then an internal standard (30 µl of 10 mg/kg rhodium) was added to the digestate and accurately diluted to 30 ml with deionized distilled water. Several blanks and samples of NIST 1568b rice flour CRM were included per batch of total element analysis.

A Thermo Scientific IC5000 ion chromatography (IC) system, with a Thermo AS7, 2x250 mm column and a Thermo AG7, 2x50 mm guard column interfaced with a Thermo ICAP Q ICP-MS in collision cell mode was used to quantify arsenic speciation. A linear gradient mobile phase was carried out over 15 minutes starting at 100% mobile phase of 20 mM ammonium carbonate and finishing at 100% mobile phase of 200 mM ammonium carbonate. The resulting chromatogram was compared with that for authentic standards; dimethylarsinic acid (DMA), i-As, monomethylarsonic acid (MMA), tetratmethylarsonium and arsenobetaine. DMA concentration series were used to calibrate the arsenic present under each chromatographic peak.

Total elements were also measured using the Thermo ICAP Q but in direct solution acquisition mode. All elements reported were present both in calibration standards and in CRM NIST 1568b with only elements with good CRM recoveries reported. Additional elements were also analyzed by bench-top XRF (Rigaku CG), including samples of NIST 1568b rice flour CRM in each batch of samples. Only elements present in the CRM and with good analytical recoveries were presented. Rice bran composition was also analyzed with a Thermo near infrared (NIR) spectroscopy. Each rice bran samples was analyzed in triplicate and the mean value was used to calculate the percentage of compositional variation of individual samples.

2.3. Statistical analyses

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

3. Results

The mean ± SE concentration and recovery of rice CRM flour NIST-1568b for arsenic species were: 0.099 ± 0.001 mg/kg and 107 ± 2% for i-As, 0.184 ± 0.007 mg/kg and 102 ± 4% for DMA, and 0.010 ± 0.001 mg/kg and 89 ± 3% for MMA, based on N = 6. The arsenic species in the rice CRM had i-As, DMA and MMA certified at 0.092 ± 0.010 mg/kg, 0.182 ± 0.012 mg/kg, and 0.0116 ± 0.0035 mg/kg, respectively. The limit of detection (LOD) for arsenic speciation, calculated from DMA calibration, was 0.002 mg/kg. All samples presented were above the LOD for DMA and i-As, however, almost half of the rice bran samples analyzed had MMA content below the LOD, and in this case ½ LOD was used in statistical analysis of the data.

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

The i-As concentration in cooked rice bran was significantly lower compared to that in the uncooked rice bran ($p<0.001$) **(Table 2)**. This study shows that greater i-As removal from cooked rice bran can be achieved with greater water-to-rice bran ratio, but only up to a certain extent (**Table 2** and **Figure 1**). The i-As concentration in cooked rice bran with 150:1 and 300:1 water-to-rice bran ratios did not differ statistically (**Table 2**). A median percentage of 68% and 76% of i-As could be removed at the highest water-to-rice bran ratios (150:1 and 300:1, respectively), and even higher than 90% in some individual samples (**Figure 1)**. The DMA concentration in cooked rice bran was significantly lower compared with that in the uncooked rice bran ($p<0.001$), however, the volume of cooking water did not affect statistically the DMA concentration in the cooked rice bran (**Table 2)**. A median percentage of 52%, 62% and 65% of DMA could be removed at 75:1, 150:1 and 300:1 water-to-rice bran ratios, respectively (**Figure 1)**. The cooking process did not affect the MMA concentration in the rice bran. The MMA traces found in the uncooked samples were still found in the cooked rice bran regardless of the volume of the cooking water tested.

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

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

4. Discussion

Rice bran has become a popular ingredient in “health-products” due to its positive nutritional aspects. However, rice bran contains high concentrations of i-As, up to 1.1 mg/kg in this study here, which needs to be reduced to make rice bran suitable for the human consumption. Using a continuous flow of arsenic-free near boiling water percolated through pure rice bran enables an i-As removal from rice bran of up to 96%, a higher percentage than that previously reported for whole-grain and polished rice samples where a maximum removal value of 85% was obtained for individual rice samples (Carey et al., 2015). This may be related to the larger cooking water-to-rice bran ratio used in this study (*i.e.* 300:1) compared to that previously tested with rice (*i.e.* 12:1). A moderation of i-As removal efficiency from rice bran was described for the higher volumes of cooking water, reaching a plateau at a cooking water-to-rice bran ratio of 150:1. The i-As removal approach described here provides a novel solution to significantly reduce the i-As concentration in pure rice bran below the UN WHO advisory level and the maximum EU i-As limit for non-parboiled milled rice (0.200 mg/kg). A patented methodology to remove arsenic in rice bran protein has been previously developed in China; however, the patent differs from the approach detailed in this study focused on i-As removal from whole rice bran instead of from the subcomponent rice bran protein. In addition, the patented approach is for an industrial setting, and combines a static cooking chemical extraction with sodium hydroxide at pH 11.5 and a centrifugation step (China Faming Zhuanli Shenqing, 2013). Conversely, the approach described here can be applied from a home/homestead to an industrial setting, and only uses pure water in a continuous novel percolation cooking technique.

The heat involved in cooking may stabilize the rice bran by destruction or inhibition of lipase – the enzyme that causes development of free fatty acids responsible for rancidity, which would save including an extra process to stabilize the rice bran (Nagendra Prasad MN et al., 2011). This remains to be tested along with the effect of the cooking process on the sensorial features of the final rice bran, *i.e.* texture and color; however, moist heat stabilization is one of the methods used in the normal rice bran processing before its use (Kim, Chung, & Lim, 2014; Lakkakula, Lima, & Walker, 2004; Patil, Kar, & Mohapatra, 2016).

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

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

The approach studied here demonstrates that the continual percolating of near boiling cooking water flow principle is an efficient i-As whole rice bran removal method. The high volumes of water used here could be greatly reduced if the cooking water was recycled through distillation by using the previously validated for i-As removal from rice grain (Carey et al., 2015).

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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 WS = Rice bran water-soluble.

Commercial RB	N	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 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)).

Water:Rice ratio	N	i-As (mg/kg)	DMA (mg/kg)	Ca (mg/kg)	Cu (mg/kg)	K (mg/kg)
Uncooked	7	0.601 (0.535 - 0.818)^{a}	0.025 (0.013 - 0.030)^{a}	515.0 (402.0 - 769.0)	10.60 (9.350 - 13.90)^{a}	15,300 (14,000 - 19,700)^{a}
75:1	7	0.374 (0.277 - 0.526)^{b}	0.012 (0.006 - 0.020)^{b}	379.0 (299.0 - 787.0)	6.980 (6.380 - 10.00)^{b}	8,430 (8,030 - 12,000)^{b}
150:1	7	0.212 (0.032 - 0.376)^{c}	0.007 (0.003 - 0.014)^{b}	489.0 (228.0 - 661.0)	6.860 (5.090 - 11.10)^{b}	6,720 (4,040 - 10,800)^{b}
300:1	4	0.167 (0.028 - 0.260)^{c}	0.006 (0.003 - 0.011)^{b}	445.5 (389.0 - 567.0)	6.195 (5.850 - 9.890)^{b}	4,805 (1,890 - 10,500)^{b}
p-value		**<0.001**	**<0.001**	0.543	**0.002**	**<0.001**

Water:Rice ratio	N	Fe (mg/kg)	Mn (mg/kg)	P (mg/kg)	S (mg/kg)	Zn (mg/kg)
Uncooked	7	84.90 (79.90 - 131.0)	276.1 (214.7 - 417.1)	18,679 (17,131 - 20,703)^{a}	1,530 (1,340 - 1,830)	60.60 (46.00 - 73.50)
75:1	7	71.50 (62.30 - 91.40)	291.2 (231.4 - 408.6)	16,238 (14,649 - 17,376)^{ab}	1,390 (1,250 - 1,840)	52.90 (38.60 - 56.00)
150:1	7	75.50 (53.40 - 149.0)	303.5 (233.5 - 427.1)	14,776 (11,064 - 17,237)^{b}	1,450 (1,090 - 2,120)	54.30 (35.90 - 84.50)
300:1	4	67.25 (51.00 - 118.0)	382.8 (319.4 - 424.8)	14,494 (11,151 - 16,536)^{b}	1,235 (1,010 - 2,090)	48.50 (34.50 - 77.40)
p-value		0.504	0.362	**<0.001**	0.635	0.601

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

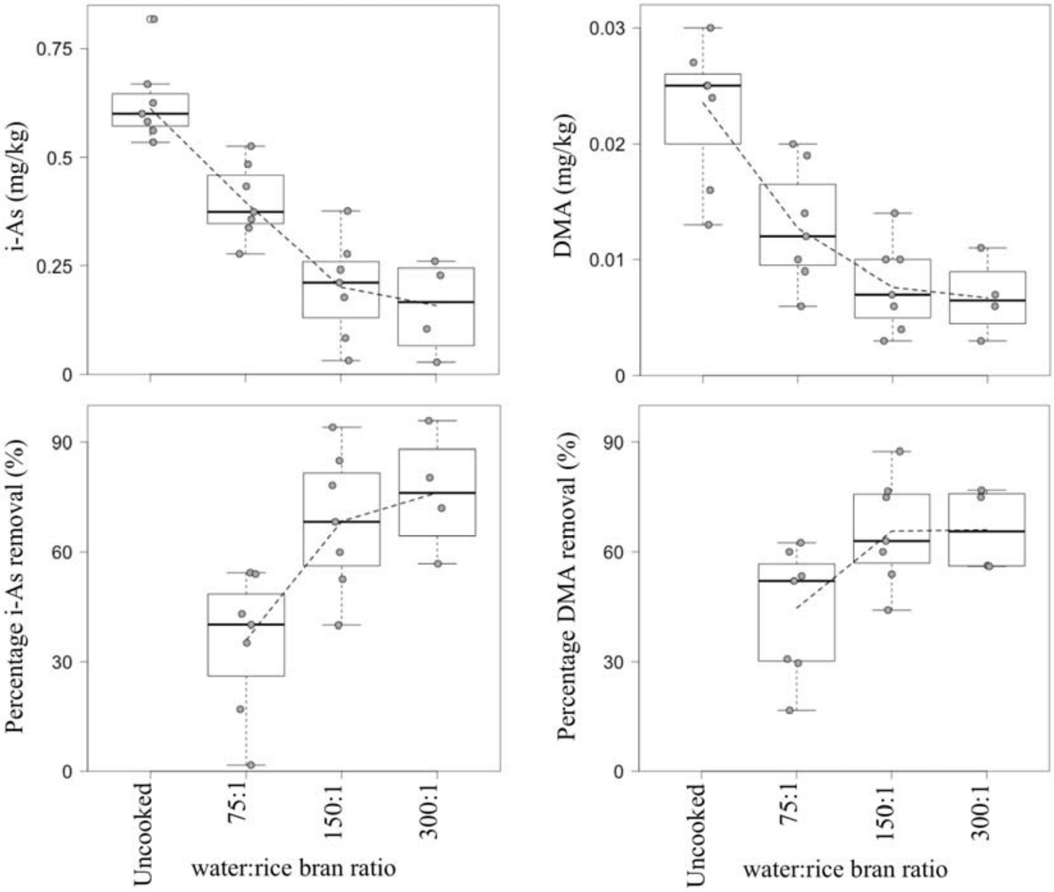

Figure 2: Percentage of compositional variation according to the water-to-rice bran ratio. Each point at 75:1 and 150:1 ratios shows the median percentage obtained from 7 rice bran samples, respectively. Each point at 300:1 ratio shows the median percentage obtained from 4 rice bran samples.