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# Assessment of influences of cooking on cadmium and arsenic bioaccessibility in rice, using an *in vitro* physiologically-based extraction test

Ping Zhuang [a,b], Chaosheng Zhang [c], Yingwen Li [b], Bi Zou [b], Hui Mo [b], Kejun Wu [b], Jingtao Wu [b], Zhian Li [b,*]

[a] College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, PR China
[b] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, PR China
[c] GIS Centre, Ryan Institute and School of Geography and Archaeology, National University of Ireland, Galway, Ireland

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ABSTRACT

The health risks associated with rice consumption may decrease if consumers use cooking practices which can reduce the bioaccessibility of metal(loid)s. The effects of cooking on the Cd and As bioaccessibility, at three contamination levels of rice, were studied. Results indicated that cooking reduced bioaccessibility of Cd and As in rice. Cooking resulted in a significant increase ($p < 0.01$) of Cd and As concentrations in the residual fraction. Low volume water-cooking of rice to dryness reduced total Cd by about 10% for rices A and B, while medium or high volume water-cooking had no effect on Cd bioaccessibility in all rice types. In contrast, low volume cooking did not remove As, but a significant decrease ($p < 0.05$) was observed when cooking with higher volumes of water. This study provides information for a better understanding of more realistic estimation of metal(loid)s exposure from rice and the possible health risks.



## 1. Introduction

There is a growing concern about cadmium (Cd) and arsenic (As) pollution in rice, since dietary intake is considered to be one of the major routes of metal(loid) exposure to humans (Sohn, 2014; Zhuang, Lu, Li, Zou, & McBride, 2014). Cd and As are considered potential carcinogens and are associated with etiology of a number of diseases, especially cardiovascular, kidney, nervous system, blood as well as bone diseases while inorganic arsenic is well known as a non-threshold, class 1 carcinogen (International Agency for Research on Cancer, 2012). Rice, used as the staple food for half of the world population, is highly efficient in Cd and As accumulation and soil-to-rice transfer compared with other cereal crops. It has been identified as a significant contributor to dietary Cd and As intakes in a number of geographical areas, including China, Japan, Bangladesh, and India (Bae et al., 2002; Sun, de Wiele, Alava, Tack, & Laing, 2012). Therefore, rice safety has emerged as an urgent issue in many countries and there is a great need to assess the health risks from ingesting Cd- and As-contaminated rice.

In general, the total amount of heavy metals in rice is commonly used as the measurement of heavy metal contamination in food matrices (Zhuang, McBride, Xia, Li, & Li, 2009). However, the ingested dose of heavy metals does not always reflect the actual level of heavy metals that is available to the consumer (Horiguchi et al., 2004). Understanding bioavailability of heavy metals in rice is helpful for estimating the amount of heavy metals which can be absorbed by the human body. Thus, there is a need to determine the oral bioaccessibility of heavy metals in rice. Oral bioaccessibility is defined as the fraction of contaminant that is released from the food matrix into the digestive juice chyme and becomes available for absorption, i.e., enters the blood stream (Oomen et al., 2002); therefore it is used as an indicator of maximal oral bioavailability of the contaminant in food (Versantvoort, Oomen, Kamp, Rompelberg, & Sips, 2005). In recent years, several *in vitro* digestion models have been proposed and extensively used to study the bioaccessibility and risk assessment of contaminants (e.g. heavy metals, organic pollutants and mycotoxins) from food, soils, toys, and herbal medicine (Oomen et al., 2002).

With regard to bioaccessibility of heavy metals in food items, various static *in vitro* models have been used to determine As bioaccessibility or bioavailability from rice (Laparra, Vélez,

* Corresponding author.
*E-mail addresses:* zhuangp@scbg.ac.cn (P. Zhuang), chaosheng.zhang@nuigalway.ie (C. Zhang), Liyw@scbg.ac.cn (Y. Li), Zoubi@scbg.ac.cn (B. Zou), Mohui@scbg.ac.cn (H. Mo), wkj966516@scbg.ac.cn (K. Wu), Wujt@scbg.ac.cn (J. Wu), lizan@scbg.ac.cn (Z. Li).

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Barberá, Farré, & Montoro, 2005; Signes-Pastor, Al-Rmalli, Jenkins, Carbonell-Barrachina, & Haris, 2012), mushroom (Llorente-Mirandes, Llorens-Muñoz, Funes-Collado, Sahuquillo, & López-Sánchez, 2016; Sun, Liu, Yang, & Zhuang, 2012) and seafood. For Cd bioaccessibility, a limited number of studies have been conducted, mostly focusing on vegetables (Intawongse & Dean, 2008; Pelfrêne et al., 2015) and seafood products (Houlbrèque et al., 2011). There is a lack of data on the bioaccessibility of Cd in rice, with only a few studies (Aziz et al., 2015) based on *in vitro* digestion. This highlights the importance of performing more bioaccessibility studies of Cd and As in rice to improve the risk assessment.

Food is generally subjected to cooking prior to consumption in order to increase the palatability of the product. In comparison to raw rice, cooked rice is more suitable for studies of human health risks as the sample must be chosen on the basis that risk assessment reflects the real situation for human exposure (Devesa, Velez, & Montoro, 2008). Numerous studies have been performed to examine the effects of common food processing procedures on the levels of heavy metals in food. It is known that the cooking process may, under certain condition, alter the concentration or speciation of contaminants in food matrices (Wang, Duan, & Teng, 2014), such as seafood (Houlbrèque et al., 2011), vegetables (Pelfrêne et al., 2015), rice (Naseri, Rahmanikhah, Beiygloo, & Ranjbar, 2014), mushroom (Llorente-Mirandes et al., 2016). This hypothetical change depends upon cooking conditions (time, temperature, and medium of cooking). Little is known about the effects of rice cooking on the bioaccessibility of Cd and its human health risk due to Cd exposure from rice intake.

The main objective of the present study was: (1) to measure the bioaccessibility of Cd and As in raw rice samples at three different contamination levels based, on *in vitro* digestion and to determine the relationship between Cd and As bioaccessibility and total concentration, (2) to study the influences of cooking on Cd and As bioaccessibility and the extent of Cd and As removal by cooking of rice in different volumes of water and (3) to determine human health risks based on the bioaccessible Cd and As from the different contamination levels of cooked rice via the ingestion pathway.

## 2. Materials and methods

### 2.1. Sampling and cooking methods

Rice (long-grain) used in the current study is known to be widely consumed by the people in southern China. Three levels of rice contamination were selected in this study: 0–0.2 (rice A; low Cd level, purchased from several public supermarkets in southern China), 0.2–1.5 (rice B; medium Cd level, purchased from local markets around a mining area), 2.0–5.0 (rice C; high Cd level, grown in a greenhouse) mg of Cd per kg of rice. For rice C, the soil was spiked with a considerable amount of Cd in the greenhouse, and the rice plants were grown for 120 d before harvest. Then the grain of rice C was harvested, dehusked and polished to obtain white rice (polished rice). The three types of rice samples were simply washed three times with double-distilled deionized (Milli-Q) water at room temperature. In all cooking experiments, the weight used was 100 g. The washed rice was cooked in a beaker for 30 min with 200 ml of double-distilled deionized water, which was subject to 2:1 (low volume) water to rice (weight) cooking until no water was left. In China, rice is generally cooked with aliquots of water in order to absorb all of it. In the experiment on effects of cooking water, the washed rice samples were subject to 4:1 (medium volume) and 6:1 (high volume) water:rice cooking, where the rice was cooked to the texture for eating. In this method of cooking, the water remaining after cooking (gruel) was discarded. Portions of this gruel and cooked rice were freeze dried to constant weight, milled to a fine powder, and then stored at 4 °C prior to analysis. Concentrations of Cd and As in raw/cooked rice and cooking water were also determined.

### 2.2. In vitro evaluation of bioaccessibility

The physiologically-based extraction test (PBET) method was modified from the previously described method (Intawongse & Dean, 2008; Ruby et al., 1993). The gastric stage was carried out using 0.5 g of raw or cooked rice samples in a 100 ml screw-cap Sarstedt tube in which 50 ml of freshly prepared gastric solution was added. The gastric solution contained 1.25 g $l^{-1}$ of pepsin, 0.50 g $l^{-1}$ of citric acid, 0.50 g $l^{-1}$ of maleic acid, 420 μl $l^{-1}$ of DL-lactic acid and 500 μl $l^{-1}$ of acetic acid dissolved in water, and the pH was adjusted to 1.5 with HCl. The mixture was incubated at 37 °C with orbital–horizontal shaking at 150 rpm for 60 min. Then the solution was centrifuged at 3000 rpm for 10 min and a 5 ml aliquot was collected from the solution and filtered through a 0.45 μm filter disk for analysis. Five millilitres of the original gastric solution was then back-flushed through the filter into the sample tube to retain the original solid:solution ratio. At the gastrointestinal stage, the amounts of 52.5 mg of bile salts and 15 mg of pancreatin were placed in the sample tube and the pH of the mixture was raised to pH 7 with saturated $NaHCO_3$. The samples were incubated at 150 rpm in a thermostatic bath maintained at 37 °C for an additional 2 h; then a second 5.0 ml aliquot was collected and filtered. The extracts were kept at 4 °C prior to analysis. The resultant sample residue (residual fraction) was further digested by aqua regia as described by Intawongse and Dean (2008).

### 2.3. Determination of Cd and As

Rice samples (0.5 g) were predigested overnight with 5 ml of nitric acid in 50 ml centrifuge tubes at room temperature. Then this solution was heated in a microwave oven (Anton-Paar PE Multiwave 3000). Duplicate analyses were performed for quality control. Reagent blanks and standard reference rice samples (GBW10010 (GSB-1) and GBW10045 (GSB-3)) were also included in each batch. The recovery rates of heavy metals in standard reference rice samples ranged from 93% to 108%. The heavy metal concentrations of rice samples and rice extracts were analyzed with an inductively-coupled plasma mass spectrometer (ICP-MS) (Agilent 7700x, Agilent Scientific Technology Ltd., USA). Blank and drift standards were run after three determinations to calibrate the instrument.

### 2.4. Data analysis

The bioaccessibilities (%) of Cd and As were calculated as a percentage and calculated per digestion using the following equation (Oomen et al., 2002):

$$\text{Bioaccessibility }(\%) = \frac{\text{Bioaccessible metal concentration}}{\text{Total metal concentration in rice}} \times 100$$

In order to evaluate a once- or long-term potential hazardous exposure to Cd and As via consumption of rice by the consumers, the established daily intake (EDI), and target hazard quotient (THQ) for Cd and As, based on the bioaccessibility data (Zhuang et al., 2009), were calculated using, the following equations, respectively:

$$\text{EDI} = \frac{\text{RC} \times \text{BC}}{\text{BW}}$$

$$THQ = \frac{EF \times ED \times EDI}{RfD \times AT} \times 10^{-3}$$

where RC is daily rice consumption (g person$^{-1}$ d$^{-1}$), BC is bioaccessible concentrations of metals in ingestion, BW is average body weight (60 kg for adults and 32.5 kg for children), ED represents the exposure duration (70 years), EF is exposure frequency (365 days per year), AT is average time for noncarcinogens (365 days year$^{-1}$ $\times$ number of exposure years, assuming 70 years in this study), $10^{-3}$ is the unit conversion factor, and RfD represents corresponding oral reference dose (1 and 0.3 μg kg$^{-1}$ day$^{-1}$ for Cd and As, respectively), as suggested by USEPA (2010). Rice consumptions of 389 g d$^{-1}$ for adults and 277 g d$^{-1}$ for children were taken from Wang (2005).

### 2.5. Statistical analyses

All statistical analyses were performed using SPSS software (Ver 18.0; SPSS, Chicago, IL, USA) and Excel 2013. All data were reported as the means or means with standard deviation (SD) from several samples of each type of rice. The means were considered to be significantly different if p values were < 0.05.

## 3. Results and discussion

### 3.1. Concentrations of Cd and As in raw and cooked rice

The total Cd and As concentrations are listed in Table 1 for the three rice types with different levels of contamination. All the selected rice samples from the mining area and the laboratory were contaminated by Cd, exceeding the maximum allowable concentration of 0.2 mg kg$^{-1}$ Cd in rice established by China (MHPRC, 2012). With respect to the raw rice A, purchased from markets in southern China, Cd concentrations in about 30% of the rice samples were above the maximum allowable value, suggesting that this rice type could be a potential contributor to dietary Cd exposure in the population with a high intake of rice. The average As values in raw rices A and B were below the maximum allowable value of 0.2 mg kg$^{-1}$ established by both the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2014) and China (MHPRC, 2012). Compared with the values recorded by Fang et al. (2014), the concentrations of Cd and As in rice collected from the market in the present study are in the range found in rice sampled from public markets from southern China.

The effects of cooking by the Chinese traditional method (water:rice 2:1) on Cd and As concentrations were evaluated for three contamination levels of rice, and the results are shown in Table 1. Cooking caused significant changes of Cd in rices A and B, with a decrease of around 10% in the cooked rice in comparison to the corresponding raw rice. However, for rice C with a high contamination level, low volume cooking did not cause a significant difference. Naseri et al. (2014) reported that cooking can reduce the concentration of Cd in rice grains. Yet, Wang et al. (2014) reported that there were no statistical differences between Cd concentrations in microwave-cooked and raw rice. Our results were in agreement with other studies. For example, it has been reported that cooking decreased Cd concentration in *Agaricus blazei Murill* (Sun, Liu, et al., 2012) and in seafood (Atta, El-Sebaie, Noaman, & Kassab, 1997). However, another study reported that cooking resulted in an increase in total Cd concentration in Chilean mussels (Houlbrèque et al., 2011). It is possible that the Cd decrease with cooking may be related to the solubilization of Cd in the leaching water because Cd is usually bound with proteins, and thermal treatment could enhance protein degradation, resulting in release of Cd with water as free salts, soluble amino acids, and bound to proteins (Perelló, Martí-Cid, Llobet, & Domingo, 2008).

Cooking slightly decreased As concentration by about 3.5–6% in all rice types analyzed in comparison to the raw rice. Our results were similar to several previous studies (Laparra et al., 2005; Sengupta et al., 2006; Sun, de Wiele, et al., 2012), which reported that cooking rice to dryness could not remove As in rice. With respect to As concentration in other foodstuffs, previous studies have suggested that high percentages of As are released from food into the cooking water, e.g. mushroom (Llorente-Mirandes et al., 2016) and mussel (Houlbrèque et al., 2011), but some studies observed increases in total As concentrations in some products cooked with contaminated water (Signes-Pastor et al., 2012). These studies revealed a reduction of heavy metal(loid)s during cooking, depending on cooking conditions (e.g. time, medium of cooking, temperature).

### 3.2. Bioaccessibility of Cd and As in rice

#### 3.2.1. Bioaccessibility of Cd and As in raw rice

The oral bioaccessible concentrations of Cd and As measured in the gastric and gastrointestinal fractions for raw rice defined by the *in vitro* PBET methods are presented in Fig. 1. Significant differences ($p < 0.05$) were found in Cd and As concentrations in gastric and gastrointestinal extracts, depending on the type of rice analyzed, with different contamination levels.

The bioaccessibility of Cd varied between 68% and 87% in the gastric fraction, with the highest being in rice C and the lowest in rice A (Fig. 1). The bioaccessibility percentages obtained in the present research were higher than those from uncooked rice (16.9%) reported by Yang et al. (2012). Compared with the values recorded in raw vegetables by Pelfrêne et al. (2015), the bioaccessibility values for the gastric and gastrointestinal fractions were lower or similar, when Cd bioaccessibility varied from 81 to 89% in the gastric phase and from 63 to 72% in the gastrointestinal phase. Amiard et al. (2008) found that the median Cd bioaccessibility in commercial shellfish was 54%. The high percentages of bioaccessibility observed during *in vitro* digestion could be explained by the fact that most of the Cd accumulates in the vacuoles of plant cells, except what is absorbed by the cell wall, so Cd is easily released from plant tissues (Fu & Cui, 2013; Hall, 2002). In the gastric phase, it has been claimed that most of the available Cd was dissolved by enzymes and a portion was still absorbed into plant tissues (Hur, Lim, Decker, & McClements, 2011). In raw rice, the present results showed that Cd bioaccessibility in the gastrointestinal fractions was significantly lower than that in the gastric fractions, which was in line with the literature (Fu & Cui, 2013;

**Table 1**
Concentrations of As and Cd in the selected raw and cooked rice samples (average ± standard deviation, mg kg$^{-1}$; Ratio = metal concentration in cooked rice/metal concentration in raw rice) for three different level of rice (rice A, B and C).

| Type | Sources | N | Cd | | | As | | |
|---|---|---|---|---|---|---|---|---|
| | | | Raw | Cooked | Ratio (%) | Raw | Cooked | Ratio (%) |
| Rice A | Market | 12 | 0.117 ± 0.031 | 0.105 ± 0.023 | 89.5 | 0.142 ± 0.014 | 0.114 ± 0.019 | 94.4 |
| Rice B | Mining area | 10 | 0.499 ± 0.17 | 0.453 ± 0.15 | 90.8 | 0.171 ± 0.040 | 0.165 ± 0.037 | 96.5 |
| Rice C | Grown in Lab | 10 | 2.792 ± 1.47 | 2.689 ± 1.62 | 96.3 | 0.258 ± 0.077 | 0.248 ± 0.089 | 96.2 |

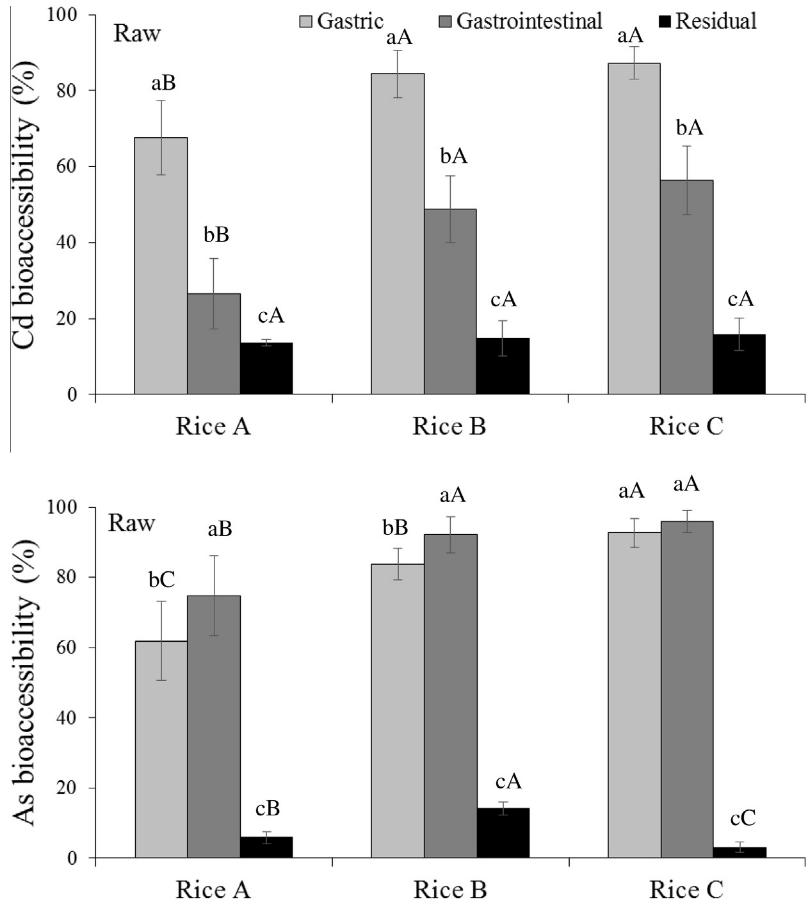


**Fig. 1.** Bioaccessibilities of Cd and As (%, in gastric, gastrointestinal and residual phases) at three contamination levels of raw rice. Lowercase letters indicate significant differences at $p < 0.05$ among the gastric, gastrointestinal and residual phase for each type of rice. Capital letters indicate significant differences at $p < 0.05$ among three types of rice at gastric, gastrointestinal and residual phases.

Intawongse & Dean, 2008; Pelfrêne et al., 2015). This may be attributed to the increase in pH and the addition of organic components such as bile extract and pancreatin in the gastrointestinal phase. At the typical pH level of the gastrointestinal phase, the increase in pH (from 1.5 in the gastric phase to 7.0 in the gastrointestinal phase) might produce precipitation and/or resorption of part of the solubilized Cd (Mounicou, Szpunar, Andrey, Blake, & Lobinski, 2002) or Cd may form insoluble complexes with the phytate that is present in the human diet (Versantvoort et al., 2005). When the concentration of Cd in rice was increased from the lowest level (rice A) to the medium level (rice B), there was a significant increase in Cd bioaccessibility in raw rice; but no difference was observed between the medium level (rice B) and the high level (rice C). It is reasonable to assume that bioaccessibility of Cd in rice is highly dependent on the sample, but the excessive levels of Cd could not be fully released from the rice matric or be converted into the insoluble form. More specifically, the Cd percentage left in the residual fraction was 14–16% of the total amounts in rices A, B and C, which were in the range of values from several vegetables measured by Intawongse and Dean (2008).

Arsenic bioaccessibilities in the gastric phase were 62%, 84% and 93% for raw rices A, B and C, respectively (Fig. 1). The different rice types had significant differences ($p < 0.05$) in bioaccessibility of As. In the literature, bioaccessibility values ranging from approximately 50–100% have been reported for As on rice (Signes-Pastor et al., 2012; Sun, de Wiele, et al., 2012) and 63–99% for inorganic As in rice (Laparra et al., 2005; Signes-Pastor et al., 2012). In raw rice, an increase in As bioaccessibility was observed when comparing gastric fractions with gastrointestinal fractions. This increase in bioaccessibility values was statistically significant ($p < 0.05$) for the gastric and gastrointestinal fractions from rice A and rice B. However, this was different for rice C, with no significant differences between bioaccessibility values in the gastric and gastrointestinal fractions ($p > 0.05$). After 2 h of intestinal digestion, the bioaccessible As fraction significantly increased up to 75% for rice A, 92% for rice B and 96% for rice C (Fig. 1). It is likely that some enzymes from the pancreas and bile contained in the simulated intestinal juices are involved in the breakdown of poly-saccharides into monosaccharide and in the cleavage of denaturalized proteins further into free amino acids and small peptides having a chain length of 2–6 amino acid residues. This makes these compounds more amenable to intestinal absorption (Sun, de Wiele, et al., 2012). These processes could further release the protein-bound As, thereby increasing the As bioaccessibility (Sun, de Wiele, et al., 2012). More specifically, the percentage of As in the gastrointestinal phase is much higher than that of Cd. These findings further indicated that the gastrointestinal phase plays an important role in the solubilization of As during the digestion process. The small concentration of As that remained in the residual fraction was 3–14% of the total As in raw rice, with the highest in rice B.

### 3.2.2. Bioaccessibilities of Cd and As in cooked rice

The oral bioaccessible concentrations of Cd and As measured in the gastric and gastrointestinal fractions, defined by the *in vitro* PBET method, are shown in Fig. 2 for the three contamination levels of cooked rice.

Compared to the data for raw rice, cooking significantly decreased the Cd bioaccessibility in rice B and C with high Cd level

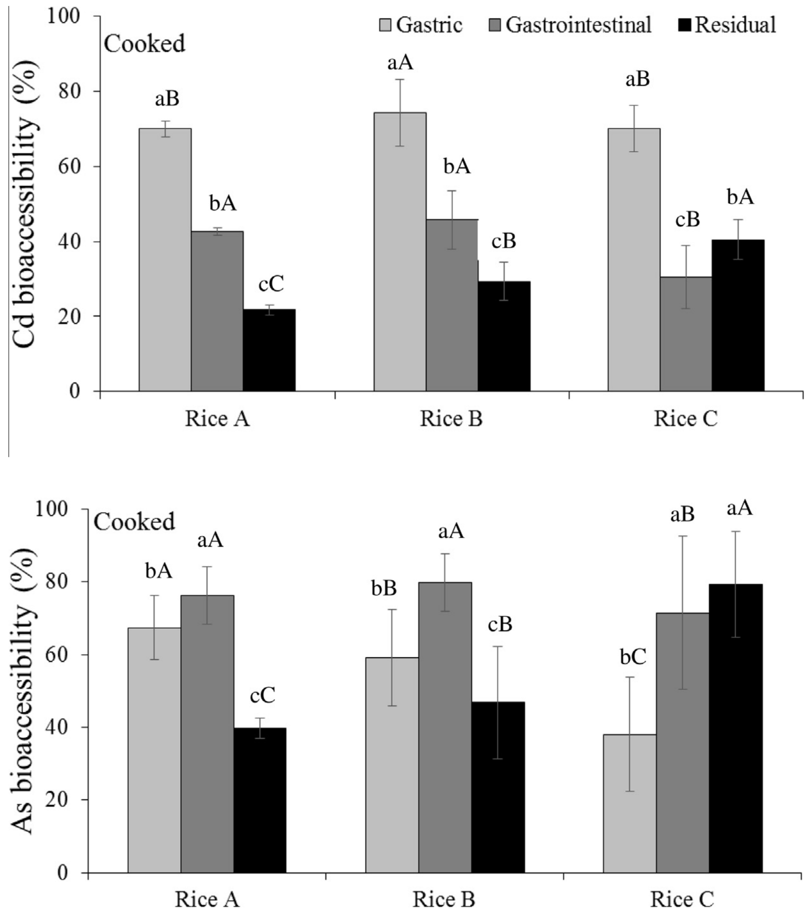


**Fig. 2.** Bioaccessibilities of Cd and As (%, at gastric, gastrointestinal and residual phases) in three contamination levels of cooked rice. Lowercase letters indicate significant differences at $p < 0.05$ among the gastric, gastrointestinal and residual phase for each type of rice. Capital letters indicate significant differences at $p < 0.05$ among three types of rice at gastric, gastrointestinal and residual phases.

in the gastric and gastrointestinal phases, while there was no or slight effect on the bioaccessibility of Cd of rice A with low Cd levels in the three phases. This result was consistent with previous studies showing that cooking significantly decreased the Cd bioaccessibility in various food matrices (Wang et al., 2014), shellfish (Amiard et al., 2008), *Agaricus blazei murill* (Sun, Liu, et al., 2012) and Chilean mussels (Houlbrèque et al., 2011). This may be explained by the fact that the heating process destroyed tissues thoroughly and led to a higher adsorption during the digestion process and lowered the metal bioaccessibility. The present results showed that the cooking treatment widened the gap between bioaccessibility in the gastric and intestinal phases (Fig. 2), which may be related to the fact that heating can reduce plant digestibility (Savoie, Charbonneau, & Parent, 1989) and some functional groups such as lysine, methionine, phenylalanine, histidine and cystine have an affinity for metal ions (Chou & Shen, 2007). The bioaccessibility of Cd in cooked rice varies from 70% to 74% in gastric extraction, and 41% to 46% in the gastrointestinal phase (Fig. 2). Wang et al. (2014) reported similar percentages of Cd bioaccessibility in cooked rice of 74%. Although the total Cd concentration in the three rice types changed drastically, the Cd bioaccessibility percentage remained relatively constant (70–74%).

Arsenic bioaccessibility in three cooked rice types varied from 38% to 67% and 72% to 80% for gastric and gastrointestinal fractions, respectively (Fig. 2). The average As bioaccessibility (ranging between 55% and 71% of two phases) in the three types of cooked rice in our investigation was similar to the values reported by Sun, de Wiele, et al. (2012) and lower than those reported in studies of Laparra et al. (2005) and Signes-Pastor et al. (2012). However, it should be noted that the latter studies focused on As bioaccessibility from rice that was cooked with As-contaminated water. Trenary et al. (2012) reported that the average percentage of As bioaccessibility for 17 rice samples was 61% (range 45–79%) measured using an *in vitro* synthetic gastrointestinal extraction protocol. However, some studies reported that cooking long grain white rice does not affect the bioaccessibility of As (Horner & Beauchemin, 2013).

In the residual fraction, cooking highly increased Cd and As bioaccessibilities in the residual phase; 22%, 29% and 41% for Cd and 40%, 47% and 38% for As in rices A, B and C were recovered, respectively (Fig. 2). In our opinion, this is probably due to the fact that cooking reduces bioaccessibility of Cd and As through binding of metals to other compounds and forming non-bioaccessible complexes in the residual fraction. To date and to the best of our knowledge, there are only a few studies on the residual fraction of metals during the *in vitro* digestion (Intawongse & Dean, 2008), in which it was found that the Cd concentration in the residual phase in several raw vegetables ranged between 16% and 38%. The insoluble residue from cocoa samples after the gastrointestinal phase was further extracted by Mounicou et al. (2002), and an additional 20 or 30% of Cd could be recovered by phytase and cellulase, respectively. Interestingly, it was unexpectedly found that, after the cooking process, bioaccessible As concentration in the residual fraction in rice C were significantly higher ($p < 0.05$) than those in both gastric and gastrointestinal phases. These portion of insoluble Cd or As in the residual fraction might be bound to some total dietary fibre constituents such as microfibres of crystalline cellulose that are difficult to destroy (Mounicou et al., 2002). We supposed that excessive Cd in this *in vitro* method could not be extracted completely by simulated gastric and intestinal juice. It is likely that Cd could bind some components into strong complexes that are insoluble in the gastrointestinal lumen. It is clear, thus, that more researches should be performed on the residual fraction for a better understanding of the metal distribution during the digestion process.

In general, the level of contamination in the food matrix has been considered as one of the factors affecting the bioaccessibility. A dose-proportional relationship between contamination level and bioaccessibility/bioavailability is taken as a basic assumption in risk assessment. In the present study, a positive relationship ($p < 0.05$) was observed between the Cd bioaccessibility in both gastric and gastrointestinal phases and the increasing total Cd concentration in three raw rice types (Fig. 3). High contamination level in raw rice could explain a large proportion of variance in the predicted bioaccessibility levels ($R^2 = 0.98$). A similar relationship has been found between the contamination level and bioaccessibility of Cd in raw rice ($R^2$ ranged 0.91 from 0.95 in the bioaccessible fraction) by Yang et al. (2012) and in vegetables ($R^2$ of 0.99 in the gastric and gastrointestinal phases) by Pelfrêne et al. (2015). More specifically, linear regression in Fig. 3 showed a statistically significant relationship ($p < 0.01$) between rice total As concentration and the percent of bioaccessible As following the gastric and gastrointestinal phases in raw rice, implying that the As bioaccessibility is concentration-dependent.

### 3.3. Bioaccessibility of Cd and As in cooked rice with different water to rice ratio

Little is known about the leaching of Cd and As from cooked rice prior to consumption, especially Cd. In general, there are two preparation methods used for cooking rice: (1) rice is cooked with excessive water and the water remaining is discarded; (2) rice is cooked with aliquots of water in order to absorb it all. The bioaccessibility of Cd and As in cooked rice with three different water to rice ratios (2:1, 4:1 and 6:1) are presented in Fig. 4.

There were no significant differences among the Cd bioaccessibilities in all cooked rice types. The bioaccessibilities of Cd were reduced to a mean of 46–61% of total Cd at all different levels of cooked rice (Fig. 4). This showed that the variations recorded among low, medium and high volume cooking were > 5% in Cd bioaccessibility for the three types of rice. These results indicate that a very low percentage of Cd could be leached into the boiling water during the cooking treatment and abundant cooking water tended not to change bioaccessible Cd in different contamination levels of rice. To date, no previous data on Cd bioaccessibility reduction in different contamination levels of rice subjected to cooking treatments have been reported in the literature; therefore, the results obtained in this study cannot be compared.

It was found in the present study that cooking rice in a large volume of water (water:rice 6:1) had the greatest effect in reducing As levels in cooked rice. The trends of average bioaccessibility

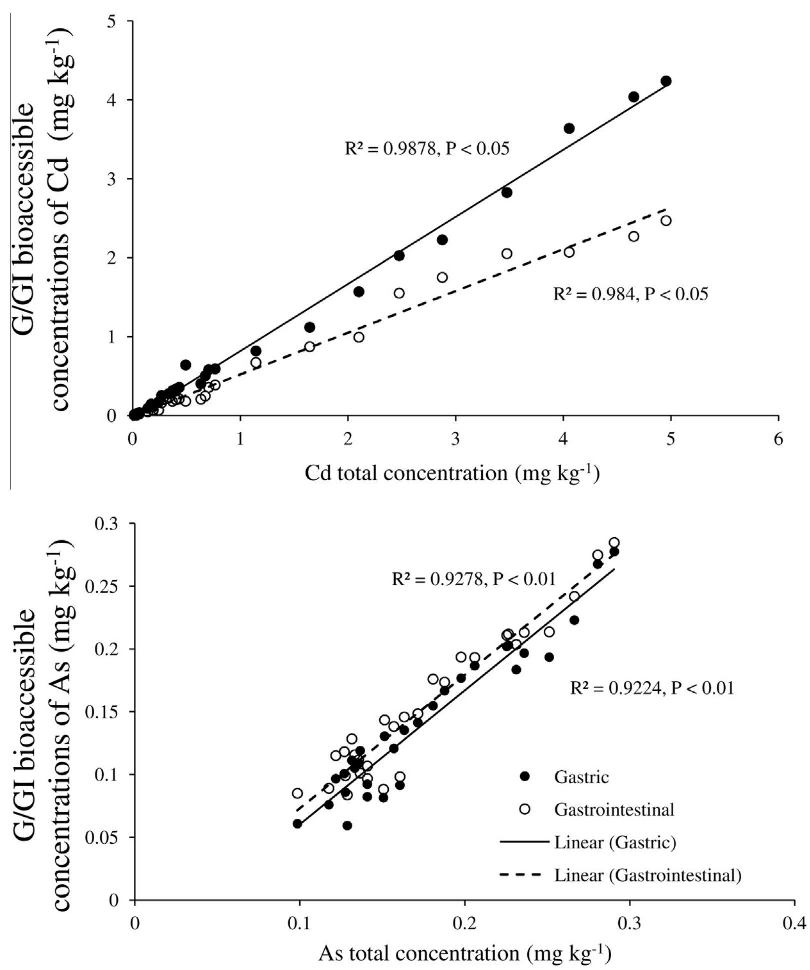


**Fig. 3.** Relationship between oral bioaccessibility and total concentrations (mg $kg^{-1}$) of Cd and As in selected rice samples.

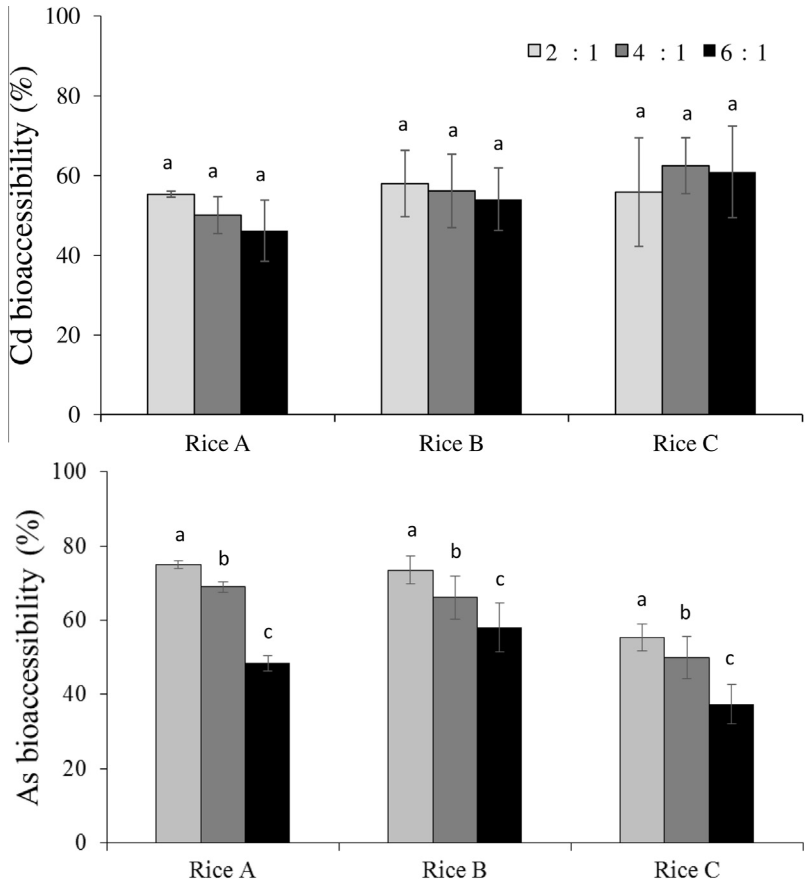


**Fig. 4.** The average bioaccessibilities of Cd and As in the digestion phases (means and SD expressed as a % of total Cd concentration) in cooked rice. Low, medium and high volume cookings are water:rice 2:1, 4:1 and 6:1, respectively. Different letters indicate significant differences at $p < 0.05$ among the three types of water volume for each type of rice.

of As in cooked rice A, B and C were in order of 2:1 > 4:1 > 6:1. Cooking rice to dryness in a 2:1 water:rice ratio, for all contamination levels of rice, resulted in a smaller loss of Cd and As from the all cooked rice, compared to that when using larger volumes of cooking water. Several studies also noted that when rice was cooked in a low volume (no water to discard), the As concentration of cooked rice did not change significantly with respect to the raw rice (Naito, Matsumoto, Shindoh, & Nishimura, 2015; Raab, Baskaran, Feldmann, & Meharg, 2009; Sengupta et al., 2006). Laparra et al. (2005) reported similar results indicating that no washing and medium volume (water:rice 4:1) cooking (until no water to discard) did not remove total As in brown and white rice. The bioaccessibility of As was reduced to a mean of 37–58% of the total from all the different levels of cooked rice by high-volume (water:rice 6:1) cooking, followed by discarding excess water (Fig. 4). Significant differences ($p < 0.05$) were found among As bioaccessibilities in 2:1, 4:1 and 6:1 water:rice ratios for the three types of rice. Compared with the results reported by Raab et al. (2009), cooking rice with a high volume (water:rice 6:1) did effectively reduce total As by 65% (ranging from 55% to 72%) of raw rice concentration, while Sengupta et al. (2006) reported that, around 57% of total As was removed when cooking with a water to rice ratio of 6:1, followed by discarding excess water. Since metal(loid)s are not evaporated or broken down to safer compounds during boiling, frying or other cooking processes, they only transfer from food matrix to the frying oil, boiling water or cooking stocks. Therefore, to reduce As concentration of cooked rice, large volumes of cooking water are effective (Raab et al., 2009).

### 3.4. Health risk assessment of Cd and As in rice

The average bioaccessible EDIs and THQs of Cd and As for adults and children via consumption of rices A and B are presented in Table 2. Based on the bioaccessibility data, the EDI values of Cd and As from rice A for adults and children were 0.34 and 0.53 $\mu g\ kg^{-1}\ day^{-1}$, and 0.44 and 0.70 $\mu g\ kg^{-1}\ day^{-1}$, respectively, which were well below the provisional tolerable daily intakes

**Table 2**
The total and bioaccessible value of estimated daily intake (EDI) and target hazard quotient (THQ) of Cd and As from consumption of cooked rice A and B samples analyzed ($\mu g\ kg^{-1}\ day^{-1}$).

| Items | Metals | EDI | | THQ | |
|---|---|---|---|---|---|
| | | Adult | Children | Adult | Children |
| *When not considering bioaccessibility* | | | | | |
| Rice A | Cd | 0.68 | 0.89 | 0.68 | 0.89 |
| | As | 0.74 | 0.97 | 2.46 | 3.24 |
| Rice B | Cd | 2.94 | 3.86 | 2.94 | 3.86 |
| | As | 1.07 | 1.41 | 3.57 | 4.69 |
| *When considering bioaccessibility* | | | | | |
| Rice A | Cd | 0.34 | 0.44 | 0.34 | 0.44 |
| | As | 0.53 | 0.70 | 1.77 | 2.33 |
| Rice B | Cd | 1.76 | 2.32 | 1.76 | 2.32 |
| | As | 0.74 | 1.37 | 2.48 | 4.58 |

The Food and Agriculture Organization/World Health Organization (FAO/WHO) (2010) recommended that the previously established provisional tolerable weekly intake (PTWI) of 15 $\mu g\ kg^{-1}$ body weight (equivalent to 2.1 $\mu g\ kg^{-1}$ body weight $day^{-1}$) for inorganic As; For Cd, the previously established provisional tolerable monthly intake (PTMI) is 25 $\mu g\ kg^{-1}$ body weight (equivalent to 0.83 $\mu g\ kg^{-1}$ body weight $day^{-1}$).

(PTDI, 0.83 μg day$^{-1}$ kg$^{-1}$ BW for Cd and 2.1 μg day$^{-1}$ kg$^{-1}$ BW for As, JECFA). For the daily average consumption of Rice B, the EDI values of Cd for adults and children were 212% and 280% of the PTDI value, respectively, while the EDIs of As were lower than the recommended PTDI. Such results indicated that the long-term large consumption of rice B produced from the mining area would result in a high exposure to Cd. The fact that cooked rice showed higher Cd and As bioaccessibility is of particular concern since this type of rice is the most commonly consumed by people in China.

As shown in Table 2, except for the THQs of Cd for rice A, the bioaccessible THQs of Cd and As from rices A and B were all more than 1, which indicated that inhabitants around the mining area were experiencing relatively high health risks. In China, cadmium- or arsenic-contaminated rice, produced in the polluted soils in the vicinity of mining areas, were sold on the market, resulting in great human health risk for the rice consumers (Sun, de Wiele, et al., 2012; Zhuang et al., 2009). The EDI and THQ values of Cd and As for children were higher than those for adults, thus the high exposure to metal(loid)s may raise some concerns about consumption by children. Given the situation of contaminated rice, the health risk of Cd and As poisoning is greatest for people who eat rice several times a day, but eating less rice is not an option in many parts of the world where the food is an irreplaceable part of the culture, diet and lifestyle.

It should be noted that absorption of Cd and As during the digestion process would be highly variable in human populations because the metal(loid) bioaccessibility of rice is influenced by many factors (Intawongse & Dean, 2008; Mounicou et al., 2002; Sun, de Wiele, et al., 2012), including nutritional characteristics, gastrointestinal tract contents, microfibres of crystalline cellulose and phytates, microbial processes, metal species, speciation and food processing method. In the present study, the digestive processes in the mouth (with simulated saliva) are neglected but may cause overestimation of Cd and As bioaccessibility from rice because rice is a highly amylaceous food. Meanwhile, the effects of microbiota on digested foods should be included in further in-depth studies so as to ensure more accurate Cd and As risk assessment.

## 4. Conclusions

This study focused on the effects of cooking on the bioaccessibility of Cd and As at three contamination levels of rice. The results indicated that cooking reduced bio-accessibility of Cd and As in rice, in contrast to a significant increase of Cd and As concentrations in the residual fraction. The results indicated that Cd bioaccessibility was higher in the gastric phase than the gastrointestinal phase, while As bioaccessibility was higher in the gastrointestinal phase than the gastric phase in both raw and cooked rice. Application of a low volume of water during cooking to dryness of rice was able to remove total Cd by about 10% for rices A and B; on the other hand, irrespective of rice type, use of medium or high volumes of water did not show any effect on Cd bioaccessibility. By contrast, use of low volume water in cooking did not remove As, but a significant decrease of As was observed when cooking with medium or high volume of water. Based on the bioaccessibility results, the established daily intake of Cd from rice B by adults and children exceeded the toxicological reference values established by the FAO/WHO. This study provides information for a better understanding of more realistic estimation of metal(loid)s exposure from rice and the possible health risks.

## Conflict of interest statement

The authors declare that no conflict of interest affects this work.

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