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Reducing the cadmium, inorganic arsenic and dimethylarsinic acid content of rice through food-safe chemical cooking pre-treatment

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1 **Reducing the cadmium, inorganic arsenic and dimethylarsinic acid**
2 **content of rice through food-safe chemical cooking pre-treatment**

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24 **Abstract**

25 Cadmium, inorganic arsenic and, potentially, dimethyl arsenic acid are carcinogens widely
26 elevated in rice. Here it was identified that the food-safe and common cadmium chelator citric
27 acid efficiently removed cadmium from intact grain via pre-soaking procedure, while also
28 reducing arsenic species. A twostep pre-soaking stage was developed whereby rice was first
29 incubated, at ambient temperature, in 1 M citric acid for 12 hours, and then in 1M calcium
30 carbonate for another 12 hours, the latter step to neutralize pH, followed by cooking. When
31 10 different individual types of rice were processed in such a way this resulted in removal
32 rates of 79% for cadmium, 81% for inorganic arsenic and a 66% for DMA. The technology is
33 particularly suitable for bulk food processing and could be deployed in the most cadmium and
34 arsenic impacted regions where rice is a staple.

35

36

37 **Keywords**

38 arsenic, cadmium, DMA, rice

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40

41 **1. Introduction**

42 Paddy rice is a major source of exposure, globally, to the carcinogen and nephrotoxin
43 cadmium (Clemens et al., 2013; EFSA, 2012; Shi et al., 2020) and the carcinogen inorganic
44 arsenic (EFSA, 2009; Carey et al., 2020). Rice can also be elevated in dimethylarsinic acid
45 (DMA) (Carey et al. 2020), which is thought to be a carcinogen (Yamamoto et al., 1995).
46 Cadmium and arsenic are specific problems for low-land rice cultivation as paddy
47 management involves anaerobic and aerobic cycling of soils, regulated by irrigation. Under
48 reduced conditions, i.e. flooding, arsenic species are mobilized in soil and assimilated by roots,
49 while it is the aerobic periods that cause cadmium assimilation and mobilization (Arao et al.,
50 2009).

51

52 There are regions of the world where cadmium and inorganic arsenic are particularly elevated
53 in rice, such as the base metal mining zone of South East China, which is also nationally a
54 major rice production region (Williams et al., 2009). Concentrations of both cadmium and
55 arsenic routinely exceed 0.2 mg/kg, the Chinese standard, in Guangdong, Hunan and Fujian
56 provinces (Cai et al., 2019; Nengchang et al., 2018; Williams et al., 2009; Xiao et al., 2018;
57 Zeng et al., 2015), the concentration that the EU state should not be exceeded for cadmium
58 (Commission Regulation, 2006) and inorganic arsenic (Commission Regulation, 2015). As the
59 populace of such regions depend on rice as a dietary staple, and there being few alternatives,
60 this makes rice production highly problematic. Thus, if cadmium and inorganic arsenic can be
61 reduced in rice then large production regions rice output can be made safer.

62

63 Post-harvest processing of rice can be used to lower grain inorganic arsenic, primarily
64 parboiling (Rahman et al., 2019) or cooking (Carey et al., 2015; Raab et al., 2009), but such

65 simple cooking technologies are ineffective for cadmium and DMA. A high water:rice volume
66 removes up to 45% (Raab et al., 2009), while percolating rice with boil water removes up to
67 70-80% (Carey et al., 2015), of grain inorganic arsenic. Inorganic arsenic is predominantly
68 present in rice as arsenite, which is uncharged and relatively soluble at neutral pH (Meharg
69 and Zhao, 2012), rice grain itself being circa. neutral. Cadmium is doubly charged and has a
70 high affinity to polar functional groups in biological matrices (Huo et al, 2016; Wu et al., 2016;
71 Zou et al., 2019). DMA has a pK_a of 6.2 (Meharg and Zhao, 2012), requiring acid conditions to
72 become neutrally charged. Therefore, more astringent methods are needed to remove
73 cadmium and DMA from rice.

74

75 Cadmium is readily complexed with dicarboxylic acids, which include cheap and common food
76 ingredients such as citric acid (Huo et al, 2016; Wu et al., 2016; Zou et al., 2019). Huo et al.
77 (2016) found that the separation of rice into starch and protein using an alkaline extraction
78 method, and then followed by washing the protein isolate with citric acid, efficiently removed
79 the cadmium from the extracted rice protein. Zou et al. (2019) found that citric acid could
80 remove cadmium from bran, while Wu et al. (2016) established that citric acid could be used
81 to reduce this element from brown rice flour. Citric acid treatment also was found to reduce
82 lead (Behrouzi et al., 2020a) and total arsenic (Behrouzi et al., 2020b) from rice grain. Here
83 we optimise procedures to remove cadmium from intact rice using citric acid, and then
84 developed processes to neutralize the subsequent acidification of pH. The consequences for
85 inorganic arsenic and DMA, and for mineral nutrients, was also determined.

86

87

88

89 **2. Materials and Methods**

90 **2.1 Experiments**

91 Packaged commercial rice grain was purchased from retail stores in Belfast, Northern Ireland.
92 Ten polished rice samples were tested including Italian, Thai and basmati. One Italian arborio
93 rice was selected on which to conduct initial optimization trials. The developed procedure
94 was then validated on the other grains. Throughout, a rice to preparation/cooking solution
95 ratio of 1:5 was used as this has been shown to very effective in decreasing inorganic arsenic
96 (Carey et al., 2015).

97

98 For the initial experiments rice (50 g) was accurately weighed from the rice packet (dry weight
99 was calculated separately), and then soaked in 250ml of a range of concentrations of both
100 citric and ascorbic acid (0, 0.0001, 0.001, 0.01, 0.1 and 1 M) in a 400 ml flat bottomed Pyrex
101 beaker for 12 hours, pre-determined by experimentation to be optimized soaking time, in
102 triplicate. After soaking, the rice samples were rinsed thoroughly with double distilled,
103 deionized Millipore water and then cooked on a traditional hotplate for approximately 10
104 mins till soft. The excess water was discarded, and samples were analysed for cadmium,
105 arsenic species, and a range of mineral nutrients and pH. A second phase of experiments
106 identified how to neutralize rice after citric acid treatment. Rice sample weights, soaking
107 ratios and preparation procedures were identical to the initial phase, but following citric acid
108 treatment and rinsing, a second 12 hour soaking stage, followed by rinse, was tested where
109 calcium carbonate (0, 0.1 and 1 M) was used, and then the rice finally cooked. Once optimal
110 cooking procedures were identified, then the 10 different rice samples were prepared. This
111 involved a 12-hour soak in 1 M citric acid, then a rinse; a 12 h soak in 1 M calcium carbonate,
112 and then a rinse, and then cooking till rice was prepared. Throughout, uncooked (i.e. straight

113 out of the packet) and traditionally cooked rice (straight from packet and cooked in a 1:5 rice
114 to purified water ratio) were also conducted to test the efficacy of the modified procedures.

115

116 **2.2 Chemical analysis**

117 For all experiments, uncooked and cooked grain rice was freeze-dried overnight. Samples
118 were then milled to a powder using a Retch PM 100 rotary ball-mill using a zirconium oxide
119 lined vessel and grinding balls, at 500 rpm for 4mins. Samples were then analysed for total
120 elements by ICP-MS and XRF, arsenic speciation by ion-chromatography-ICP-MS, and pH.

121

122 For ICP-MS total elemental analysis, milled samples were accurately weighed (0.1 g) into 50ml
123 polypropylene centrifuge tubes. To each tube, 2ml of concentrated 69% Aristar nitric acid was
124 added and left to sit overnight. Analytical grade hydrogen peroxide (2ml) was then added and
125 allowed to outgas for 15mins. Samples were prepared in batches of 40 and included a blank
126 and a rice CRM, NIST 1568b rice flour which is certified for a wide range of both trace and
127 macro elements. Microwave digestion in a CEM MARS 6 instrument was then carried out
128 under conditions 95^oC for 5 min held for 10 min, 135^oC for 5 min held for 10 min and 180^oC
129 for 5 min held for 30 min. Total elements were then measured using the Thermo ICAP Q ICP-
130 MS in direct acquisition mode, with rhodium as an internal standard. All elements reported
131 in results were present in the existing in calibration standards (SPEX Europe), as well as in
132 CRM NIST 1568b. Elements with adequate CRM recoveries (50-150%) were reported, along
133 with LoDs (Table 1).

134

135 For inorganic arsenic speciation, ~0.1 g of powdered rice was accurately weighed into 50ml
136 polypropylene centrifuge tubes to which 2ml of dilute 69% Aristar nitric acid was added and

137 left overnight. Samples were prepared in batches of 40 that included a blank and CRM NIST
138 1568b Rice flour which is certified for arsenic species. Microwave digestion was conducted
139 under similar conditions to the table above. Following digestion, the digestate was then
140 diluted to 10ml with distilled water and 700 ul was transferred to a 2ml polypropylene vial,
141 to which 0.7 ul of analytical grade hydrogen peroxide was added. The rice solution was then
142 run on a Thermo Scientific IC5000 Ion Chromatography system with a Thermo AS7, 2x250 mm
143 column (and a Thermo AG7, 2x50 mm guard column) with a gradient mobile phase (A: 20 mM
144 ammonium carbonate, B: 200 mM ammonium carbonate- starting at 100% A, changing to
145 100% B, in a linear gradient over 15 mins) interfaced with the Thermo ICAP Q ICP-MS which
146 monitors m/z^+ 75, using helium gas in collision cell mode. The resulting chromatogram was
147 compared with authentic standards DMA, inorganic arsenic, monomethylarsonic acid (MMA),
148 trimethyl arsenic oxide (TMAO), tetramethyl arsonium (TETRA) and arsenobetaine (AB).

149

150 A bench top XRF (Rigaku CG) was used to analyse additional elements using the milled rice
151 samples. Batches of 9 samples were analysed in triplicate with CRM NIST 1568b rice flour.
152 Approximately 3 g of each rice sample was weighed into prepared XRF cups lined with proline
153 film and pressed using a PANAPRESS. Following XRF analysis, elements present in the CRM
154 with good analytical recoveries (50-150%) were reported (Table 1).

155

156 To measure the rice pH after pre-soaking step and subsequent cooking, a 2:1-part water to
157 rice ratio slurry was made and the pH meter swirled until a steady pH reading was obtained.

158

159 Half limit of detection (LoD) was used for elemental concentrations below LoD. LoDs are
160 reported in Table 1. Statistical analysis was conducted using two-way ANOVA in MiniTab 18
161 as well as sample t-test in where applicable.

162

163 **3. Results**

164 Cadmium in the rice investigated ranged by over an order-of-magnitude, from 0.006 to 0.077
165 mg/kg (Figure 1). Total arsenic in the grain had a similarly large range, from 0.02 to 0.095
166 mg/kg. Chemical analysis of rice grains before and after pre-soaking treatment and cooking
167 showed that citric acid pre-soaking resulted in a maximal 81 % removal of cadmium at 1M
168 concentration ($P<0.0001$), with the efficacy of removal increasing as acid concentration rose
169 (Fig. 1). Decrease in cadmium was linear with \log_{10} increase in citric acid concentration
170 ($P<0.0001$). Citric acid inhibited total arsenic removal, as compared to the control, with a
171 linear increase with \log_{10} increase in citric acid ($P<0.0001$). However, even at 1 M citric acid,
172 the highest concentration, 1/3 of total arsenic was removed as compared to the uncooked
173 grain. The pH after soaking in citric acid showed a progressive decrease from a pH of 6.4 to
174 2.5 (Fig. 1).

175

176 The use of calcium salts for pH neutralization of organic acid treated grain was then
177 investigated. Trials found that the rice required a 12-hour soak for maximum pH
178 neutralization to occur, with calcium carbonate being the most effective (Fig. 2). The pH
179 increased linearly with time. Soaking the citric acid treated rice in calcium carbonate linearly
180 ($P=0.0001$) increased pH up to 7.5 at 12 hours.

181

182 The optimal procedures for removing cadmium were a soaked for 12 hours in 1 M citric acid,
183 thoroughly rinsed, and then soaked for 12 hours in 1 M calcium carbonate, followed by
184 subsequent boiling in water (Fig. 3). For the 10 rice types this sequence, on average caused
185 cadmium to be reduced $>60\%$, with a standard error of 4.6 %, of the starting raw rice grain.
186 Standard traditional cooking of rice resulted in a slight enhancement of cadmium

187 concentration (5 %), while treatment with citric acid alone caused an 80 % reduction in
188 cadmium. The calcium carbonate soak caused cadmium in citric acid treatment to be doubled,
189 probably due to trace cadmium concentrations in the calcium carbonate, given that the rice
190 in the citric acid treatment was very low in cadmium. Median changes in grain cadmium,
191 comparing water, citric acid and citric acid plus carbonate were significant ($P=0.0001$) when
192 analysed using Kruskal-Wallis analysis. For total arsenic, cooking in 5:1 water to rice ratio
193 reduced arsenic to 66% of uncooked grain, but further reductions were seen when a citric
194 acid pre-soak was used (46%) and citric acid and calcium carbonate in combination (38%).
195 These medians were significantly different from each other at $P=0.05$.

196

197 To explore the arsenic data further, speciation was conducted (Figure 4). Inorganic arsenic
198 dominated species, with inorganic arsenic averaging 0.08 mg/kg, and DMA 0.033 mg/kg.
199 Cooking in a 5:1 water to rice ratio reduced inorganic arsenic by ~60%, from 0.08 to 0.033
200 mg/kg, with further reductions on citric acid treatment (to 0.02 mg/kg) and citric acid plus
201 calcium carbonate (0.015 mg/kg), a maximum 82% reduction, on average. These percentage
202 differences were highly significant when tested by Kruskal-Wallis analysis ($P<0.0001$). The
203 DMA did not alter concentration on cooking alone, but a 50% reduction was observed on citric
204 acid, and a 66% reduction on calcium carbonate treatment, from a starting concentration of
205 0.032 mg/kg ($P<0.0001$).

206

207 Five-to-one water to rice cooking ratios decreases phosphorus by a $2/3$, zinc by $1/3$ and
208 potassium by $5/6$ across the 10 rice types tested, with all these differences being significant
209 at $P<0.0001$ (Figure 5). Citric acid pre-soak strips out more potassium, zinc and sulphur, but
210 not phosphorus and calcium to any major consequence. The calcium carbonate soak has little

211 effect on all nutrients examined except for calcium, which was fortified in cooked grain by 2-
212 orders of magnitude ($P < 0.0001$). Calcium carbonate also restored pH of the rice from a mean
213 level of 2.4 in citric acid alone treated rice to 7.3 when the citric acid treatment was followed
214 by a subsequent calcium carbonate soak ($P < 0.0001$).

215 **4. Discussion**

216 Here we showed that simple soaking treatments in citric acid and then calcium carbonate
217 reduced cadmium and inorganic arsenic by ~ 80 % (60 % for cadmium after pH neutralization),
218 and DMA by 2/3, while fortifying with calcium by 2-orders of magnitude. This means that rice
219 from highly impacted mining regions, typified by the S.E. China mining belt (Cai et al., 2019;
220 Nengchang et al., 2018; Williams et al., 2009; Xiao et al., 2018; Zeng et al., 2015), can be made
221 much safer for human consumption.

222

223 The loss of essential nutrients observed here, as compared to conventional cooking, conforms
224 with the study by Mwale et al. (2018) which reported that the use of excess water in rice
225 cooking for arsenic removal resulted in a significant loss of all essential elements except iron,
226 selenium and copper. Similarly, Carey et al. (2015) highlighted a 53 % potassium loss following
227 the use of percolating boiling water for rice cooking to remove arsenic. Fortifying nutrients
228 into grain is common practise, and in many regions of the globe is used as standard to improve
229 the health of the populace (Dwyer et al., 2015). We propose that such fortification can be
230 conducted at the neutralization stage as it was very effective for calcium here.

231

232 It is well understood that inorganic arsenic is readily stripped from rice by pre-soaking in and
233 cooking in large volumes of water to rice, while DMA and cadmium are not (Carey et al. 2015;
234 Raab et al., 2009). So, having procedures to remove DMA, and particularly, cadmium from
235 rice is an important advance. The pK_a for DMA is 6.2, and this may explain its enhanced
236 mobility when the pH is dropped, i.e. it becomes fully protonated and, thence, neutral and
237 mobile (Meharg and Zhao, 2012). Caruso et al. (2001) found that DMA and inorganic arsenic
238 could be decreased in freeze-dried apple samples that were treated with a solution including

239 acetic (extraction efficiency range 79% to 117%). For inorganic arsenic a low pH per se does
240 not enhance mobilization due to a pK_a of 9.2 for arsenite (Meharg and Zhao, 2012), so the
241 reason why lower pH mobilizes arsenite is not known in the experiments reported here.

242

243 Cadmium is readily complexed by dicarboxylic acids, such as citrate (Huo et al., 2016; Wu et
244 al., 2016; Zou et al., 2019) and this, along with the drop to a low pH, explains its enhanced
245 mobility and leaching under the protocols developed here. Wu et al. (2016) trialled citric,
246 malic and tartaric acid for the removal of cadmium from brown rice and found citric acid to
247 be optimal, removing up to 94 %. They noted that quality of brown rice was not affected.
248 However, they only used up to 0.06 M, as opposed to 1 M here that removed only up to 80 %
249 but causing a considerable drop in pH. Presumably, this was due to Wu et. (2016) using flour
250 as opposed to intact grain, with intact grain being more intractable. A very highly liquid:solid
251 ratio was optimal, 12:1, with lower ratios extracting less. They did not test for mineral
252 nutrients. Huo et al. (2016) removed 95 % of cadmium from rice protein extracts using citric
253 acid at 0.5 M citric acid in a 6:1 ratio. They had trialled a wide range of acids, food safe and
254 non-food safe, (hydrochloric, lactic, maleic, malic, oxalic, phosphoric and tartaric), and found
255 that concentrations above 0.2 M were required to be effective, with enhanced removal up to
256 0.6 M. Liquid:solid ratio had to be above 6 for effective removal. pH and loss of mineral
257 nutrients were not recorded. Similarly, 0.15 M citric acid at a 10:1 liquid:solid ratio was
258 effective at removing 94 % of cadmium from rice bran (Zou et al., 2019). The overall chemical
259 structure of the bran was not greatly affected, while vitamin E compounds and rice analogues
260 were slightly enriched. The vitamin B suite was not analysed, and neither were mineral
261 nutrients or pH. They found that citric acid opened the structure of the rice (using SEM), and
262 that there was little difference in FTIR spectra. We conducted SEM and FTIR on our samples,

263 including the calcium carbonate treated samples, and concluded the same that treatment did
264 not greatly affect the structure of grain (data not shown). As well as acids, neutral deep
265 eutectic solvents, which act as micelles, were used to efficiently extract cadmium (>90%) from
266 rice flour, again with a large solvent:solid ratio, 12:1, being most effective (Huang et al., 2018).
267 The consequences on arsenic species, and nutrients and vitamins were not explored.

268
269 Previous approaches to cadmium removal from rice have used microbial fermentation.
270 Incubation with *Bacillus* and yeast species resulted in 81% removal of cadmium Zhang et al.
271 (2017). However, it required 60 hours of fermentation for optimum cadmium removal and
272 resulted in a decrease in protein content. Similarly, Zhai et al. (2019) found that fermentation
273 of rice with *Lactobacillus plantarum* strains resulted in a 93% cadmium removal, reducing
274 protein, lipid and ash contents of rice grain. Although effective at cadmium removal, these
275 post-harvest approaches are labour and capital intensive and thus may not appeal to rice
276 producers, manufacturers and consumer. It is like that fermentation is effective in cadmium
277 removal due to increased acidity and to the opening up of rice structure, making cadmium
278 more leachable.

279
280 A more radical approach to reducing cadmium exposure from rice grain was developed by
281 Sun et al. (2020). They proposed to reduce gut availability of cadmium, derived from rice, by
282 adding components to food that with absorb cadmium in the gut using an *in vivo* mouse
283 model. These included tannic acids, titanium dioxide and zinc glutamate, amongst others,
284 achieving a 10-98% reduction in gut bioavailability of cadmium. However, their findings may
285 be limited in translation to humans in that human gut microbiology is more complex, and that
286 dietary interventions rely on palatability/acceptability.

287

288 The procedures outlined here have their limitations, and studies still needs to be conducted
289 on food acceptability/palatability, and potential products for the rice cooked in this way may
290 need developing. Refinement of these procedures lies in the hands of food technologists.
291 However, we have established that cadmium, in tandem with inorganic arsenic and DMA, can
292 be removed using food-safe chemical procedures. Also, we have ascertained that subsequent
293 neutralization is also a very useful step, not just for readjusting pH, but also for any potential
294 fortification (calcium in this case).

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425

426 **Table 1.** Recoveries of elements and arsenic species from CRM NIST 1568b, rice flour, along
427 with limits of detection (LoD).

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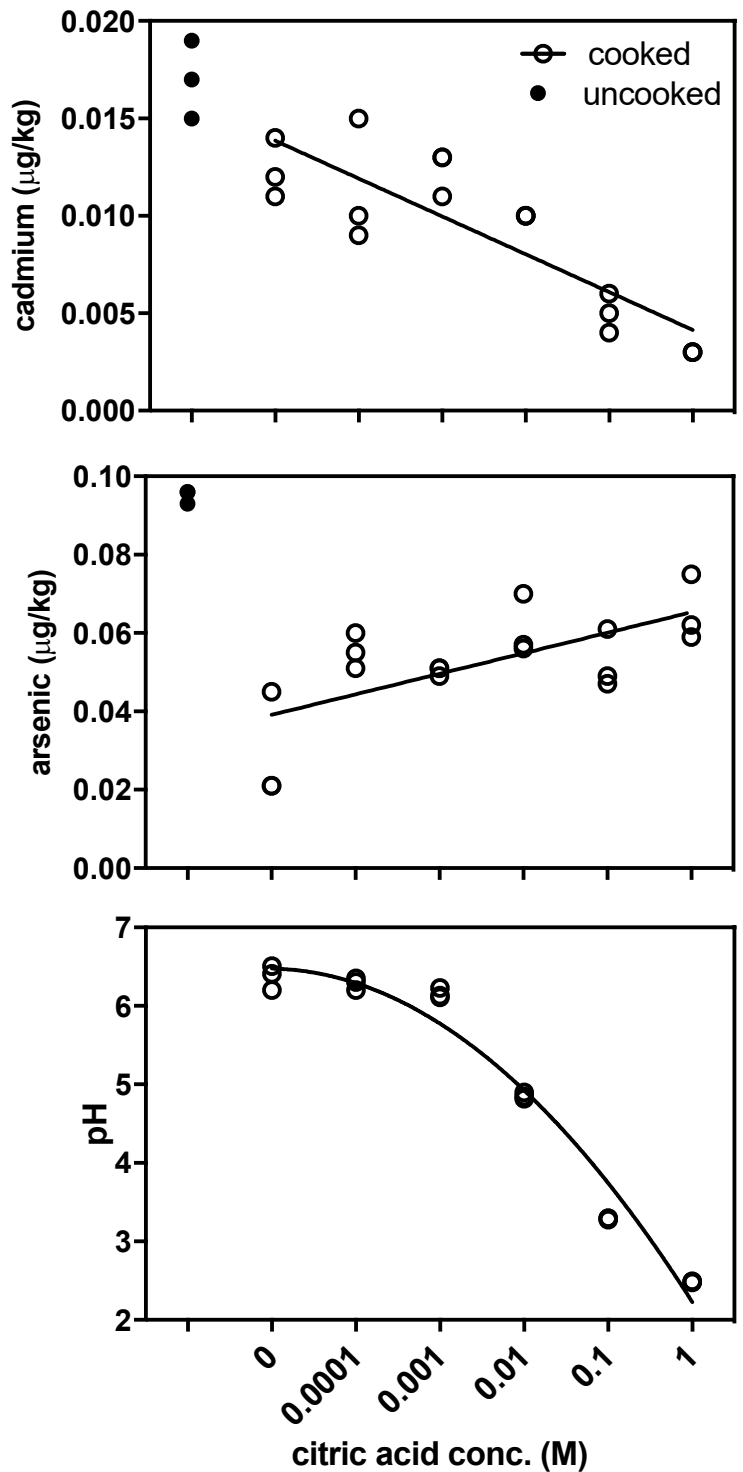
element	CRM reported conc. (mg/kg)	CRM recovery conc. (mg/kg)	CRM recovery (%)	S.E.M	L.o.D.
cadmium	0.0224	0.0162	72.3	0.002	0.006
arsenic	0.285	0.222	77.8	0.018	0.03
potassium	1282	1026	80	4.206	-
zinc	19.4	17.7	91	1.298	1.785
calcium	118	113	96	5.004	-
sulphur	1200	1020	85	3.086	-
phosphorus	1530	1591	104	86.801	19.953
inorganic arsenic	0.090	0.0830	92.2	0.001	0.0004
DMA	0.180	0.155	86.1	0.002	0.0004

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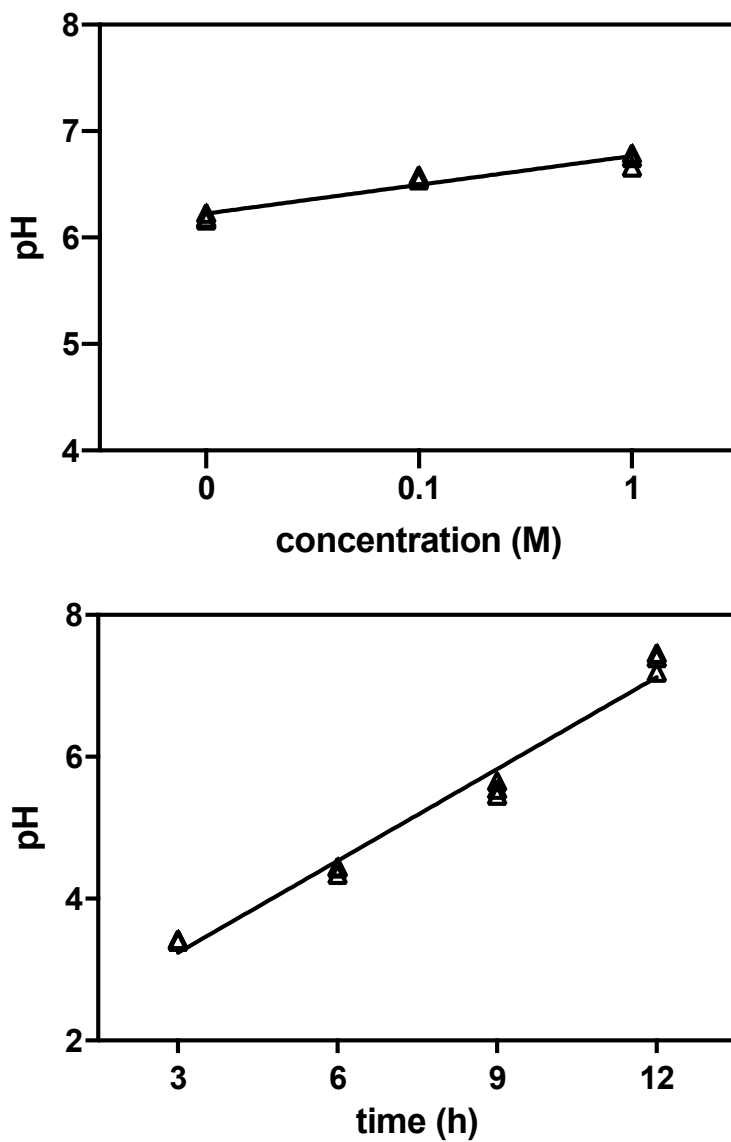
432

433 **Figure 1.** The effect of 12 hour soaking in molar concentrations of citric and ascorbic acid
 434 and then subsequent cooking in pure water, on cadmium and arsenic content and pH of
 435 Arborio rice. Each acid concentration was conducted in triplicate. Individual samples are
 436 shown.



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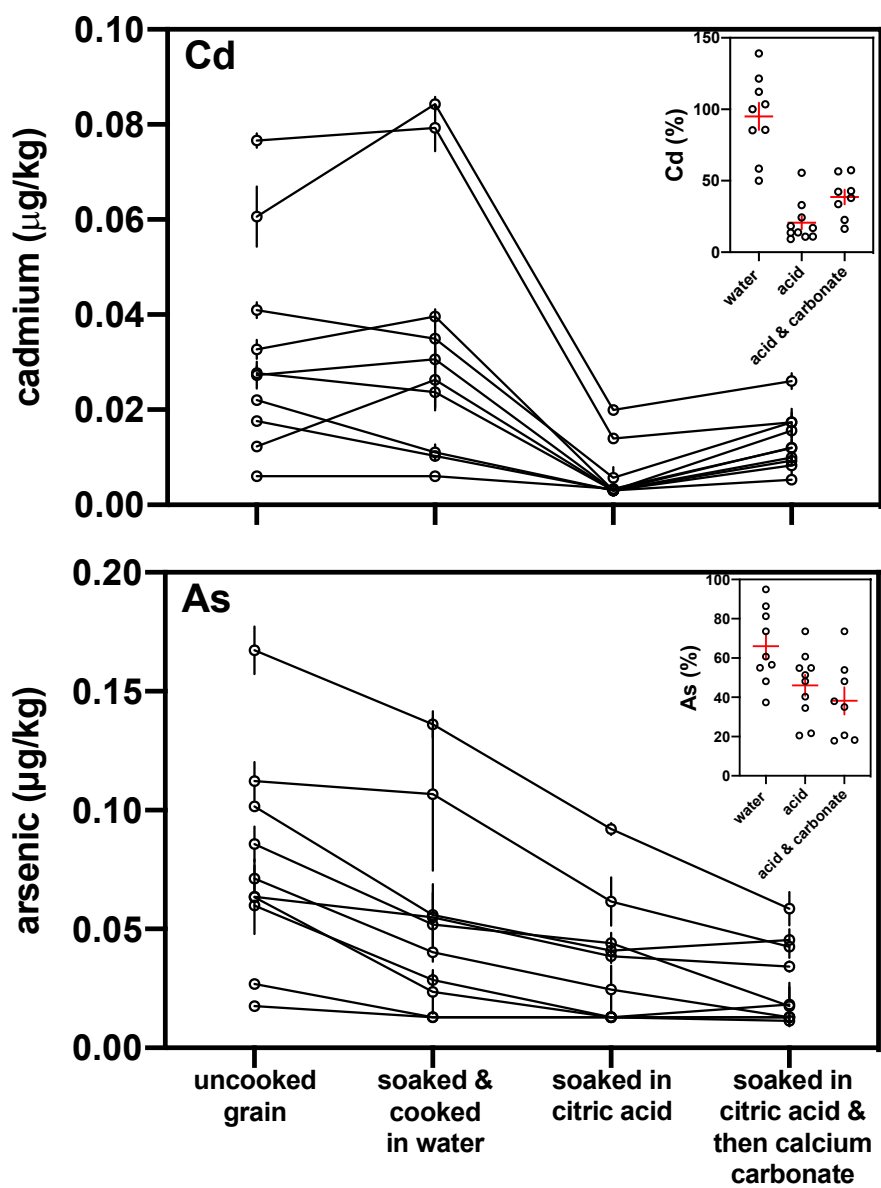
438 **Figure 2:** The effect of calcium carbonate, calcium chloride and calcium citrate on the pH
439 levels of rice grain after pre-soaking with the calcium salts for 12 hours and subsequent
440 boiling. Each salt concentration was conducted in triplicate. Individual samples are shown.
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446 **Figure 3:** The effect of rice pre-soaking treatments on 10 polished rice types after a 12-hour
 447 pre-soak in citric acid, and another 12-hour soak in calcium carbonate and subsequent boiling,
 448 with a 12-hour pre-soaking in water as control, on the cadmium and inorganic arsenic acid
 449 content. The insert shows the median and 25th percentiles for remaining cadmium. Means
 450 and standard errors are shown in the main graph, averages in the insert.
 451

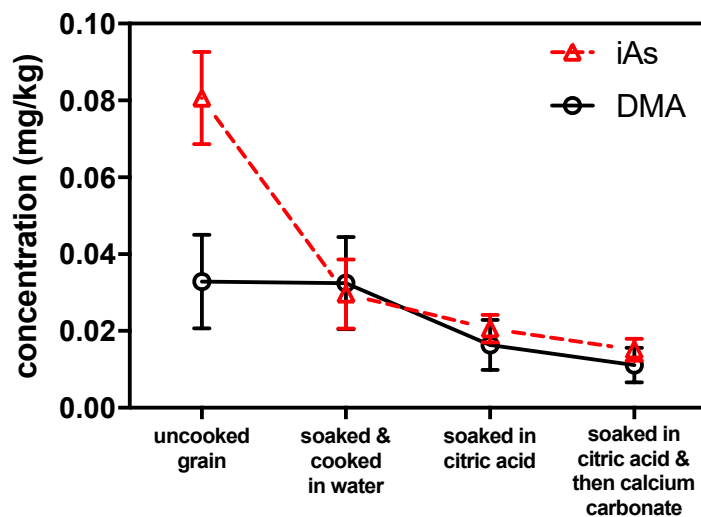


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453

454 **Figure 4:** The effect of treatment of polished rice types after a 12-hour pre-soak of white rice
455 grain in citric acid, and another 12-hour soak in calcium carbonate and boiling, with a 12-hour
456 pre-soaking in water as control on the arsenic species. Each aligned point represents triplicate
457 analysis and an average, plus and minus the standard error, presented.

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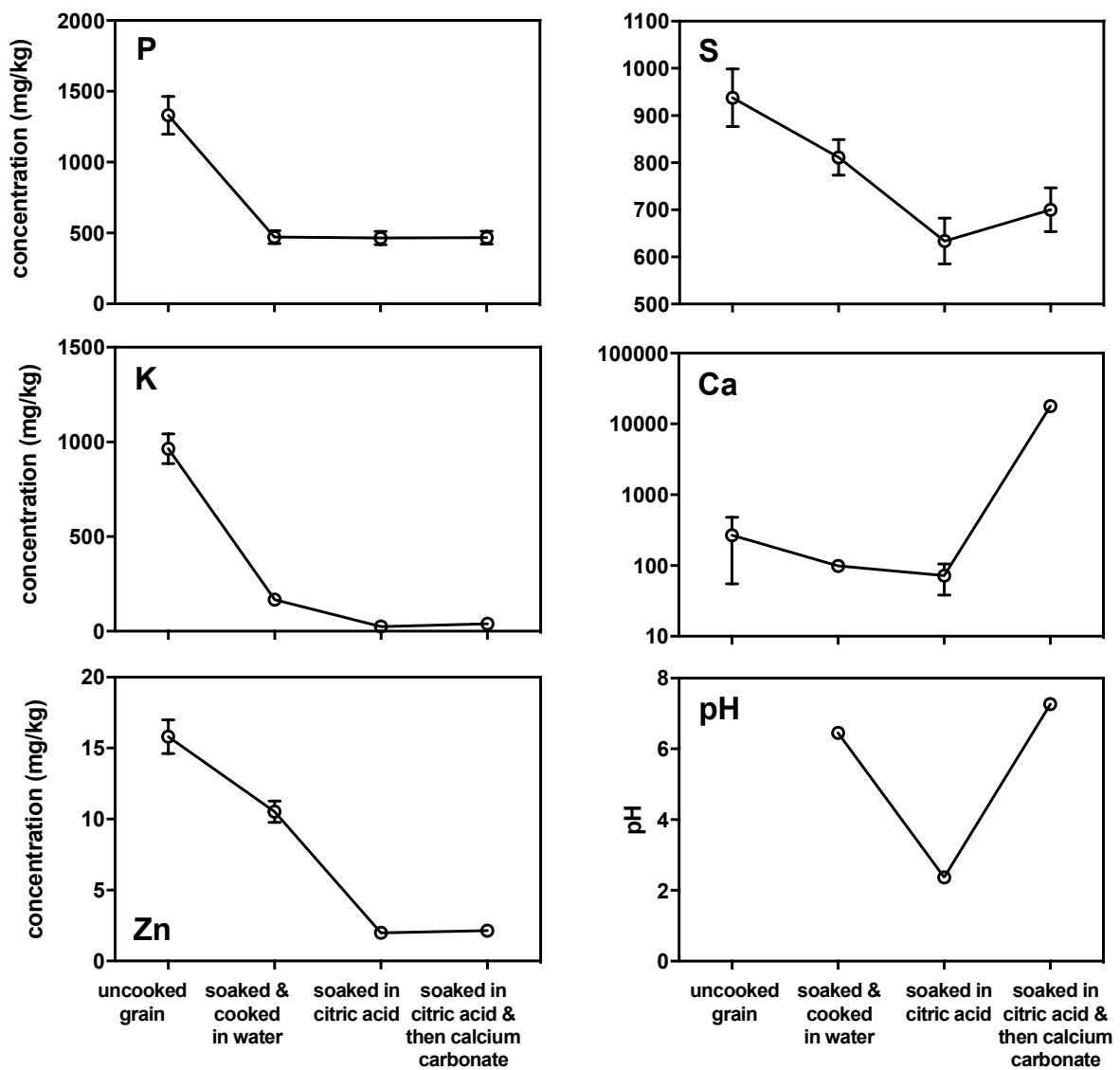
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462 **Figure 5:** Multi-elemental content (potassium, zinc, calcium, sulphur, chlorine and
 463 phosphorus) of polished rice grains. Rice samples were soaked in citric acid for 12 hours and
 464 then in calcium carbonate for another 12 hours with subsequent boiling with a 12-hour pre-
 465 soaking in water as control and analysed in triplicate. Each point represents triplicate analysis
 466 plus and minus standard error of the mean..

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