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Essential micronutrient and toxic trace element concentrations in gluten containing and gluten-free foods

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ABSTRACT

For individuals following a gluten-free (GF) diet, rice is commonly the major grain. People following a GF diet have a higher arsenic burden than the general population. We conducted a multielemental market basket study of GF and gluten containing ingredients and prepared foods (Mn, Fe, Ni, Cu, Zn, Cr, Co, Se, Cd, Sb, Pb, total As, As species, total Hg and methylmercury). Foods containing rice were significantly higher in As, Hg and Pb and lower in Se, Fe, Cu and Zn. Wheat-based foods were higher in Cd. Mercury concentrations were low (< 3.5 ng/g); speciation was predominantly methylmercury. Arsenic and mercury in rice were correlated. GF foods contained significantly more As and Hg. Eating a wide variety of GF grains may reduce contaminant exposure and increase micronutrient status compared to a rice-based GF diet.

1. Introduction

Grains have long been a staple of the human diet, being an excellent source of carbohydrate. The most widely consumed grains – rice, maize and wheat – provide 60% of the world's food energy intake (FAO., 1995). Like other plants, these staples can accumulate non-essential and potentially toxic trace elements from natural or human input to the soil. It is well-established that the concentration of arsenic in rice grain is higher than in the grains of other cereals such as wheat, oats and barley (Cubadda, Jackson, Cottingham, Van Horne, & Kurzius-Spencer, 2017). This is due in equal parts to the practice of rice paddy farming and the physiological requirement of rice for silicon, for which arsenite is a chemical analog. Flooding rice paddies promotes the dissolution of iron oxide minerals in the soil that otherwise sequester arsenic; the released arsenic is efficiently taken up by rice plants via silica transport systems and translocated to the rice grain (Punshon et al., 2017). Compared to environmental factors, it is the cultivar of rice that has the greatest influence on the amount of arsenic that accumulates in the rice grain (Norton et al., 2012). Grain arsenic concentration varies widely between different cultivars, which has given rise to breeding efforts to develop cultivars with low arsenic accumulation characteristics.

Inorganic arsenic, the most acutely toxic form (Le et al., 2000) and consequently the form regulated in food and water, tends to accumulate in the nutrient-rich outer layers of the rice grain (tegumen, pericarp and aleurone layers) known as the bran. Brown rice, therefore, usually contains higher concentrations of inorganic arsenic than white, in which the bran layers have been removed or 'polished' (Meng et al., 2014). Food products made primarily from rice bran can be particularly high in inorganic arsenic (Sun et al., 2008).

The EU has introduced regulations specifically for white rice of 0.2 μg/g for inorganic arsenic and rice products aimed at infants have a lower limit of 0.1 μg/g (EU, 2015). Because of their lower acute toxicity to humans, the organic forms of arsenic are not targeted for regulation. The US FDA has also set an action limit of 0.1 μg/g for inorganic arsenic in infant rice cereals (FDA, 2016). The regulatory focus on foods aimed at infants and young children reflects their heightened developmental vulnerability and higher exposure to arsenic (ingestion normalized to body weight) compared to adults (European Food Safety Authority, 2009). Concerns about arsenic exposure from rice-based diets have been raised for Asian populations and for individuals following a gluten-free (GF) diet where processed food products (such as bread and pasta) use rice as a staple grain instead of wheat (Meharg, Norton, Deacon, Williams, Adomako, Price, et al., 2013). A recent statistical analysis of the National Health and Nutritional Examination Study (NHANES) database (2009–2014) of urine and blood concentrations measured in the US population found that urinary arsenic was significantly higher in individuals that self-reported as being on a GF diet (n = 74) compared with the total individuals sampled (n = 7471) (Bulka, Davis, Karagas, Ahsan, & Argos, 2017).

Other potentially toxic elements have also been found in rice. Cadmium can reach levels of concern (Meharg et al., 2013) when rice is grown aerobically. In China, mercury contamination of rice grown on

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contaminated soils can contribute to human mercury exposure (Rothenberg, Windham-Myers, & Creswell, 2014). Arsenic and mercury are among the top three contaminants of concern on the Agency for Toxic Substances and Disease Registry's National Priority list; together with cadmium (at number 7) they are taken up by plants, and are developmental neurotoxins (Grandjean & Herz, 2015).

Food, in general, is the main source of cadmium exposure for non-smokers. In 2012 the European Food Safety Authority reviewed cadmium exposure in the European population and concluded that children and adults at the 95th percentile of exposure could be consuming cadmium levels in excess of health based guidelines (0.001 mg/kg/day) (European Food Safety Authority, 2009). Subsequently maximum allowable limits for cadmium in certain foods were adjusted down to reduce exposure to the general public. Wheat can be a major source of cadmium to diet and, like arsenic uptake in rice, cadmium uptake in wheat is dependent on the variety of wheat grown (Harris & Taylor, 2004).

There has been a recent focus on mercury uptake by rice and subsequent human exposure; this is primarily an issue with rice grown on mercury polluted soils, and again growing rice anaerobically exacerbates the problem, because sub-oxic conditions promote methylation of mercury to methylmercury, the most toxic form. Hence, when fish consumption is low, rice products can be the major source of methylmercury to the diet (Rothenberg et al., 2016). A recent market basket analysis of infant rice products found that rice cereals and rice teething biscuits were on average 61 and 92 times higher in methylmercury respectively than cereals made with wheat or oats (Rothenberg, Jackson, Carly McCalla, Donohue, & Emmons, 2017). Provisional tolerable weekly intake (PTWI) limits for methylmercury exposure from food were established in the EU by the Joint Expert Committee on Food Additives (JECFA) of 1.3 µg mercury/kg body weight (0.18 µg per day) (JECFA, 2007). The US EPA oral reference dose (RfD) for daily intake of methylmercury is 0.1 µg/kg body weight (USEPA, 2001). Analysis of data from NHANES, mentioned above, also found higher blood mercury (inorganic and methylmercury) concentrations in people following a GF diet (Bulka et al., 2017). Increased rice consumption is one possible explanation.

Numerous market basket studies have measured the concentration and speciation of arsenic in whole grain rice, food containing rice, and ingredients processed from rice. This has provided the basis for the food-arsenic regulation mentioned above. Databases of arsenic concentrations and speciation in many rice-containing food products are available (FDA, 2016) and it is clear that some iterations of a GF diet could potentially provide a higher arsenic exposure (Munera-Picazo, Ramirez-Gandolfo, Burlo, & Carbonell-Barrachina, 2014) than is considered safe in terms of increases in life-time cancer risk. Elevated cadmium, lead and nickel concentrations were also found (Orecchio et al., 2014). However, side-by-side comparisons of the concentration of arsenic and other relevant elements in GF and non-GF products – needed to inform a dietary arsenic exposure risk assessment – have not been extensively carried out. In addition to the 1% of the US population who have celiac disease based on seroprevalence, GF diets are also necessary for those with non-celiac gluten sensitivity and wheat allergies. Despite concerns about the nutritional adequacy of the GF diet (Theethira & Dennis, 2015), it has gained a reputation as being more beneficial for health and weight maintenance than diets containing gluten. Surveys conducted in 2013 indicated that almost 25% of the US population had adopted a GF diet (DiGiacomo, Tennyson, Green, & Demmer, 2013). In line with this, the GF food retail market more than doubled in value between 2011 and 2016 (Group, 2013), a trend which is expected to continue.

In this study, we measured the concentration of arsenic, mercury, lead, cadmium and other elements including micronutrients manganese, iron, zinc, copper and selenium, in locally-available rice grains and rice-containing products and compared them to metal concentrations measured in equivalent wheat-based products. Arsenic and mercury speciation was determined in rice grains and rice-containing products, because total concentrations of these elements were high enough in these products to allow reliable speciation analysis and/or exceed proposed regulatory limits. We examined the elemental content of other readily available GF flours and grains such as amaranth, oat, sorghum, almond and coconut. We show that, consistent with observations of mercury in blood of GF diet followers (Raehsler, Choung, Marietta, & Murray, 2017; Vici, Belli, Biondi, & Polzonetti, 2016), rice-containing products appear to be a source of methylmercury at very low concentrations (up to 3.5 ng/g) in comparison with products made from wheat or other grains.

2. Methods and materials

2.1. Sample procurement and preparation

Sixty-seven food products were purchased from local food stores in Hanover and West Lebanon (New Hampshire, USA) and from on-line suppliers from late 2016 through mid 2017. The choice of food products was intended to compare readily available GF cooking ingredients and staple prepared food products and their gluten-containing counterparts. Products were grouped into flours, whole grain rice, and prepared foods. Designation of the products as GF, organic or enriched were made on the basis of information on the packaging. We purchased 19 different rice grains, three rice flours, and 19 non-rice flours (including corn, corn masa, whole wheat, all-purpose wheat, sprouted wheat, spelt, millet, oat, buckwheat, chickpea, coconut, and almond, amaranth) as well as other popular grains (black chia seed and tricolor quinoa). The GF and non-GF prepared food products consisted of gluten free pastas, breads, cakes (Table 1). Most GF products contained rice as one of top 3 listed ingredients.

Whole grains were not rinsed or cooked prior to being prepared for analysis, and were ground in a clean, dry coffee grinder. Flour and powder samples were acid digested without further preparation. Moist prepared food samples were freeze-dried and homogenized prior to analysis. The final results were corrected so they reflected the wet weight concentration. Foods were analyzed for Mn, Fe, Ni, Cu, Zn, Cr, Co, As, Se, Cd, Sb, Hg, and Pb (Table 1). Whole grain rice and rice flour were subject to speciation analysis for arsenic (inorganic As, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA)) and mercury (methylmercury and total mercury).

2.2. Sample digestion for total metals analysis

Samples were acid digested by closed vessel microwave assisted digestion; 5 ml of 9:1 Optima HNO_3:HCl was added to 0.25 g of sample and the digestion temperature was ramped to 220 °C over 15 min and held for a further 20 min. Following digestion, the sample was diluted to 50 ml with deionized water.

For arsenic speciation, 0.25 g of sample was heated to 100 °C in 2% HNO_3 following our previous methods (Jackson, 2015). Methylmercury analysis was performed by species specific isotope dilution, acid extraction, ethylation, purge and trap concentration, gas chromatographic separation and ICP-MS analysis following our previous methods (Taylor, Jackson, & Chen, 2008).

2.3. Elemental analysis

Samples digested for total elemental analysis were analyzed by collision cell inductively coupled plasma mass spectrometry (ICP-MS) (Agilent, 7900x) operated in helium gas mode for all elements and no gas mode for lead and mercury. For digestions and extractions, one sample per batch (where a batch is denoted as ≤20 samples) was digested/extracted in duplicate and spiked with analyte and taken through the digestion/extraction process. Additionally, one spiked blank (fortified blank), reagent blank and standard reference material

Table 1
Mean (SD) elemental concentration of food products, grouped by product categories and type of grain as determined by ICP-MS. Results of one-sample t-tests or ANOVA statistical tests are indicated: ns not significant; *p-Value < .05; ** < .01 and *** < .0001. Tukey's means comparison test results are indicated by superscript lettering. nc = not certified. Italicized SRM recoveries indicate reference values supplied on SRM certification. The method detection limit was based on the higher value of either the instrument detection limit or 3σ of the digestion blanks. The method detection limit also accounts for the sample digestion dilution and reflects the concentration limit in the food product.

Product	N	Mn	Fe	Ni	Cu	Zn	Cr	Co	As	Se	Cd	Sb	Hg	Pb
Grain type		μg/g					ng/g							
Method detection limit		0.01	0.3	0.02	0.2	0.3	27	0.4	11	21	0.3	2.5	0.5	5
Below detection limit [N (%)]		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	25 (38)	0 (0)	15 (23)	7 (11)	0 (0)	39 (59)	18 (27)	23 (35)
% SRM recovery (n = 4) Mean (SD)		101 (4)	97 (4)	nc	98 (4)	97 (2)	nc	100 (7)	110 (3)	94 (5)	92 (3)	nc	89 (9)	106 (15)
Flour		ns	ns	ns	ns	**	ns	*	**	ns	ns	ns	**	ns
Rice	4	22.5 (18.2)	31.2 (19.2)^{b}	0.7 (0.8)^{ab}	2.4 (1.6)^{b}	14.9 (6.8)^{b}	54 (31)	30.6 (29.9)^{ab}	112 (92)^{a}	117 (57)	26 (35.6)	1.8 (1.0)	2.2 (1.1)^{a}	4 (2)
Wheat	6	25.9 (25.4)	38.0 (15.6)^{ab}	0.3 (0.3)^{b}	3.3 (2.0)^{ab}	20.1 (13.4)^{b}	10 (9)	12.8 (17.0)^{b}	11 (4)^{b}	375 (1 6 4)	45 (23.5)	4.8 (5.8)	0.9 (0.9)^{b}	3 (3)
Other	12	31.7 (21.6)	72.4 (29.5)^{a}	2.3 (2.1)^{ab}	10.3 (9.3)^{a}	38.0 (15.5)^{a}	52 (84)	108.5 (103.9)^{a}	13 (5)^{b}	199 (1 8 4)	31 (25.3)	5.3 (3.7)	0.6 (0.2)^{b}	8 (6)
Whole grain rice		**	**	ns	ns	*	ns	*	*	ns	ns	ns	ns	ns
White	5	7.8 (3.1)^{b}	3.7 (4.4)	0.4 (0.4)	2.3 (1.5)	13.1 (6.0)^{b}	48 (1 0 0)	9.3 (5.9)^{b}	94 (89)^{a}	92 (71)	38.0 (42.7)	2.2 (1.2)	1.4 (1.1)	19 (25)
Brown	6	28.9 (2.7)^{a}	11.5 (1.5)	0.4 (0.2)	3.1 (1.0)	22.5 (4.3)^{a}	18 (14)	26.4 (15.9)^{a}	183 (46)^{a}	104 (1 0 3)	27.0 (28.6)	1.7 (1.2)	1.8 (1.2)	8 (6)
Enriched white	7	9.3 (3.3)^{b}	19.4 (17.8)	0.2 (0.1)	1.8 (0.4)	12.3 (4.0)^{b}	58 (59)	12.1 (4.3)^{b}	177 (69)^{a}	137 (81)	10.9 (4.1)	6.9 (13.8)	1.9 (1.0)	21 (23)
Prepared Foods & Pasta		ns	**	*	**	*	**	**	***	*	ns	ns	**	**
Rice	18	20.1 (22.4)	13.3 (5.2)	0.4 (0.2)	2.0 (0.9)	14.3 (5.1)	189 (1 0 6)	23.5 (16.9)	141 (82)	115 (91)	15.9 (11.3)	2.9 (1.6)	3.4 (2.0)	22 (12)
Non-rice	8	13.9 (11.0)	26.1 (14.4)	0.2 (0.1)	3.4 (1.6)	20.9 (8.4)	111 (1 1 3)	6.7 (5.5)	15 (12)	340 (3 2 3)	52.4 (42.7)	1.7 (1.7)	1.0 (0.4)	7 (3)
All products combined		ns	***	***	***	**	ns	***	***	**	**	ns	***	*
Rice	38	17.9 (17.2)^{b}	14.0 (11.8)^{c}	0.3 (0.3)^{b}	2.2 (1.1)^{b}	15.0 (5.9)^{b}	108 (1 1 0)	19.3 (15.0)^{a}	147 (78)^{a}	114 (84)^{b}	21.3 (24.2)^{b}	3.3 (5.9)	2.5 (1.7)^{a}	18 (16)^{a}
Wheat	15	17.7 (18.1)^{b}	32.0 (15.8)^{b}	0.2 (0.2)^{b}	3.2 (1.7)^{b}	20.2 (10.0)^{b}	66 (98)	9.1 (11.0)^{b}	13 (9)^{b}	333 (2 6 9)^{a}	46.5 (36.2)^{a}	2.9 (3.9)	0.9 (0.6)^{b}	5 (4)^{b}
Other	13	33.1 (20.1)^{a}	70.7 (31.4)^{a}	2.4 (2.0)^{a}	10.4 (9.2)^{a}	38.1 (15.3)^{a}	55 (83)	113.8 (99.7)^{c}	13 (4) ^{b}	207 (1 7 7)^{ab}	31.5 (24.5)^{ab}	5.2 (3.8)	0.6 (0.3)^{b}	8 (6)^{ab}
Gluten Content		ns	ns	**	ns	ns	ns	**	***	**	ns	ns	**	ns
Contains gluten	13	16.5 (18.9)	28.8 (16.8)	0.2 (0.2)	2.9 (1.7)	18.2 (10.2)	85 (1 3 3)	9.5 (11.8)	18 (19)	307 (2 5 6)	37.8 (27.9)	3.1 (4.1)	1.0 (0.7)	7 (7)
GF	55	21.5 (18.5)	27.6 (29.1)	0.8 (1.2)	4.1 (5.4)	20.6 (12.8)	90 (97)	38.8 (60.5)	112 (90)	149 (1 4 6)	26.7 (29.3)	3.6 (5.4)	2.0 (1.7)	15 (15)

per batch were taken through the digestion/extraction process. For analysis, additional quality control involved duplicate analysis of digested/extracted samples and analyte spikes of digested/extracted samples. For total analysis by ICP-MS, the instrument was calibrated using NIST-traceable multi-element standards prepared on the day of analysis. The calibration was verified using second source NIST-traceable standards after the calibration and repeatedly every 10 samples.

Method detection limits and standard reference material recoveries (%) are given for all elements in Table 1. We also analyzed mercury using the direct mercury analyzer (Milestone, Shelton, CT). This data was used for total mercury and % recovery of the NIST 1568b was 86 (± 3)% (n = 4).

2.4. Arsenic speciation

Arsenic species were analyzed by anion exchange chromatography coupled to reaction cell ICP-MS (Agilent 8800, Santa Clara, CA) as described previously (Jackson, 2015). The system was calibrated with arsenic species, arsenite, dimethylarsinic acid (DMA), monomethylarsonic (MMA) and arsenate and NIST 1568b which is certified for inorganic arsenic (sum of arsenite (3+) and arsenate (5+)), DMA and MMA. Recovery of Inorganic arsenic was 108 (± 23)% and organic arsenic (DMA + MMA) was 93 (± 6)%. Method detection limits for arsenic species were 3 ng/g.

2.5. Mercury speciation

Methylmercury concentrations were determined using an automated methylmercury analyzer (MERX-M, Brooks Rand Instruments, Seattle, WA) coupled to ICP-MS and quantified by isotope dilution as described previously. Duplicates of SRMs NIST 1566b Oyster Tissue, certified at 0.0132 μg/g MeHg, and NIST 2976 mussel tissue, certified at 0.0272 μg/g MeHg, were analyzed and recovery ± % relative difference was 118 ± 2.6% and 102 ± 7.1%. Detection limits for methylmercury were 0.12 ng/g.

2.6. Statistical analysis

We conducted one-way analysis of variance (ANOVA) or one-sample t-tests as appropriate on data for the metal concentrations in food products, using $\log_{10}$ transformation to normalize metal concentration data, which tended to be left skewed. For data measurements below the limit of detection, we assigned a value of half of the instrument detection limit. Means comparisons were conducted using Tukey's statistical tests. We used non-parametric Spearman's statistical test to examine correlations between total arsenic concentrations (all species combined), inorganic arsenic (arsenite + arsenate), organic arsenic (DMA and MMA) and the measured total mercury and methylmercury concentrations.

3. Results and discussion

Table 1 summarizes the total elemental concentrations and results of one-sample t-tests and ANOVA statistical analysis on the food and product groups analyzed in this study.

3.1. Arsenic

The total arsenic concentration in whole grain rice, rice flour and processed foods containing rice was significantly higher than non-rice flours and processed foods based on other grains [one-sample t (39.0) = 10.71, $p < 0.0001$] as summarized in Fig. 1. As shown in numerous studies, rice and rice products contain elevated arsenic concentrations compared to non-rice foods (Carbonell-Barrachina et al., 2012). Our speciation analysis of arsenic found that inorganic arsenic made up on average 63 (± SD 15)% of the total arsenic across all rice-containing foods sampled in this study (N = 38).

Between the three rice grain types (brown, white and enriched white) total arsenic concentrations were significantly different ($p = 0.0335$; F = 4.2320), with brown rice and enriched white rice having a higher concentration of arsenic than non-enriched white. The inorganic arsenic concentration ($p = 0.0029$, F = 8.8611) and the percent of inorganic arsenic ($p = 0.0029$, F = 8.8380) was also significantly higher in brown rice than enriched white or non-enriched white ($p = 0.01$; F = 6.0126). Inorganic arsenic accumulation in bran layers of the rice grain (Carey et al., 2011) is a likely explanation for the higher inorganic arsenic concentrations measured in brown rice. However, the reason why enriched white rice grain contains higher organic arsenic concentrations than brown or non-enriched white rice is not clear, and may be an artifact of the small sample size of each grain type (where certain suppliers had more than one grain in each product type).

We found that only one whole grain rice sample measured below detection limits for arsenic (2 ng/g), an upland rice grown under non-flooded conditions. This cultivation technique is an effective method for limiting arsenic uptake in rice because arsenic remains bound to the soil and not available for plant uptake (Hu et al., 2013). However, this rice sample also contained the highest concentration of cadmium of all the grains and food products tested (0.12 μg/g, compared to a mean of 0.02 μg/g cadmium for whole grain rice in this study), which again has been shown to be a consequence of growing rice aerobically. Under aerobic soil conditions cadmium is not sequestered as the insoluble sulfide and is more available for uptake (Tai, Li, McBride, & Yang, 2017).

The average inorganic arsenic content of rice and rice products measured in this study was 93.9 ng/g: with and an individual serving of either grain or pasta estimated to be 50 g, a serving could contain an average of 4.6 μg of inorganic As. An individual following a GF diet eating 3–4 servings of rice and rice products could conceivably consume 14–18 μg of inorganic arsenic per day.

3.2. Mercury

Mercury concentrations were very low, < 4 ng/g for all foods tested. This concentration level is much lower than fish and other seafood, which is generally the major source of mercury to diet. The US FDA reported mean levels of mercury in fish that ranged from 45 ng/g for anchovies to > 1000 ng/g for tuna, halibut and other high trophic level fish (FDA, 2017). While much lower than fish, the mercury concentrations in the foods we tested were of interest because of recent biomonitoring studies and data analysis of the NHANES database, which showed that self-reporting rice eaters and people following a gluten free diet had higher levels of blood mercury than non-rice eaters or those not on a GF diet (Bulka et al., 2017; Raehsler et al., 2017).

When data for all products was grouped by the constituent grain type, food containing rice had significantly higher mercury concentrations than those based on wheat or other grains [one-sample t (56.3) = 6.49, $p < 0.0001$] (Table 1). Within the whole grain rice category, mercury concentrations did not significantly differ between white, brown or enriched white rice, suggesting that mercury does not differentially accumulate in the bran layers in the way that arsenic does.

Methylmercury concentration of rice grains was highly correlated with the independent measure of total mercury and suggested that essentially all the mercury in these rice grains was methylated (Fig. 2). Studies of rice grown in Hg-contaminated soils around artisanal gold mining operations have found high levels of total mercury and methylmercury in rice grains (Rothenberg et al., 2014). These studies have mostly focused on rice grown in contaminated soils; however, more recent studies have shown that even in non-contaminated soils, rice is a source of mercury to diet (Hong, Yu, Liu, Cheng, & Rothenberg, 2016). Like arsenic, the presence of methylmercury in rice is due to the

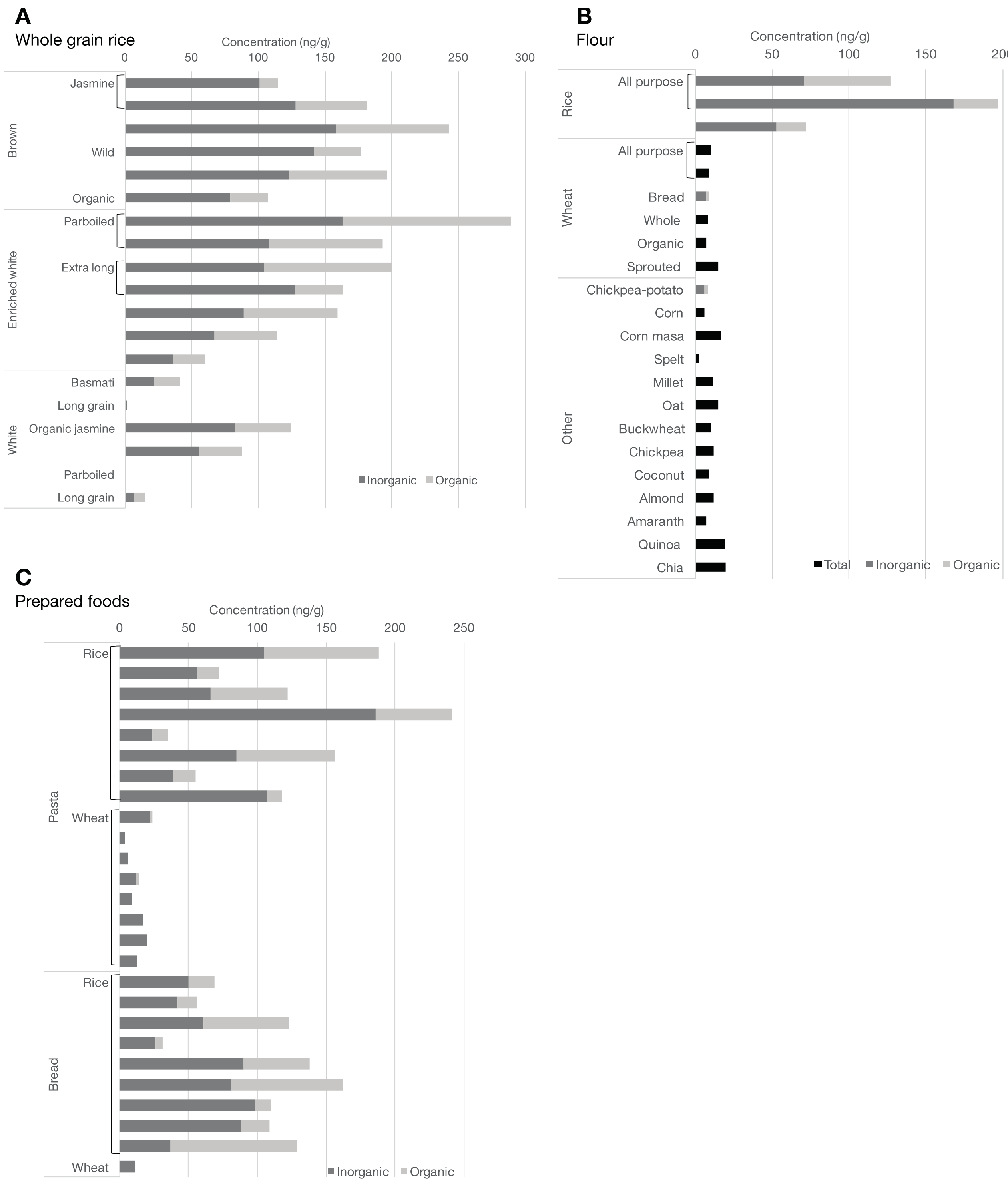

Fig. 1. Concentration of arsenic (ng/g) in a range of gluten-free foods and their non gluten-free equivalents, including A: whole grain rice, B: flours and C: pasta and bread.

practice of growing rice in flooded soils, because methylmercury formation is microbially driven and favored in slightly reducing environments where iron-reducing and sulfate-reducing bacteria predominate (Rothenberg & Feng, 2012). Although levels of mercury and methylmercury in rice are low, rice can be a significant source of these toxicants to the human diet because rice is a staple food (Rothenberg et al., 2014). Considerable concern has been raised about inorganic arsenic levels in infant rice cereal, because very young children are exposed to higher body burdens of inorganic arsenic and early life is a particularly sensitive window of exposure. Similar arguments could be raised for methylmercury exposure through rice cereal during developmentally-sensitive life stages (Hong et al., 2016), and perhaps additional concern is warranted for combined metalloid exposure from rice consumption. Using an average MeHg concentration of 2.5 ng/g, for an adult (60 kg) on a GF-diet consuming 3–4 rice-based servings (50 g) per day, an individual's MeHg exposure would be 0.006–0.008 µg/kg body weight. - This is over an order of a magnitude lower than JECFA and EPA recommended daily limits and therefore methylmercury exposure from

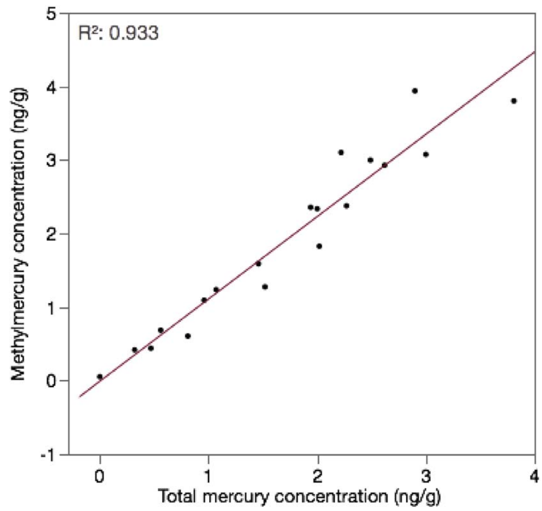

Fig. 2. Correlation between independent measures of total mercury concentration and methylmercury concentrations in rice products.

uncontaminated rice to adults is unlikely to pose any risk.

As with arsenic, the whole grain rice sample grown aerobically had undetectable mercury (below 0.5 ng/g), which is unexpected because chemically, mercury availability is believed to be similar to cadmium in its increased availability for uptake when not sequestered as the insoluble sulfide. This suggests that it is actually the methylation of mercury that may be of most importance in plant uptake.

We also found that, among the whole grain rice products tested (N = 19), total arsenic and mercury concentrations were strongly correlated (Table 2). Inorganic and organic forms of arsenic were equally correlated with total mercury, whereas the correlation between organic arsenic and methylmercury was marginally stronger than inorganic arsenic and methylmercury. A strong correlation between arsenic and methylmercury concentrations of rice based infant products was also recently found in a recent market basket analysis (Rothenberg et al., 2017).

3.3. Other contaminant elements

Cadmium concentrations were significantly higher in wheat-based prepared foods compared to rice or other grains and in gluten containing compared to GF foods. Increased uptake of cadmium in wheat compared to other grains is well recognized, and wheat is a main source of cadmium to the human diet (Arduini, Masoni, Mariotti, Pampana, & Ercoli, 2014). As noted earlier, rice can also take up cadmium, especially when grown aerobically, and, indeed, the highest cadmium containing food we measured was an aerobically grown rice which had a concentration of 0.12 µg/g. Due to relatively high uptake of cadmium and high consumption levels the European Food Safety Authority identified cereals and cereal products as the highest source of cadmium to the diet (EFSA, 2009).

Table 2
Results of Spearman's non-parametric statistical test between measured concentrations of arsenic species (total[a], inorganic[b], organic[b]) and total mercury[a] and methylmercury[c] in whole grain rice (N = 19).

Arsenic	Total Hg		MeHg	
	Spearman's ρ	*p*-Value	Spearman's ρ	*p*-Value
Total	0.7309	< 0.0001	0.6368	0.0034
Inorganic	0.5083	0.0001	0.5088	0.0311
Organic	0.5296	< 0.0001	0.6257	0.0055

[a] Measured by ICP-MS.
[b] Measured by LC-ICP-MS.
[c] Measured by direct mercury analysis.

Concentrations of lead in all rice containing foods were significantly higher than those based on wheat and, when the spelt flour was omitted from the 'other' grain category, rice was significantly higher than either of the other two grain types. Also, within the food products category, Rice products were higher in Pb than the wheat-based products.

3.4. Nutrient elements

Rice and rice based products were significantly lower in the nutrients iron, nickel, copper, zinc and cobalt compared to wheat flours and food products and the other (rice-free) GF flours. The superior nutritional quality of pseudocereals (e.g. quinoa, buckwheat and amaranth) are well known (Alvarez-Jubete, Arendt, & Gallagher, 2010), having a higher protein content than wheat, and constituting a good source of calcium, magnesium and iron. Despite seven of the rice grain products being fortified with ferric orthophosphate, their iron concentrations were comparatively lower than wheat flours and foods containing other non-rice GF flours. We observed that iron concentrations in enriched rice were highly variable in comparison with brown rice. Of the seven enriched white rice grain products analyzed, only three products had iron concentrations that exceeded 12 µg/g – the highest iron concentration measured in non-enriched white rice. One suggestion for this variation would be the specific method use to apply micronutrients to rice grain. Brown rice is known to have a higher nutrient content than white rice due to the intact bran layers, and we found that zinc, iron and manganese concentrations were significantly higher in brown rice than in white rice, with brown rice also having a higher average copper concentration.

Wheat was significantly higher in selenium than rice or the other GF flours. Wheat grown in the US readily accumulates selenium from Se-rich US soils (Reilly, 2006), whereas rice does not take up comparable concentrations of this micronutrient. Copper was significantly higher in non-rice flours and wheat pasta than rice pasta or grain. In fact, concentrations of Fe, Cr, Co, Ni, Cu and Zn in non-rice GF flours were all significantly higher than rice. When non-rice GF flours were combined with the rice data to create a GF food category for comparison with the wheat-based non-GF foods then only Se remained significantly higher in the NGF foods; in other words, the nutrient deficiencies of rice were negated by pooling the data with other GF grains, which were generally higher in nutrient content.

A multi-elemental market basket study of 27 GF foods purchased in and around Palermo City (Italy) found that the nutritional quality of the foods, in comparison with recommended dietary intake levels for the essential micronutrient elements, was relatively poor (Orecchio et al., 2014).

4. Conclusions

Rice and rice products were significantly higher in As, Hg and Pb and lower in Se, Fe, Cu and Zn than foods based either on wheat or non-rice GF grains. Wheat flours and wheat-based foods were higher in Cd than rice and rice-based products. We found a strong correlation between arsenic and mercury in rice and rice based products that warrants further investigation. Assuming that the concentrations of metals measured in these products are indicative of their bioavailable concentrations upon ingestion, our study reinforces nutritional advice to those concerned about dietary arsenic exposure as part of a GF diet, namely to consume a wide variety of grains, which would reduce arsenic, and mercury exposure while supplying essential micronutrients.

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