



Total arsenic, inorganic arsenic, and other elements concentrations in Italian rice grain varies with origin and type



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ABSTRACT

Rice is comparatively efficient at assimilating inorganic arsenic (As_i), a class-one, non-threshold carcinogen, into its grain, being the dominant source of this element to mankind. Here it was investigated how the total arsenic (As_t) and As_i content of Italian rice grain sourced from market outlets varied by geographical origin and type. Total Cr, Cd, Se, Mg, K, Zn, Ni were also quantified. As_t concentration on a variety basis ranged from means of 0.18 mg kg^{-1} to 0.28 mg kg^{-1} , and from 0.11 mg kg^{-1} to 0.28 mg kg^{-1} by production region. For As_i concentration, means ranged from 0.08 mg kg^{-1} to 0.11 mg kg^{-1} by variety and 0.10 mg kg^{-1} to 0.06 mg kg^{-1} by region. There was significant geographical variation for both As_t and As_i ; total Se and Ni concentration; while the total concentration of Zn, Cr, Ni and K were strongly influenced by the type of rice.

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1. Introduction

Arsenic (As) is found in virtually all environmental matrices (Fitz and Wenzel, 2002). The most common species of As in terrestrial habitats are arsenite ($As(III)$) and arsenate ($As(V)$), inorganic forms, and monomethylarsonic (MMA) acid and dimethylarsinic acid (DMA), organic forms (Smith et al., 1998; Smedley et al., 2002). Food and drinking water are the major routes of As exposure for humans (Mahmudur et al., 2009). Furthermore, in recent decades, millions of people have suffered from arsenic poisoning as a result of arsenic-contaminated groundwater being used as drinking water, for crop irrigation, and in food cooking in Southeast Asia (Carbonell-Barrachina et al., 2009). As_i has been evaluated by the International Agency for Research on Cancer (IARC) as group 1 carcinogen (IARC, 2004). No standards for As in food currently exist in the EU or US, a major gap in food health policy (Francesconi, 2007).

Rice (*Oryza sativa*) is the dominant staple food for over half of the world's population, especially in developing Asian countries, contributing ~70% of the energy provided by their daily food intake (Phuong et al., 1999; Torres-Escribano et al., 2008). Unfortunately, rice is much more efficient at assimilating arsenic into the grain than other staple cereal crops (Meharg et al., 2009). The As

levels in the rice grain are influenced by cultivation method, genetic variation, soil biogeochemistry and cooking (Meharg and Zhao, 2012). Most foods with elevated As content are of marine origin where speciation is dominated by organic As species (As_o) which have much lower toxicities as compared to As_i (Mass et al., 2001; Styblo et al., 2000). Rice, however, appears to be an exception because it contains high As_i concentrations, typically between 0.05 and 0.4 mg arsenic/kg (Sun et al., 2008a; Meharg et al., 2009). In addition to As_i , MMA and DMA have been detected in samples of rice (Heitkemper et al., 2001; Williams et al., 2005; Nardi et al., 2009; D'Amato et al., 2004; Lamont, 2003). Previous investigations have determined that rice is the primary food source of As exposure in non-seafood diets in EU (Robberecht et al., 2002) and US (Tao and Bolger, 1999), and that rice is a dominant source of As_i to humans, with exception of those regions with elevated As_i in drinking water (Sun et al., 2008b).

Rice production is heavily concentrated in Asia, with just four countries, China, India, Indonesia, and Bangladesh, accounting for nearly 70% of global production (FAO, 2011). In the European Union, Italy is the leading rice producer with approximately 50% of the total EU harvest (Italian Grain and Feed Report, 2012). Rice cultivation in Italy is mostly located in the northern regions and extends over about 240,000 ha which represent only 1.4% of the total arable area (16,800,000 ha). Rice cultivation is primarily based in the Po Valley. About 52% of the rice area is in Piemonte, mostly in Vercelli and Novara provinces, and about 41% in Lombardia, for the most part in Pavia and Milano (Istat, 2012). The majority of the planting is

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with japonica subspecies (Russo and Callegarin, 2004). Climatic conditions in Italian rice plantations are generally inadequate for authentic indica varieties (Lupi et al., 2010). The most famous and cultivated species are Carnaroli, Arborio and Vialone Nano used in traditional risotto, while other species like Roma are used in confectionary (Padovani et al., 2006). The dimensions of a typical rice farm in Italy are about 48 ha and the average rice yield ranges from 5.0 tons/ha to 6.9 tons/ha, according to the variety (Lupi et al., 2010).

Italian domestic rice consumption is around 40% of total production, and the remaining part is exported to the EU, especially to France, Germany, and UK (Italian Grain and Feed Report, 2012). In Italy, high As concentrations have been detected in the soil of Campania, Lombardia, Puglia, and Calabria. Furthermore, As concentrations above $50 \mu\text{g L}^{-1}$ have been found in groundwater of several regions (Campania, Lazio, Toscana, Emilia-Romagna, Lombardia, Veneto, and Sardegna) (Cubadda et al., 2010).

Here a survey of Italian commercial rice purchased in Italian supermarkets is presented to investigate total As (As_t) and As speciation. Also, other elements of nutritional and toxicological interest Cr, Cd, Se, Mg, K, Zn, Ni were analyzed using samples (101) of commercial rice collected in supermarket stores in the city of Napoli, Milano and Cosenza during 2012. The principal aim of this study was to quantify and qualify how Italian rice varied in As content dependent on regional origin and variety.

2. Materials and methods

2.1. Samples preparation to total elemental quantification

The origin and varieties of samples analyzed are outlined in Table 1. All samples were dried overnight in an oven at 70°C and ground by a mill ball. Subsequently, samples were processed as described in a published methodology (Williams et al., 2007b). Briefly, summarizing this analysis, approximately 0.2 g of the milled samples were accurately weighed into 50 mL polypropylene digest tube (Corning, NY) to which 2.5 mL of concentrated Aristar nitric acid (HNO_3) was added. The mixture was left overnight, then 2.50 mL of hydrogen peroxide (H_2O_2) was added and then the samples were digested in a microwave oven (CEM Mars 5, CEM Corp., Matthews, NC). The temperature for digest was increased first to 55°C for 15 min, then to 75°C for 15 min and finally to 95°C for 30 min.

Microwaved samples were cooled to room temperature and then diluted to 50 mL with ultrapure deionized water obtained from a Milli-Q system (Millipore, Billerica, MA), after rhodium ($10 \mu\text{g}^{-1}$) was added as internal standard. In each

analytical batch one reagent blank and one certified reference material (CRM) (rice flour NIST SRM 1568a) were included. A quadruple ICP-MS 7500 (Agilent Technologies) was used to determine metal and trace element (As, Cd, Cr, Zn, Se, Ni). Samples were randomized before the analysis. Standards were run after every 30 samples. The concentration of samples was determined using a seven-point calibration (from 0.1 to 100.0 mg L^{-1}) calculated from a multi-element standard solution (Claritas PPT). Atomic Absorption Spectroscopy (AAS) was used to analyze Mg and K, using a Perkin Elmer AAnalyst 100.

2.2. Samples preparation for As speciation

The procedure for speciation extraction followed that described in Zhu et al. (2008a,b). Around 0.2 g of rice was accurately weighed for each milled samples into a 50 mL polypropylene digest tube and 10 mL of 1% Aristar HNO_3 were added and was left overnight. Then, the samples were extracted using a microwave oven with the same conditions as previously described for samples preparation for ICP-MS and atomic adsorption analysis. When the samples were cooled to room temperature, 1.5 mL samples were centrifuged at 10,000 g for 10 min and 900 μL of supernatant was mixed with 100 μL of H_2O_2 . The samples were left overnight at 4°C before analysis. Quality controls of CRM and blanks were run with each extract batch. Arsenic speciation was quantified by HPLC (HP1100, Agilent Technologies) coupled to the ICP-MS (Sun et al., 2008a,b). Chromatographic separation utilized a pre-column (11.2 mm, 12–20 μm Hamilton, Reno, NV, USA) and a PRP-X100 10- μm anion-exchange column (150 \times 4.1 mm, Hamilton). The mobile phase consisted of 6.66 mM ammonium hydrophosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) and 6.66 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using ammonia 3%. Retention time for the As species was determined using a species standards mix of $10 \mu\text{g L}^{-1}$ containing arsenite, arsenate, DMA and MMA, and DMA standards ($0-0.5-2.5-5.0-10.0-25.0 \mu\text{g L}^{-1}$), used to calibrate the instrument. The species standards mix was run after every 20 samples.

3. Results and discussion

3.1. CRM recovery

The CRM mean and standard error As_t recovery was $0.27 \pm 0.02 \text{ mg kg}^{-1}$ compared to a certified value of 0.29 mg kg^{-1} (Raab et al., 2009). Limit of detection (LOD) was 0.016 mg kg^{-1} . No rice CRM certified speciation is available; so rice flour CRM speciation data obtained here was compared with that previously reported by Raab et al. (2009) for As_i 0.09 , which was 0.09 ± 0.007 here and As_o 0.18 mg kg^{-1} , which was $0.17 \pm 0.02 \text{ mg kg}^{-1}$ here. CRM and Spike recoveries for others elements are reported in Table 2.

3.2. Total As concentration

It was found that the highest mean concentration of As_t was for Vialone Nano rice varieties (0.28 mg kg^{-1} , 5 samples), whereas, the lowest total mean concentration was found in Ribe rice samples (0.18 mg kg^{-1} , 21 samples). According to the region of provenience, it was found that the highest total concentration was in Emilia Romagna region (0.28 mg kg^{-1} 3 samples) and the lowest mean in Calabria (0.11 mg kg^{-1} 5 samples), the range for the entire data set being $0.07-0.47 \text{ mg kg}^{-1}$ (Table 2). Meharg et al. (2009) analyzed 38 samples of Italian rice. They found As_t concentration $\sim 0.15 \text{ mg kg}^{-1}$; lower than rice from French Camargue production region (0.28 mg kg^{-1}), the highest value found commercially. Also they analyzed US produced rice and they found a mean $\sim 0.25 \text{ mg kg}^{-1}$ for As_t concentration. The overall range found for the individual samples was from 0.01 mg kg^{-1} found in Egyptian and Indian rice to 0.82 mg kg^{-1} in Spanish rice (Meharg et al., 2009). Zavala et al. (2008) found US rice had a mean As_t of 0.20 mg kg^{-1} . D'Illo et al. (2002) analyzed Italian rice varieties and found the following results: 0.20 mg kg^{-1} (Arborio); 0.14 mg kg^{-1} (Carnaroli); 0.28 mg kg^{-1} (Ribe); 0.12 mg kg^{-1} (Ribe parboiled); 0.22 mg kg^{-1} (Vialone Nano); 0.18 mg kg^{-1} (Originario). The study presented here, in comparison, had Ribe at 0.18 mg kg^{-1} and parboiled at 0.20 mg kg^{-1} mean value. Here also, for As_t concentration means of 8 different varieties varied significantly ($P = 0.03$) when analyzed by one way analysis-of-variance (ANOVA). For the region

Table 1

The origin and varieties of rice samples analyzed.

| Variety | N. samples | Region of provenience |
|-------------------------------------|------------|-----------------------|
| Arborio (japonica, very fine) | 29 | 19 Lombardia |
| | | 8 Piemonte |
| | | 1 Emilia Romagna |
| | | 1 Calabria |
| | | 1 Campania |
| Carnaroli (japonica, very fine) | 17 | 10 Lombardia |
| | | 6 Piemonte |
| | | 1 Calabria |
| Ribe (japonica, fine) | 21 | 11 Lombardia |
| | | 8 Piemonte |
| | | 1 Emilia Romagna |
| | | 1 Calabria |
| | | 1 Campania |
| Ribe/Roma parboiled (japonica) | 10 | 6 Lombardia |
| | | 4 Piemonte |
| Roma (japonica, very fine) | 8 | 4 Lombardia |
| | | 3 Piemonte |
| | | 1 Emilia Romagna |
| Vialone Nano (japonica, semi fine) | 5 | 3 Lombardia |
| | | 2 Piemonte |
| Originario (japonica, common) | 3 | 2 Piemonte |
| | | 1 Lombardia |
| Others (mixture of these varieties) | 8 | 5 Lombardia |
| | | 2 Calabria |
| | | 1 Piemonte |

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