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36	Keywords separated by ' - '	Arsenic - Rice - Ecuador - Spain - Cooking - Arsenic species
37	Foot note information	<p>Rice can reach high arsenic concentrations, and the cooking process can substantially reduce its concentration</p>

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## Electronic supplementary material

**ESM 1**  
(DOCX 50 kb)

**ESM 2**  
(DOCX 24 kb)

1  
3  
2

RESEARCH ARTICLE

4

**Effect of cooking on arsenic concentration in rice**

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Q1 6

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9

**Abstract**

This study assessed the effect of rinsing and boiling on total content of As (tAs) and of its inorganic and organic forms in different types of rice (polished and brown) from Spain and Ecuador. Rice was subjected to five different treatments. The results showed that the treatment consisting of three grain rinsing cycles followed by boiling in excess water showed a significant decrease in tAs content compared with raw rice. Regarding As species, it is worth noting that the different treatments significantly reduced the content of the most toxic forms of As. The estimated lifetime health risks indicate that pre-rinsing alone can reduce the risk by 50%, while combining it with discarding excess water can reduce the risk by 83%; therefore, the latter would be the preferable method.

**Keywords** Arsenic · Rice · Ecuador · Spain · Cooking · Arsenic species

**Introduction**

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Q5 21

Arsenic (As) intake through the consumption of drinking water and rice (*Oryza sativa*) with high contents of this element constitutes a severe public health issue for almost half the world's population. This issue is particularly dramatic in South and Southeast Asian countries such as Bangladesh, where 35–77 million people are at risk of arsenic poisoning (Rahman et al., 2018).

As is considered a class I carcinogen by the IARC (2012), and the main source of exposure is contaminated drinking water (Rahman et al., 2018, Davis et al., 2017, Rasheed et al., 2018). However, in countries where rice is a staple foodstuff (i.e., Southeast Asia, Latin America), rice is also a major source of As intake due to its capacity for As accumulation (Rahman et al., 2018, Williams et al., 2007a,b, Davis et al., 2017).

The predominant species of arsenic in rice grain are inorganic forms (AsIII, AsV) and dimethylarsinic acid (DMA) (Zhu et al., 2008, Signes-Pastor et al., 2016), with inorganic forms showing a much higher toxicity (Meharg and Zhao, 2012). Moreover, the toxicity of As has been observed to depend on other factors such as the amount of rice consumed (Mandal et al., 2019), type of rice (polished, brown, organic, etc.) (Segura et al., 2016, Yim et al., 2017, Meharg et al., 2008, Zhu et al., 2008), body weight (USEPA, 1989), and factors influencing the toxicity of the chemical, including genetic polymorphisms, life stage, gender, nutritional status, and concurrent exposure to other agents or environmental factors (NRC, 1999). More recently, the cooking method has also been observed to significantly influence As intake (Rahman et al., 2018).

Most studies on total arsenic (tAs) and its chemical forms have been performed mainly on raw rice grains (see e.g., Raber et al., 2012, Otero et al., 2016, Nunes & Otero, 2017, Chen et al., 2016, Dos Santos et al., 2017); however, its presence in cooked rice is worth considering, since this is the form in which it is consumed by the population (Jitaru et al., 2016).

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**Hypothesis** Rice can reach high arsenic concentrations, and the cooking process can substantially reduce its concentration

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56 It has been shown that the concentration of total arsenic  
 57 content and of arsenic species may be altered during the pre-  
 58 preparation of food for human consumption. The number of stud-  
 59 ies on this regard is still limited and the observed uncertainty is  
 60 high, as shown in Table S1 (Supplementary Material—main  
 61 studies published over the last decades about the effect that  
 62 cooking methods may have on arsenic content of rice). These  
 63 studies considered the following sources of variability: (i) the  
 64 arsenic content in the water used for cooking (no arsenic; low  
 65 arsenic content; high arsenic content); (ii) the effect of rinsing  
 66 the raw rice; and (iii) water-to-rice ratio (ranging between 1:2  
 67 and 1:10). Percentage of total arsenic remaining after cooking  
 68 was the main indicator, with only a small fraction including  
 69 speciation. Very large intra-study uncertainty is common,  
 70 sometimes with percentages differing up to four times be-  
 71 tween the lowest and highest reported values (see, e.g.,  
 72 Althobiti et al. (2018)). Inter-study uncertainty is even higher,  
 73 with difference in percentages of up to nine times for rice  
 74 cooked under the same conditions.

75 Despite these uncertainties, some general conclusions are  
 76 possible. Rinsing the rice before cooking can reduce total  
 77 arsenic content between 78 and 97% of that of the untreated  
 78 rice (Gray et al., 2015; Sharafi et al., 2019a), and between 75  
 79 and 91% for inorganic arsenic (Gray et al., 2015). When low  
 80 arsenic water is used for cooking and the excess water is  
 81 discarded (namely for water-to-rice ratios above 1:4), the  
 82 cooked rice will have less total and inorganic arsenic than in  
 83 the unprepared rice (Mandal et al., 2019; Sharafi et al.,  
 84 2019b). The mean arsenic content of cooked rice decreases  
 85 as the water-to-rice ratio increases, as long as excess water is  
 86 discarded, indicating a clear dilution effect. Cooking with ex-  
 87 cess water is without doubt the best method to reduce expo-  
 88 sure to arsenic. Ratios of 1:6 and above can reduce total and  
 89 inorganic arsenic concentrations to about half of those in raw  
 90 rice.

91 When water rich in arsenic is used for cooking, the final  
 92 product will be enriched in the substance, as almost all the  
 93 arsenic present in contaminated cooking water may be  
 94 retained during boiling of rice (FAO/WHO, 2011). In this  
 95 case, the different studies show high variability, being strongly  
 96 affected by the studied concentrations in cooking water.

97 If the rice is cooked until all water evaporates at boiling  
 98 temperature, no relevant alteration in arsenic concentrations  
 99 should be observed, apart from a small conversion of species  
 100 (Gray et al., 2015; Raab et al., 2009). Conversion to other  
 101 arsenicals during food preparation has been observed and  
 102 may be significant after cooking at temperatures above  
 103 150 °C (Van Elteren and Šlejkovec, 1997), which may occur  
 104 in some cooking treatments in which the food surface is in  
 105 direct contact with the source of heat (grilling, frying, or bak-  
 106 ing) (Devesa et al., 2008).

107 The above observations agree with those of Bundschuh  
 108 et al. (2012) and Cubadda et al. (2017) who identify the

cooking method as maybe the most important process affect- 109  
 ing both total arsenic concentrations and arsenic speciation. 110  
 More research in this area is still necessary, with specific focus 111  
 on iAs and on ready-to-eat food, since preparation and 112  
 cooking can significantly affect bioaccessibility (Cubadda 113  
 et al., 2017). 114

As mentioned, recent studies have mainly analyzed the 115  
 presence of total As (tAs) and of its inorganic forms (iAs) after 116  
 different rice-rinsing and rice-cooking processes (Mandal 117  
 et al., 2019), but few studies have considered organic species 118  
 of As (oAs), which are considered less toxic than iAs 119  
 (Rasheed et al., 2018). 120

Taking into account that As toxicity depends essentially on 121  
 the concentration of its chemical forms, the main aim of this 122  
 study was to determine the effect of grain rinsing and boiling 123  
 on the concentration of tAs and of its inorganic (iAs) and 124  
 organic (oAs) forms in market basket samples of rice from 125  
 Ecuador and Spain. For this purpose, five treatments were 126  
 applied to brown and polished rice samples, combining rins- 127  
 ing in different volumes of water and different cooking 128  
 methods (boiling with or without excess water). After each 129  
 treatment, As was extracted and contents of tAs and of its 130  
 inorganic (AsIII, AsV) and organic (dimethylarsinic acid, 131  
 DMA; monomethylarsonic acid, MMA; and arsenobetaine, 132  
 AsB) forms were determined. 133

**Materials and methods** 134

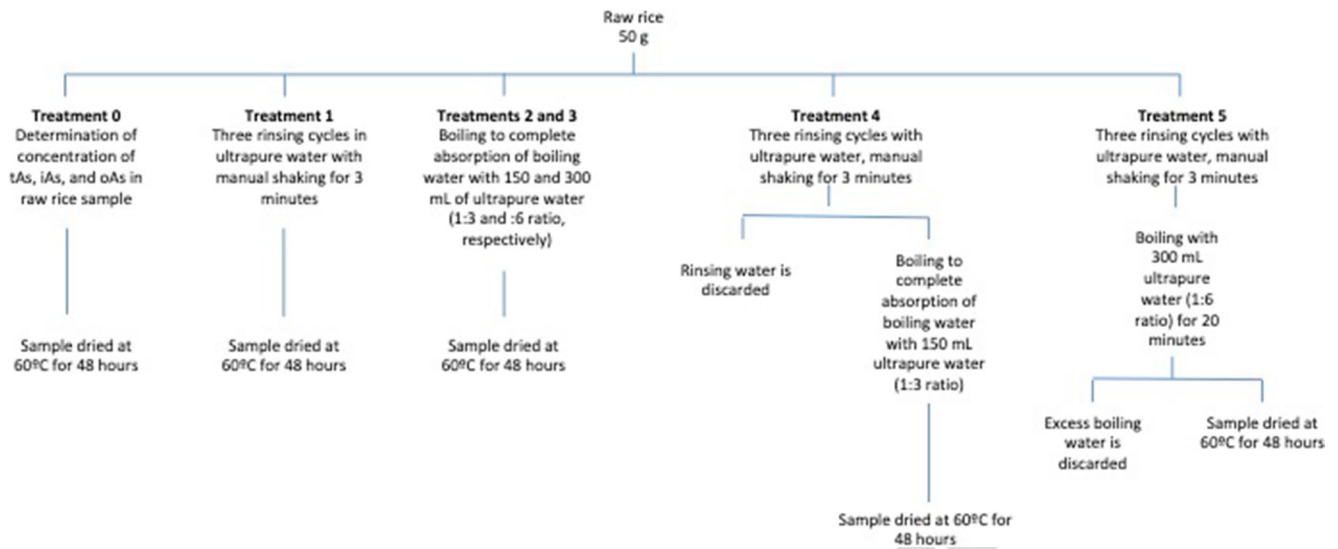
**Treatment of rice grains** 135

In practical studies, the statistics of the data are not known a 136  
 priori, so the sample size is chosen parsimoniously to accom- 137  
 modate the available resources while complying with objec- 138  
 tives of the experiment. Small sample sizes can increase the 139  
 likelihood of a type II error skewing the results, which de- 140  
 creases the power of the study; but as the sample size grows 141  
 above a certain size, the power of the test also increases, iden- 142  
 tifying small, impractical effects. As so, the option here was to 143  
 start with a parsimonious sample size. 144

Seven market basket samples of rice from Ecuador (2 sam- 145  
 ples) and Spain (5 samples), of which 4 samples corresponded 146  
 to polished rice and 3 to brown rice, were subjected to six 147  
 different treatments to determine the effect of rinsing and 148  
 cooking methods on concentration of tAs and its species. 149

Treatments were designed considering previous studies 150  
 (Jitaru et al., 2016, Mihucz et al., 2007, Naito et al., 2015, 151  
 Raab et al., 2009; Fig. 1). For this, 50 g of rice was subjected 152  
 to the following treatments: 153

- Treatment T0: Concentrations of tAs and its inorganic 154  
 (iAs) and organic forms (oAs) in raw rice samples were 155  
 determined. 156



**Fig. 1** Rinsing and cooking treatments applied to rice samples

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- 157 • Treatment T1: Samples were subjected to three rinsing  
158 cycles with 300 mL Milli-Q water with manual shaking  
159 for 3 min.
- 160 • Treatment T2: Samples were directly boiled in 150 mL  
161 Milli-Q water (1:3 ratio) to complete absorption of the  
162 boiling water, with no prior rinsing.
- 163 • Treatment T3: Similar to treatment 2, but using 300 mL  
164 Milli-Q water (1:6 ratio).
- 165 • Treatment T4: Samples were subjected to three rinsing  
166 cycles with 300 mL (1:6) Milli-Q water with manual shak-  
167 ing for 3 min; rinsing water was then discarded, and rice  
168 was subjected to boiling to complete absorption in 150 mL  
169 (1:3) Milli-Q water.
- 170 • Treatment T5: Samples were subjected to a rinsing process  
171 similar to the one described in the previous item (treatment  
172 T4), and the rice was subsequently boiled in excess Milli-  
173 Q water (300 mL) for 20 min at 150 °C, discarding excess  
174 boiling water.

175  
176 After each treatment, samples were dried at 65 °C to con-  
177 stant weight (~48 h), ground in an agate mortar, and stored in  
178 polyethylene bags at room temperature until analysis.

179 **Determination of As concentration in grain rice**

180 The content of tAs was determined in 0.5–1.0 g of previously  
181 ground samples. Samples were digested in a mixture of HNO<sub>3</sub>  
182 and H<sub>2</sub>O<sub>2</sub> (Suprapur) (Meharg and Rahman, 2003): 5 mL  
183 HNO<sub>3</sub> (65%), 1 mL H<sub>2</sub>O<sub>2</sub> (33%), and 5 mL Milli-Q water  
184 (w/v), and were left to rest overnight. Tubes were subsequently  
185 placed in a sample preparation block (Perkin Elmer SPB 48–  
186 50) at 95 °C for 3 h. The extract was filtered by 0.20 μm. The

total As content was determined by an ICP-MS system 187  
(Agilent Technologies, Palo Alto, CA, USA). 188

Partitioning of As was carried out on 0.50 g of sample (dry 189  
weight), and it was extracted with 15 mL of 0.28 M HNO<sub>3</sub> 190  
(65%, Merck) by heating the samples at 95 °C for 90 min. The 191  
samples were then centrifuged at 10,000 rpm at 4 °C for 192  
20 min. The supernatant was then filtered through a 193  
0.45-μm filter and conserved at –20 °C until analysis. 194  
Inorganic As (iAs: ΣAsIII, AsV) and organic As (oAs: Σ 195  
(DMA, MMA, AsB)) were analyzed by HPLC (Varian 196  
Prostar, Spectralab Scientific, Toronto, Canada) coupled to 197  
an ICP-MS system (Varian 820-MS). 198

Concurrently, the certified reference material (CRM) 199  
1568b, rice flour, by NIST (USA), was analyzed. Mean values 200  
obtained for the different arsenic species were iAs: 0.109 ± 201  
0.038 mg/kg; DMA: 0.218 ± 0.093 mg/kg; and MMA: 0.018 202  
± 0.001 mg/kg, corresponding to a 107%, 113%, and 118% 203  
percentage of recovery, respectively. The detection limit (DL) 204  
was 3.75 μg/kg for inorganic forms and 1.35–4.35 μg/kg for 205  
organic forms. The mean content and percentage of recovery 206  
of tAs in the CRM (n = 4) was 0.349 ± 0.102 mg/kg and 207  
123%, respectively. 208

209 **Methodology for ELTR estimation**

Estimated daily intakes (EDI) for lifetime exposure were cal- 210  
culated by assessing the amount of iAs to which an individual 211  
is exposed per day and per kilogram of body weight (Eq. 1). 212Q7

$$EDI = \frac{iAs \cdot IR}{BW} \quad (1)$$

where iAs is the concentration of inorganic arsenic in rice 213  
(μg/kg), IR is the ingestion rate (kg/day), and BW is the body 215Q8

216 weight (kg). Ingestion rates for adults were obtained from the  
 217 Food and Agriculture Organization “Food balance sheets  
 218 (FAO, 2018). Anthropometric data were obtained from  
 219 National Institute of Statistics in Ecuador (INEC, 2014) and  
 220 from the recent nutrition study for the Spanish population  
 221 (Bartrina and Rodrigo, 2018).

222 No provisional tolerable daily intake is currently accepted  
 223 for inorganic arsenic: the World Health Organization conclud-  
 224 ed that the former value of 2.1  $\mu\text{g}/\text{kg}$  day was no longer  
 225 considered health-protective (FAO/WHO, 2011). Estimated  
 226 lifetime health risks (ELTR) were calculated; these are propor-  
 227 tional to EDI, and the proportionality coefficient is known as  
 228 the cancer slope factor (CSF), equal to  $1.5 \times 10^{-3}$   
 229  $(\mu\text{g}/\text{kg}$  day) $^{-1}$  (USEPA, 1995):

$$\text{ELTR} = \text{EDI} \times \text{CSF} \quad (2)$$

232  
 230

### 233 Statistical analysis

234 One- or two-way analyses of variance with Fisher’s HSD post  
 235 hoc test were performed to test differences between means,  
 236 with a significance level of  $\alpha = 0.10$ . This significance level  
 237 was deliberately chosen, instead of the more traditional 0.05,  
 238 as the authors were willing to accept a 10% chance of incor-  
 239 rectly finding that an innocuous and very cheap treatment is  
 240 beneficial for human health when it is not. From a statistical  
 241 point of view,  $\alpha = 0.05$  or 0.1 is equally valid (Koch and Link,  
 242 1971; Gibbons and Coleman, 2001).

243 The Doornik-Hansen test was used to test normality.  
 244 Concentration values were log-transformed before statistical  
 245 testing.

## 246 Results

### 247 Effect of rinsing and/or boiling on total arsenic 248 content

249 Significant differences in tAs concentrations were found  
 250 between treatments ( $F(5,36) = 2.4$ ,  $p < 0.10$ ) for polished  
 251 rice. Cooking with no previous rinsing and without remov-  
 252 ing excess water (treatments T2 and T3) did not signifi-  
 253 cantly reduce the content of tAs in cooked rice compar-  
 254 ed with raw rice (Fisher’s HSD,  $n = 36$ ,  $p > 0.10$ ).  
 255 Contrarily, rinsing before cooking can efficiently reduce  
 256 tAs concentrations in cooked rice (Fisher’s HSD,  $n = 36$ ,  
 257  $p < 0.10$ ). The different treatments ordered by increasing  
 258 efficiency would be  $T2 > T4 > T5$  (Fig. 2).

259 **Treatment T0** The concentrations of tAs in rice from Spain  
 260 (polished rice 0.163–0.234 mg/kg,  $n = 4$ ; brown rice 0.231–  
 261 0.438 mg/kg,  $n = 2$ ) were higher than for those from Ecuador

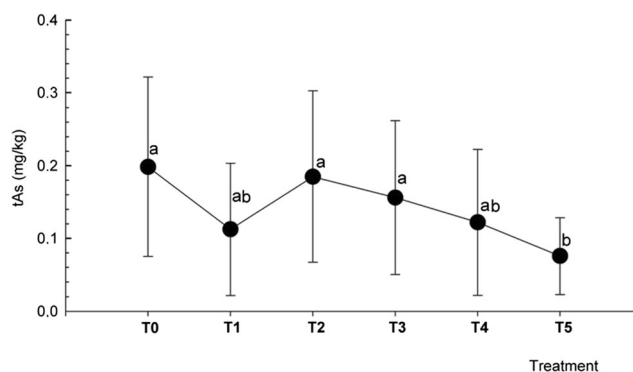


Fig. 2 Decreases in total arsenic concentration by type of treatment for the whole set of samples. Different letters indicate significant differences at the  $p < 0.10$  level

(polished rice 0.090 mg/kg, integral rice 0.165 mg/kg,  $n = 1$ ). 262  
 These results are consistent with those obtained in previous 263  
 studies suggesting that rice from Spain usually shows high tAs 264  
 contents (see, e.g., Meharg and Zhao, 2012, Signes-Pastor 265  
 et al., 2016, Torres-Escribano, et al., 2008). Nevertheless, it 266  
 is worth noting the low concentrations of tAs obtained for 267  
 Spanish organic rice (0.067 mg/kg) (Table 1). By type of rice, 268  
 the concentration of tAs in raw polished rice (T0) ( $0.138 \pm$  269  
 $0.076$  mg/kg $^{-1}$ ) was lower than in brown ( $0.278 \pm$  270  
 $0.142$  mg/kg) (Table 1). 271

**Treatment T1 (rinsing only)** Treatment T1, along with treat- 272  
 ment T5, was the process that removed the greatest amount of 273  
 tAs. The concentration of tAs in polished rice after rinsing 274  
 (T1) ranged between 0.024 and 0.097 mg/kg, while for T5, 275  
 it ranged between 0.02 and 0.075 mg/kg. These values corre- 276  
 spond to a 39% and 59% reduction, respectively (Table 1). 277  
 The concentration of tAs ranged between 0.025 and 278  
 0.097 mg/kg for polished rice and between 0.095 and 279  
 0.310 mg/kg for brown rice. 280

The treatment consisting in boiling with 150 mL to com- 281  
 plete absorption with no previous rinsing (T2) showed an 282  
 8.4% reduction in tAs (range 4–18%). The highest percentage 283  
 of reduction (17%) was found for one polished rice sample 284  
 from Spain. The concentration of tAs after treatment T2 285  
 ranged between 0.081 and 0.193 mg/kg for polished rice and 286  
 between 0.151 and 0.423 mg/kg for brown rice. 287

The treatment consisting in boiling with 300 mL to com- 288  
 plete absorption with no previous rinsing (T3) led to a 30% 289  
 reduction in the concentration of tAs (range 7–49%). The 290  
 concentration of tAs ranged between 0.084–0.122 mg/kg for 291  
 polished rice and between 0.113–0.362 mg/kg for brown rice. 292

Rinsing and boiling to complete absorption (T4) de- 293  
 creased tAs content by 53% in polished rice, with the 294  
 highest percentage of reduction (74%) found in rice from 295  
 Spain. The concentration of tAs ranged between 0.060 296  
 and 0.074 mg/kg for polished rice and between 0.128 297  
 and 0.0338 mg/kg for brown rice. 298

**Table 1** Total As concentration (mg/kg) by treatment

Site	Type of rice	Treatment					
		T0—raw rice	T1—only washing	T2—cooked to dryness (1:3)	T3—cooked to dryness (1:6)	T4—washing and cooked to dryness (1:3)	T5—washing and cooked with excess water (1:6)
Ecuador	Brown	0.165	0.101	0.151	0.113	0.128	0.081
	Polished	0.090	0.097	0.810	0.084	0.065	0.075
Spain	Polished	0.233	0.080	0.192	0.122	0.060	0.049
	Brown	0.231	0.095	0.219	0.171	0.128	0.076
	Brown	0.437	0.310	0.422	0.362	0.338	0.184
	Polished	0.163	0.080	0.153	0.084	0.074	0.042
	Polished	0.067	0.025	0.073	0.072	0.061	0.020

Rinsing and boiling in excess water (T5) was the treatment with the greatest reduction in tAs content, with a mean value of 63% and significantly lower tAs content compared with raw grain for this set of samples (Fig. 2). The greatest percentage of reduction was found in polished rice from Spain (79%). The concentration of tAs decreased to values of 0.020–0.075 mg/kg for polished rice and 0.076–0.184 mg/kg for brown rice.

**Effect of rinsing and/or cooking on the content of forms of arsenic**

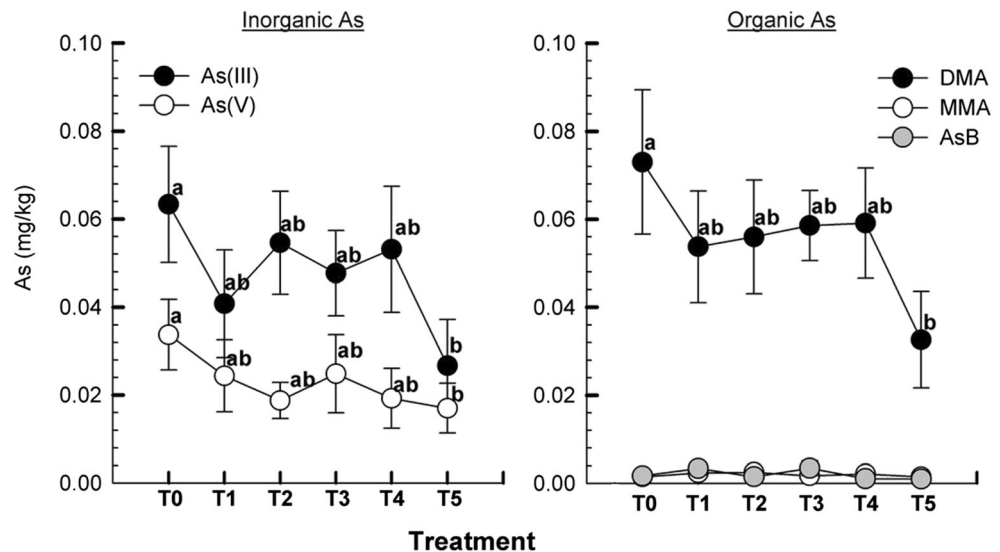
The statistically significant differences found among arsenic concentrations in rice were due to changes in contents of both iAs and oAs between raw rice (T0) and rice subjected to treatment T5 ( $F(5,247) = 3.1, p < 0.01$ ; Fisher's HSD,  $n = 247, p < 0.10$ ) (Fig. 3).

**Treatment T0** The content of iAs forms varied substantially both between countries and between types of rice (Table 2 and Table S2). The concentration of iAs was higher in

Ecuadorian than in Spanish rice, while the concentration of oAs was higher in brown rice from Ecuador (0.166 mg/kg,  $n = 1$ ), followed by polished rice from Ecuador (0.135 mg/kg,  $n = 1$ ), brown rice from Spain (median value 0.118 mg/kg,  $n = 2$ ), and polished rice from Spain (median value 0.059 mg/kg,  $n = 4$ ).

The predominant iAs form was As(III), with values ranging from 0.027 to 0.131 mg/kg, except in brown rice from Ecuador, where As(V) was slightly higher. The concentration of As(V) ranged between 0.023 and 0.088 mg/kg for the whole set of samples. The highest oAs concentration was found in brown rice from Spain (0.127 mg/kg), whereas the lowest was found in polished rice from Ecuador (0.024 mg/kg); meanwhile, the concentration of oAs in polished rice from Spain showed similar values to those found in brown rice from Ecuador (0.062 and 0.068 mg/kg, respectively). The predominant oAs form was DMA, whose concentrations represented 85–100% of oAs. MMA and AsB showed very low concentrations, which were below the detection limit in most cases (Table 2, Table S1, and Fig. 3).

**Fig. 3** Decrease in the concentration of arsenic forms by type of treatment for the whole set of samples. Different letters indicate significant differences at the  $p < 0.01$  level





**Table 2** Concentration of As species by rice milling step (brown and polished) and by treatment

	As(III)		As(V)		DMA		MMA		AsB	
	Brown	Polished	Brown	Polished	Brown	Polished	Brown	Polished	Brown	Polished
t2.1	Mean ± SD	0.09 ± 0.04	0.05 ± 0.04	0.026 ± 0.004	0.10 ± 0.07	0.05 ± 0.03	0.003 ± 0.002	< LOD	0.005 ± 0.004	< LOD
t2.2	Median	0.078	0.040	0.025	0.065	0.051	0.003	< LOD	0.005	< LOD
t2.3	Mean ± SD	0.11 ± 0.09	0.02 ± 0.01	0.014 ± 0.007	0.08 ± 0.05	0.04 ± 0.01	0.003 ± 0.002	0.002 ± 0.002	0.006 ± 0.005	0.003 ± 0.002
t2.4	Median	0.070	0.020	0.013	0.052	0.038	0.002	0.002	0.006	0.002
t2.5	Mean ± SD	0.08 ± 0.03	0.05 ± 0.03	0.014 ± 0.004	0.08 ± 0.04	0.04 ± 0.02	0.003 ± 0.002	0.001 ± 0.001	0.001 ± 0.001	0.003 ± 0.001
t2.6	Median	0.075	0.034	0.013	0.060	0.042	0.003	0.001	0.002	0.002
t2.7	Mean ± SD	0.07 ± 0.04	0.04 ± 0.03	0.013 ± 0.006	0.07 ± 0.04	0.05 ± 0.02	0.007 ± 0.007	0.001 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
t2.8	Median	0.067	0.033	0.011	0.074	0.046	0.007	0.001	0.002	0.002
t2.9	Mean ± SD	0.09 ± 0.04	0.05 ± 0.04	0.015 ± 0.008	0.08 ± 0.04	0.03 ± 0.02	0.005 ± 0.004	< LOD	< LOD	0.001 ± 0.001
t2.10	Median	0.067	0.054	0.015	0.062	0.021	0.005	< LOD	n.a	0.002
t2.11	Mean ± SD	0.04 ± 0.02	0.02 ± 0.04	0.009 ± 0.005	0.07 ± 0.05	0.02 ± 0.03	0.003 ± 0.002	< LOD	0.001 ± 0.001	< LOD
t2.12	Median	0.039	0.006	0.008	0.043	0.017	0.004	< LOD	0.002	< LOD

LOD limit of detection, SD standard deviation, n.a. not analyzed

**Treatment T1** The mean concentration of iAs was 0.031 mg/kg ( $n = 4$ ) for polished rice from Spain and 0.159 mg/kg ( $n = 2$ ) for brown rice, corresponding to a mean percentage of reduction of 50% and 8%, respectively (Supplementary Table 1, Fig. 3). The concentration of iAs in samples from Ecuador was 0.040 mg/kg ( $n = 1$ ) in polished rice and 0.142 mg/kg ( $n = 1$ ) in brown rice, corresponding to a 70% and 14.5% reduction, respectively. Mean reduction in iAs content with treatment T1 was 40% both for AsIII and for AsV. The concentration of oAs (DMA) was reduced by 15–37%. Content of oAs was higher in rice from Spain (polished 0.045 mg/kg; brown 0.104 mg/kg) than in rice from Ecuador (polished 0.028 mg/kg; brown 0.058 mg/kg) (Table 2).

**Treatment T2** Reduction in iAs ranged between 14.5 and 49%, with higher iAs content in rice from Ecuador (polished 0.105 mg/kg; brown 0.097 mg/kg,  $n = 1$ , Supplementary Table 1) than in rice from Spain (polished 0.042 mg/kg,  $n = 4$ ; brown 0.109 mg/kg,  $n = 2$ ). The mean percentage of reduction after this treatment was 21.5% for AsIII and 47% for AsV; therefore, the concentrations of AsIII reached higher values than AsV in all the studied types of rice. The content of oAs (mainly DMA) decreased by 24–38%, with median values of 0.052 mg/kg for polished rice from Spain, 0.106 mg/kg for brown rice from Spain, 0.052 mg/kg for brown rice from Ecuador, and 0.018 mg/kg for polished rice from Ecuador (Fig. 3, Table 2, Supplementary Table 1).

**Treatment T3** The results for iAs concentration were similar to those found with treatment 2. The content of iAs decreased by 20–39%, while oAs decreased by 4–31%. The concentration of iAs was higher in rice from Ecuador (polished 0.108 mg/kg, brown no data, Supplementary Table 1) than in rice from Spain (median values: polished 0.038 mg/kg,  $n = 4$ ; brown 0.124 mg/kg,  $n = 2$ ). The percentage of reduction was 25% for AsIII and 52% for AsV. The concentration of oAs ranged between 0.058 mg/kg for polished rice from Spain and 0.082 mg/kg for brown rice from Spain. For polished rice from Ecuador, the concentration of oAs was 0.025 mg/kg.

**Treatment 4** The reductions of iAs ranged between 8 and 65%. The median concentration of iAs was 0.029 mg/kg ( $n = 4$ ) in polished rice from Spain and 0.117 mg/kg ( $n = 2$ ) in brown rice from Spain, corresponding to a 55% and 8% decrease, respectively. For Ecuadorian rice, the percentages of reduction of iAs reached values around 20%, with concentrations of 0.131 mg/kg for brown rice and 0.103 mg/kg for polished rice. Reductions in AsIII reached mean percentages of 29%, while for AsV, it was 46%. The percentages of reduction for oAs were similar to those for iAs (3–48%), with mean concentrations of 0.059 mg/kg in polished rice from Spain, 0.102 mg/kg I in brown from Spain, 0.013 mg/kg in polished rice from Ecuador, and 0.056 mg/kg in brown rice from Ecuador.

388 **Treatment 5** This treatment showed the highest percentages of  
 389 reduction of As (iAs 29–90%, oAs 4–85%), with significantly  
 390 lower concentrations of iAs forms and DMA compared with  
 391 those found in raw grain rice (Fig. 3). The median concentra-  
 392 tion of iAs in rice from Spain was 0.006 mg/kg for polished  
 393 rice and 0.064 mg/kg for brown rice, while in rice from  
 394 Ecuador, it was 0.096 mg/kg for polished rice and 0.086 mg/kg  
 395 for brown rice. This was the only treatment in which the per-  
 396 centage of reduction was higher for AsIII (62.5%) than for  
 397 AsV (51%).

398 Mean oAs concentrations were 0.044 mg/kg in polished  
 399 rice from Spain and 0.089 mg/kg in brown rice from Spain.  
 400 For Ecuadorian rice, the obtained oAs concentrations were  
 401 0.006 mg/kg for polished rice and 0.034 mg/kg for brown rice.

402 **Estimated daily intakes and estimated lifetime health**  
 403 **risks**

404 The low concentrations of iAs in Spanish rice and the relatively  
 405 low importance of rice in the diet of the Spanish population  
 406 (22.8 g raw rice/day; FAO, 2018) result in an estimated daily  
 407 intake (EDI) of polished rice of 19.4 ng/kg day for raw rice  
 408 (T0); 10.0 ng/kg day for T1; 9.6 ng/kg day for T2–T4; and  
 409 3.2 ng/kg day for T5. The corresponding ELTR values are in  
 410 the same order as above:  $2.9 \times 10^{-5}$ ,  $1.5 \times 10^{-5}$ ,  $1.4 \times 10^{-5}$ , and  
 411  $4.8 \times 10^{-6}$ . Pre-rinsing the rice or cooking it using the traditional  
 412 method in Spain (T2–T4) seems to be sufficient to reduce ELTR  
 413 by 50% compared with the assessed concentration in raw rice.  
 414 The T5 cooking method reduces ELTR by 83% compared with  
 415 T0 and therefore constitutes the preferable method.

416 **Discussion**

417 **Arsenic in cooked rice**

418 The results of tAs concentration in rice from Ecuador and  
 419 Spain are consistent with previously published data that sug-  
 420 gest that iAs content in rice from Spain is usually high (Torres-  
 421 Escribano et al., 2008, Meharg and Zhao, 2012, Signes-Pastor  
 422 et al., 2016); in fact, it was higher than in rice from Ecuador.  
 423 However, the predominant species of As in Spanish rice is  
 424 DMA, whose toxicity is lower than that of inorganic forms  
 425 of As (Suriyagoda et al., 2018).

426 Content of tAs and iAs was also higher in brown than in  
 427 polished rice, consistently with the fact that As is mainly con-  
 428 centrated in the outermost portion of the grain (pericarp and  
 429 aleurone layer), which is removed in polished rice (Meharg  
 430 et al., 2008, Zhu et al., 2008, Carey et al., 2010).

431 Preliminary washing (treatment 1) removed 39–59% of the  
 432 total arsenic, 40% of the inorganic arsenic, and between 15 and  
 433 37% of organic forms. These values are about 10% above the  
 434 values reported by other authors (see Table S1). Raab et al.

(2009) investigated total arsenic and inorganic arsenic in different 435  
 rice types (basmati, long-grain, polished (white), and wholegrain 436  
 (brown)) after being cooked in non-contaminated water. The 437  
 effects of rinse washing, low water volume (rice-to-water ratio 438  
 of 1:2.5), and high water volume (rice-to-water ratio of 1:6) 439  
 cooking were investigated. Rinse washing was effective at re- 440  
 moving about 10% of the total and inorganic arsenic from 441  
 basmati rice, but was less effective for other rice types. 442  
 Sengupta et al. (2006) tested the three major rice-cooking proce- 443  
 dures in practice globally, using low arsenic water (tAs < 444  
 0.003 mg/L). Preliminary washing removed 28% of the rice 445  
 arsenic. The results were not influenced by water source (tube 446  
 well, dug well, pond, or rain), cooking vessel (aluminum, steel, 447  
 glass, or earthenware), or the absolute weight of rice or volume of 448  
 water. Naito et al. (2015) studied the traditional Japanese rice 449  
 cooking method by cooking washed rice until dry (rice-to-water 450  
 ratio of 1:1.4). Again, rinse washing was effective at removing 451  
 16–24% of tAs and 12–29% of iAs. 452

The most commonly used rice-cooking method in Spain 453  
 and in Ecuador is using a volume of water that will result in 454  
 all the water being absorbed or evaporated (Torres-Escribano 455  
 et al., 2008). Rice cooked by boiling to complete absorption 456  
 (treatments 2 and 3) constituted the least effective treatment to 457  
 remove As from rice (Fig. 3), which is also consistent with 458  
 results by other authors (Sengupta et al., 2006, Torres- 459  
 Escribano et al., 2008, Raab et al., 2009; Ackerman et al., 460  
 2005) (see Table S1 for a more exhaustive list). Contrarily, 461  
 this cooking method may even result in an additional increase 462  
 in As content with respect to raw rice if the boiling water has 463  
 an abnormally high content, as occurs in many South Asian 464  
 countries (e.g., Bangladesh and India) (Meharg and Zhao, 465  
 2012; Mandal et al., 2019). 466

Significant decreases in tAs and iAs content in rice grain 467  
 were only obtained when rice was rinsed and cooked in excess 468  
 water (1:6 ratio; treatment 5). The mean percentage of total 469  
 arsenic removed for the whole set of samples (62%) is in 470  
 agreement with results obtained by previous studies under 471  
 similar rinsing and cooking conditions: 57% (Sengupta 472  
 et al., 2006), 54% (Mihucz et al., 2007), and 65% removal 473  
 (Raab et al., 2009). Nevertheless, it is also worth highlighting 474  
 that simply rinsing rice grains before cooking leads to a sub- 475  
 stantial removal of tAs, particularly of iAs, going from a ratio 476  
 of  $iAs/tAs_{raw-rice} = 0.49$  to  $iAs/tAs_{T1} = 0.44$  for the whole set 477  
 of samples. This is mainly due to the fact that iAs is accumu- 478  
 lated in the outermost portion of the grain, while DMA is 479  
 found in the inner endosperm (Carey et al., 2010). Raab 480  
 et al. (2009) also found that rinsing and cooking with excess 481  
 water specifically reduces iAs but has no effect on DMA. 482

483 **Risk assessment: estimated excess lifetime risk**

484 Previous studies have estimated daily intake (EDI) for the 484  
 Ecuadorian population as a whole, which is almost twice that 485

486 of Europe but from one-half to one-third that of Brazil,  
 487 Bangladesh, and India. Estimated excess lifetime risk  
 488 (ELTR) for adults was  $3.0 \times 10^{-4}$ , while for infants, it varied  
 489 between  $10 \times 10^{-4}$  in rural areas and  $20 \times 10^{-4}$  in urban areas  
 490 (Nunes & Otero, 2017). Nevertheless, these estimations were  
 491 based on iAs content in raw grain. However, considering the  
 492 percentage of iAs that is lost with each treatment, EDI and  
 493 ELTR decreased substantially when calculated for cooked  
 494 rice. Thus, simply rinsing rice grains before cooking reduced  
 495 ELTR by 50%, while rinsing and cooking in excess water led  
 496 to an 83% decrease. This scenario is more realistic and less  
 497 dramatic than calculations based on As contents in raw grain.

498 **Conclusions**

499 Rinsing and boiling rice in excess water and simply rinsing  
 500 rice grains with As-free water are two efficient methods to  
 501 significantly reduce As intake in the population. According  
 502 to our results, the rinsing of rice before cooking can reduce  
 503 the content of total and of inorganic arsenic by a substantial  
 504 amount (up to 40–59% of total arsenic and 40% of inorganic  
 505 arsenic). When rinsing and cooking in excess water are used,  
 506 the reductions are even more pronounced, of up to 62% for  
 507 total and inorganic arsenic. This observation can have signif-  
 508 icant impacts on risk estimates as exposure to the hazard is  
 509 reduced by the same amount.

510 In summary, rinsing rice grains before cooking can reduced  
 511 health risk by 50%, while rinsing and cooking in excess water  
 512 can promote a reduction of 83% in the risk. This scenario is  
 513 more realistic and less dramatic than calculations based on As  
 514 contents in raw grain.

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