1 **Inorganic arsenic removal in rice bran by percolating cooking water**

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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.

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27

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

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

57 Previous studies have shown that i-As in rice is quite soluble in cooking water, and

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**

73 Commercial rice bran samples (16) were purchased, including pure rice bran ($n = 14$)

74 and rice bran water-soluble ($n = 2$) products. An off-the-shelf coffee percolator by

75 Andrew James, with no adaptation, was used to cook rice bran, as per Carey *et al.*

76 (2015). This type of coffee-maker provides a continual stream of percolating, near

77 boiling, water through a filter unit. Here, the water reservoir was filled with 1.5 L of

78 deionized water, which took 15 min. to fully discharge through the filter unit. In the

79   metal-mesh filter unit 20 g of bran was placed. The bran samples were then cooked in

80   1- 4 15 min. cycles with the reservoir re-filled at the end of each cycle, water-to-rice

81   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 µl 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

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 $107 \pm 2\%$ for i-As, 0.184 ± 0.007 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 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

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

184 the cooking water tested.

185

186 When a range of trace- and macro- elements were analyzed between uncooked and

187 cooked rice bran with different volumes of cooking water, only copper ($p = 0.002$),

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

204 had a negative correlation coefficient of -0.63, with a median percentage reduction of

205 sugar content of 35%, 57%, and 82% according to the level of percolating cooking

206 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

230   home/homestead to an industrial setting, and only uses pure water in a continuous

231   novel percolation cooking technique.

232

233   The heat involved in cooking may stabilize the rice bran by destruction or inhibition

234   of lipase – the enzyme that causes development of free fatty acids responsible for

235   rancidity, which would save including an extra process to stabilize the rice bran

236   (Nagendra Prasad MN et al., 2011). This remains to be tested along with the effect of

237   the cooking process on the sensorial features of the final rice bran, *i.e.* texture and

238   color; however, moist heat stabilization is one of the methods used in the normal rice

239   bran processing before its use (Kim, Chung, & Lim, 2014; Lakkakula, Lima, &

240   Walker, 2004; Patil, Kar, & Mohapatra, 2016).

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 removed, these could be refortified if necessary.

256

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

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

261 rice grain (Carey et al., 2015).

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334

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

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

337   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)

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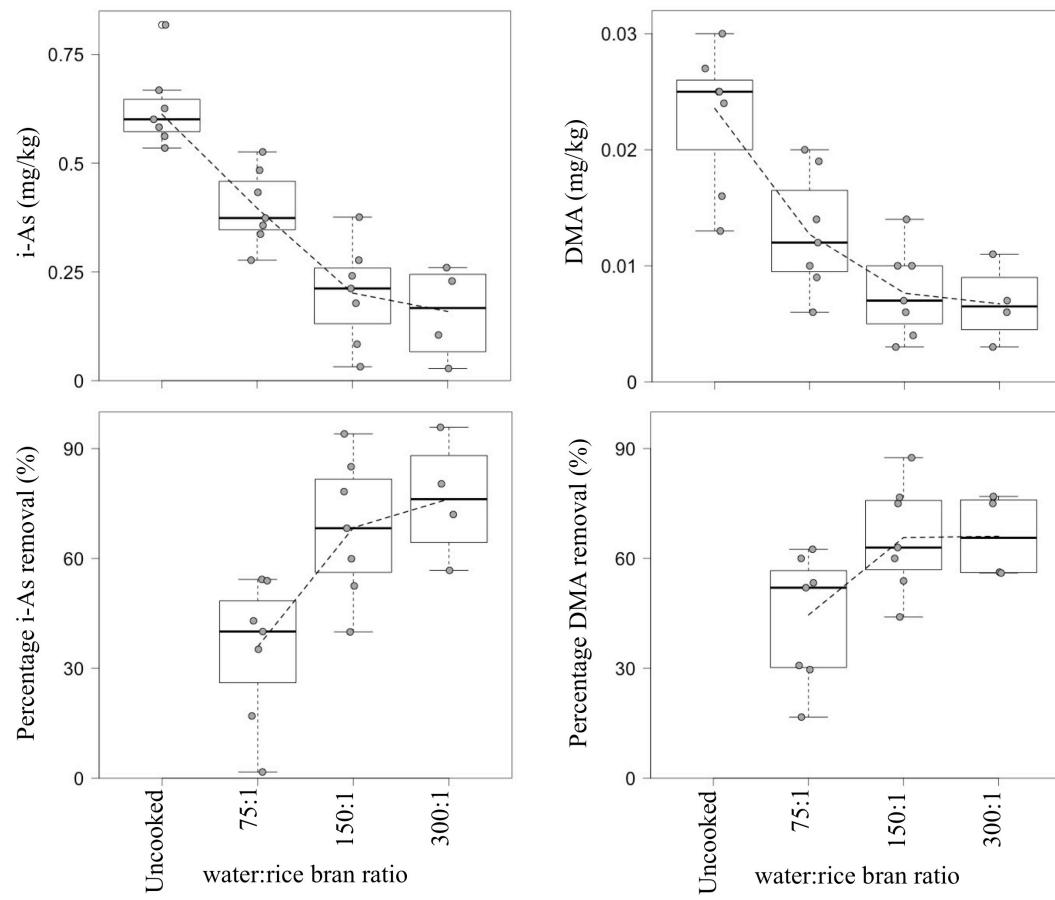
Figure 2: Percentage of compositional variation according to the water-to-rice bran ratio. Each point at 75:1 and 150:1 ratios shows the median percentage obtained from 7 rice bran samples, respectively. Each point at 300:1 ratio shows the median percentage obtained from 4 rice bran samples.