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Assessment of the effect of cooking on speciation and bioaccessibility/cellular uptake of arsenic in rice, using *in vitro* digestion and Caco-2 and PSI cells as model

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ABSTRACT

In vitro digestion/Caco-2 or pig small intestinal epithelium cell line (PSI) uptake models were used to study the bioaccessibility and cellular uptake of arsenic (As) in cooked white rice and brown rice. The arsenite(AsIII), was the predominant species in cooked rice and in its bioaccessible fractions. The percentage of total As bioaccessibility in white rice (75%) was slightly higher ($p = 0.061$) than that in brown rice(66%). However, there was no difference in the inorganic As (iAs) bioaccessibility between white rice (95%) and brown rice (96%). In Caco-2 cell monolayer, total As retention was 7-31%, transport was 4-25%, and uptake (sum of retention and transport) was 16-38%. In PSI cell model, the retention, transport, and uptake of tAs were 10-28%, 14-31%, and 29-50%, respectively. In both cells, the cellular uptake of tAs in brown rice was 1.4-1.5 folds lower ($p < 0.05$) than that of white rice. These results indicate that the cellular uptake of As can be affected by nutritional compositions. These in vitro screening methods can serve as preliminary screens to predict the relative impact in rice matrix having different As species and processing conditions, although more research efforts should be applied to validating the existing *in vitro* methods

1. Introduction

Arsenic (As) is widely known to cause harm to humans and animals (Manju et al., 1998). According to the International Agency for Research on Cancer, As and As compounds are classified as carcinogenic for humans (Group 1), (Rousseau et al., 2005). Arsenic exists in various chemical forms, in which inorganic As (iAs) species containing arsenite (AsIII) and arsenate (AsV) are far more toxic than pentavalent organic variants, and AsIII is much more poisonous than AsV (Edmonds and Francesconi, 1993). Previous research has reported that iAs is detrimental to the urinary bladder, lungs, and skin (Rousseau et al., 2005). However, monomethylarsonous acid (MMA^{III}), an intermediate in iAs methylation, is known to be more toxic (Petrick et al., 2000; Styblo et al., 2000). In their cytotoxicity assays using LDH, K^+, and XTT, the following order of toxicity in Chang human hepatocytes was reported: MMA^{III} > AsIII > AsV > MMA^{V} = DMA^{V} (Petrick et al., 2000). On the other hand, another paper showed that dimethylmonothioarsinic acid ($DMMTA^{V}$) may be one of the most toxicologically potent arsenic species (Naranmandura et al., 2011).

Regarding human health, drinking-water and crops irrigated with contaminated water have been recognized as major sources of As exposure, and rice and rice-based foods have been considered as leading dietary sources of iAs exposure because these foods contain relatively high levels of iAs species (Meharg et al., 2008; Signes-Pastor et al., 2009). Therefore, it is necessary to determine the bioaccessibility and cellular uptake of As to predict the possible impact on human health from ingesting rice contaminated with different As species. As such, cooked rice is more suitable to estimate the cellular uptake of As because it reflects real habits for dietary intake.

Bioaccessibility is defined as the fraction of contaminant that is discharged from food into the digestive juice chyme and has the potential to be absorbed by the small intestine during digestion (Fu and Cui, 2013). Percentage solubility is calculated as the amount of soluble compound relative to the total amount of As in the test samples. In recent years, various *in vitro* digestion models have been proposed and are generally used to estimate bioaccessibility due to their lower cost, time, ease of control, energy savings, and independence from physiological effects. Additionally, these allow for better reproducibility and can thus provide a cost-effective approximation of the *in vivo* situation (Etcheverry et al., 2012). A method proposed by the Netherlands National Institute for Public Health and the Environment (RIVM) has been used to assess the bioaccessibility of heavy metals in soil and food

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materials and the bioaccessibility of mycotoxins and steryl ferulates (Mandak and Nyström, 2012; Oomen et al., 2003; Versantvoort et al., 2005).

In the human epithelial cell line Caco-2, cells are originally derived from a colon carcinoma, and Caco-2 has been widely used as an *in vitro* model of the intestinal epithelial barrier for transport and retention studies of various substances (Balimane et al., 2000). This characterized cell line represents the morphological and biochemical characteristics of enterocytes (polarity, tight junctions, specific transport systems, and enzymes) after differentiation in culture (Hidalgo et al., 1989; Rousset, 1986). However, the tight junctions of the Caco-2 cells are tighter than those in the small intestine, which may lessen the permeability of drugs or compounds with significant paracellular absorption (Lennernäs et al., 1996; Saitoh et al., 2004; Takenaka et al., 2014). Due to the lower cellular uptake of the Caco-2 cell model compared to that of *in vivo* models, small intestinal epithelial cells may represent a more appropriate *in vitro* cell model for assessing the small intestinal epithelial barrier. In this study, pig small intestinal epithelium cell line (PSI) cells (kindly provided by Professor Dr. Wilhelm Holzapfel, Handong University, Republic of Koreas) were also used to investigate the bioaccessibility and cellular uptake of total As and iAs in cooked rice. The PSI cells are normal mature small intestinal epithelial cells, and they have non-tumor and untransformed features (Trapecar and Cencic, 2012).

A few studies have been conducted to estimate the bioaccessibility and cellular uptake of As in cooked rice employing the Caco-2 cell model (Laparra et al., 2005a, 2005b). Data, which are still lacking in speciation analysis of a gastric and gastric-intestinal solution with white and brown rice, were collected in our study. Additionally, *in vitro* digestion was coupled with the Caco-2 or PSI cell model to assess bioaccessibility/uptake of total and different As species in cooked white and brown rice, in which the water used for cooking the rice was not spiked with iAs, and both AsIII and AsV were analyzed. To the best of our knowledge, no study has assessed the cellular uptake of As derived from cooked rice by small intestinal cells. With cooked white rice and brown rice grains, the following aspects were determined: i) their total As level, ii) Arsenic speciation in cooked rice via high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) analysis, iii) Arsenic bioaccessibility for dietary exposure in rice after simulating human *in vitro* digestion using an RIVM model, iv) Arsenic speciation present in bioaccessible fractions, and v) cellular uptake of As in bioaccessible fractions using the Caco-2 and PSI cell models.

2. Materials and methods

2.1. Samples and cooking procedure

The two types of rice (short-grained) used in this study were domestic white rice (polished rice, n = 5) and brown rice (unpolished or husked rice, n = 5). Approximately 50 g of the raw rice sample were simply washed three times with an equal weight of deionized water at 20 °C. The washed rice was then cooked with 100 mL of deionized water (1:2 ratio) for white rice and 150 mL deionized water (1:3) for brown rice, according to the corresponding cooking methods (Omar et al., 2015). The cooked samples were lyophilized and milled to a fine powder using a grinder (NSG-1002SS, Hanil Electric, Seoul, Korea).

2.2. Reagents and certified reference materials

All solutions used in this study were prepared using ultrapure deionized water obtained from a YL WPS System (Young Lin Instrument Co., Ltd., Gyeonggi, Korea). Enzymes, including α-amylase, pepsin, pancreatin, mucin, lipase, bovine serum albumin, and organic solutions were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of the analytical or ultrapure grade.

Standard solutions of As were used to determine the total As. The reagents used in the As speciation analysis were as follows: sodium arsenite (Fisher Scientific, Massachusetts, USA), sodium arsenate dibasic heptahydrate and cacodylic acid (Sigma-Aldrich, St. Louis, USA), and disodium methyl arsonate hexahydrate (Chemservice, Pittsburgh, USA). External calibration standards were prepared daily from standard stock solutions. The calibration was in the range of 0.25–10 μg/L for each As species, and this standard solution was checked every 15 samples. The results were in agreement within ± 10% of the original value. The quality control procedure was carried out using certified reference material (CRM). The CRM was white rice flour NIST SRM 1568b (National Institute of Standards and Technology, Gaithersburg, USA). The certified mass fraction values for arsenic species in SRM 1568b were as follows: DMA (0.180 ± 0.012 mg/kg, as As), MMA (0.0116 ± 0.0035 mg/kg, as As), and inorganic As (0.092 ± 0.010 mg/kg, as As).

2.3. In vitro digestion method

The bioaccessibility of As in the cooked rice samples was estimated by applying the *in vitro* digestion model suggested in the RIVM method (Versantvoort et al., 2004) with a slight modification. Digestive juices were prepared according to reported methods (Oomen et al., 2003; Wei et al., 2012) (Supplementary Table 1). Cooked white rice and brown rice were artificially digested with four different digestive juices including saliva, gastric juice, duodenal juice, and bile. The digestive procedure was performed in triplicate and was composed of three steps for the mouth, stomach, and intestine. Food digestion and absorption of compounds occur mostly in the small intestine, so the large intestinal tract was not considered (Versantvoort et al., 2005). The digestive juices were prepared by adding enzymes and proteins into the solutions and adjusting the corresponding pH. Potassium thiocyanate is known as a toxic compound and complexing agent, so it was excluded from our experiment (Mandak and Nyström, 2012). A schematic representation of the simulated *in vitro* digestion model is presented in Fig. 1. Briefly, the prepared cooked rice samples were subjected to simulated gastric digestion by incubating them in 4 mL of saliva juice for 5 min in a 55-rpm shaking incubator at 36.5 ± 0.5 °C. Subsequently, 8 mL of gastric juice were added, and then the mixture was incubated for 2 h to obtain the gastric (G) fraction. Finally, 8 mL of duodenal juice and 4 mL of bile were simultaneously added. After that, the mixture was rotated for another 2 h. The gastrointestinal (GI) fraction was heated for 15 min at 90 °C to inactivate the digestive enzymes. The digestion tubes of G and GI fractions were then centrifuged for 15 min at 3000 *g*, yielding the chyme. The supernatants were filtered through nylon filters (SmartPor, 0.22 μm pore size, Port Washington, USA) before performing the analysis, and these samples were analyzed for total As and inorganic As

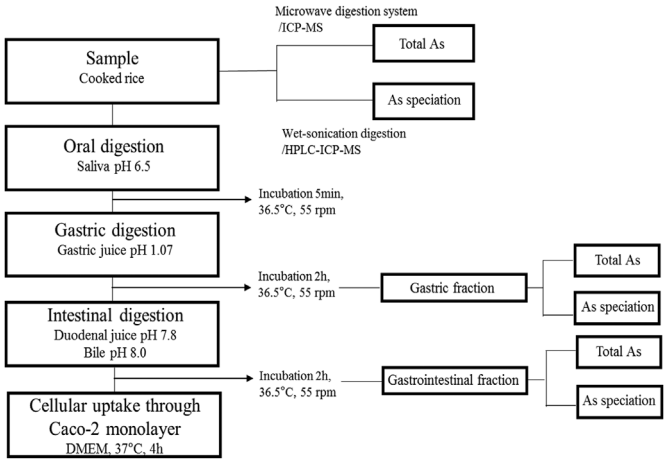

Fig. 1. Schematic representation of *in vitro* digestion methods for arsenic (As) bioaccessibility determination.