Analytical Methods

Extraction and determination of arsenic species in leafy vegetables: Method development and application

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ABSTRACT

Extraction of arsenic (As) species in leafy vegetables was investigated by different combinations of methods and extractants. The extracted As species were separated and determined by HPLC-ICP-MS method. The microwave assisted method using 1% HNO_3 as the extractant exhibited satisfactory efficiency (>90%) at 90 °C for 1.5 h. The proposed method was applied for extracting As species from real leafy vegetables. Thirteen cultivars of leafy vegetables were collected and analyzed. The predominant species in all the investigated vegetable samples were As(III) and As(V). Moreover, both As(III) and As(V) concentrations were positive significant ($p < 0.01$) correlated with total As (tAs) concentration. However, the percentage of As(V) reduced with tAs concentration increasing probably due to the conversion and transformation of As(V) to As(III) after uptake. The hazard quotient results indicated no particular risk to 94.6% of local consumers. Considerably carcinogenic risk by consumption of the leafy vegetables was observed.

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1. Introduction

Arsenic (As) considered as one of the most dangerous carcinogens is ubiquitous in natural environment. There are two main sources of As: natural occurrence and anthropogenic contamination, the latter are mostly originated from mining activities, fertilizer application, arsenical pesticide usage and fossil fuel combustion (Mandal & Suzuki, 2002). In the last decades, the environment of China has been seriously polluted by As contaminations with rapid urbanization and industrialization. The survey of agricultural soils in China showed that nearly 10% of the study areas have been considerably polluted by As and heavy metals (Chen et al., 2008; Li, Wang, Allinson, Li, & Xiong, 2009). Elevated As concentration in agricultural soils may inevitably reduce crop yield and finally threaten human health. Vegetables as an important component of human diet could take up As from contaminated agriculture soil and accumulate it in their edible parts (Sipter, Rózsa, Gruiz, Tátrai, & Morvai, 2008) and also inedible parts. Ingestion of vegetables became a main route of As exposure for human beings. Long-time excessive intake of As might result in serious health risk to human and induce cancers including skin, lung and kidney (Chen, Chen, Wu, & Kuo, 1992). The Chinese maximum contaminant levels (CMCLs) of total As in fresh vegetables

regulated by China Food Standard Agency is 0.5 mg/kg (China Food Standard Agency., 2012).

The toxicity and bioavailability of As in vegetables is highly dependent on its chemical speciation. The predominant As species found in vegetables and terrestrial plants were arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Amaral, Nobrega, & Nogueira, 2013; Rehman et al., 2016). Also arsenobetaine (AsB) and arsenocholine (AsC) were occasionally detected (Mihucz et al., 2007). As(V) transfer from soil to plants was shown to share the common pathway of phosphate due to their analogous molecular structures (Zhao, Ma, Meharg, & McGrath, 2009). As(III) as well as MMA and DMA were described to be taken up probably through aquaporin channels (Li et al., 2009; Ma et al., 2007). Compared to organic forms, inorganic As (iAs) including As(III) and As(V) are more toxic and mobile. However, MMA and DMA considered as the metabolites of iAs have been identified to be possible promoters of cancer (Kenyon & Hughes, 2001). Therefore, separation and identification of As species in vegetables are important for investigation of their accumulation and metabolism.

Extraction is a critical step in As speciation studies due to the difficulty in extracting As from complex matrices without altering their species (Ma, Wang, Tang, & Yang, 2016; Zhao, Li, Xu, Luo, & Ma, 2015). There are two main requirements for the extraction procedure: satisfy acceptable extraction efficiency and preserve the integrity of original As species. In order to achieve these objectives, various extractants, such as water, water/methanol mixture,

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phosphate buffer, hydrochloric acid (HCl), trifluoroacetic acid (TFA), phosphoric acid (H_3PO_4) and nitric acid (HNO_3), have been employed for As extraction from vegetables and terrestrial plants by physical shaking, sonication or microwave assisted method (Amaral et al., 2013; Quaghebeur, Rengel, & Smirk, 2003; Schmidt, Reisser, Mattusch, Popp, & Wennrich, 2000). The diversity in the methods indicates that there is no consensus between authors about the best conditions to extract As species from plants. The extraction efficiency depended on the predominant As species in the sample matrix. Although mixtures of water/methanol are effective to extract organic As from marine tissues (Whaley-Martin, Koch, & Reimer, 2012), the extraction efficiency of iAs substantially decreased (Amaral et al., 2013). Consequently, it is not appropriate to apply the mixtures in As extraction from vegetables of which the predominant species are iAs. Acid extractants were widely used for As extraction from plants due to the ability of these solvents to break the strong bond between As(III) and thiol groups in cell walls, lipids, lignin or insoluble cellulose (Munoz, Velez, & Montoro, 1999). Relatively high extraction efficiency was obtained through introducing TFA or HCl as extractant, whereas interconversion between As(III) and As(V) occurred during the extraction procedure (Abedin, Cresser, Meharg, Feldmann, & Cotter-Howells, 2002; Munoz et al., 1999). Phosphoric acid at different concentrations was also popular in extracting As species from biological and environmental matrices (Larios, Fernandez-Martinez, LeHecho, & Rucandio, 2012; Orero Iserte, Roig-Navarro, & Hernández, 2004). However, the distortion of chromatographic peaks was observed when the concentration of H_3PO_4 present in the extracted solution was greater than 0.25 M (Ellwood & Maher, 2003). Phosphate buffer with similar chemical properties to As(V) was applied to extract As species from plants and environmental solids (Lomax et al., 2012; Sun, Ma, Yang, Lee, & Wang, 2015). This extractant could provide a mild extraction condition to prevent interconversion among As species. Furthermore, the extract solution of 1% HNO_3 has been proved successfully in As species extraction from plant tissue samples including Chinese herbal medicine, rice flour and shoots (Liu et al., 2013; Zhu et al., 2008).

Extraction of As species in terrestrial plant tissues have been investigated in many reports (Mir et al., 2007; Quaghebeur et al., 2003; Schmidt et al., 2000; Zhao et al., 2015). However, few studies focused on As speciation in leafy vegetables. The goal of the present study was to develop a simple and effective method to extract As species from leafy vegetable samples. Considering the advantages of various extractants and methods, water, phosphate buffer and HNO_3 were employed as extractants using shaking, sonication or microwave assisted method to evaluate the extraction efficiency and species integrity. The As speciation was performed in different cultivars of leafy vegetables taken from markets using the optimized method followed by HPLC-ICP-MS method. The potential health risk to consumers from exposure to iAs in vegetables was also estimated.

2. Materials and methods

2.1. Chemicals and standards

The 1000 mg/L stock solutions of As(III) and As(V) were obtained from O2SI smart solutions (Charleston, USA). Stock solutions of MMA and DMA were prepared by dissolving methylarsonic acid mono sodium salt hydrate and cacodylic acid sodium salt (Dr. Ehrenstorfer, Ausberg, Germany) in ultrapure deionized water (18.2 MΩ cm, Direct-Q 3, Millipore SAS, France), respectively. Stock solutions of AsB and AsC were diluted from concentrated corresponding standard solutions (CRM/RM information center, Beijing, China) in deionized water, respectively. All the stock solutions

were kept at 4 °C. The intermediate stock solutions and working solutions were prepared freshly by dilutions of corresponding stock solutions in deionized water at room temperature. Nitric acid (HNO_3, 70% wt, ⩾99.999% metals basis), ammonium hydroxide ($NH_3 \cdot H_2O$, 28%, ⩾99.999% metals basis), hydrogen peroxide (H_2O_2, 30% wt, guaranteed reagent grade), ammonium bicarbonate (NH_4HCO_3, ⩾99.995% metals basis) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$, ⩾99.99% metals basis) were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Certified reference material (CRM) GBW10049 green onion obtained from CRM/RM information center (Beijing, China) was employed to evaluate extraction efficiency. The reference total As value in GBW10049 is 0.52 ± 0.11 mg/kg.

2.2. Sample collection and preparation

Thirteen leafy vegetable cultivars including leaf lettuce (*Lactuca sativa* var. *longifolia* Lam.), spring onion (*Allium fistulosum* L.), celery (*Apium graveolens*), bok choy (*Brassica rapa* subsp. *chinensis*), napa cabbage (*Brassica rapa* subsp. *pekinensis*), coriander (*Coriandrum sativum* L.), lettuce (*Lactuca sativa*), purple-stem mustard (*Brassica campestris* L. var. *purpurea* Bailey), spinach (*Spinacia oleracea*), garlic chives (*Allium tuberosum*), rapini (*Brassica rapa* var. *rapifera*), turnip green (*Brassica rapa* subsp. *rapa*) and choy sum (*Brassica rapa* subsp. *Parachinensis*) were purchased from four markets in Changsha City, located in Southern China. The detail of sampling information was listed in Table S1. The collected samples were washed thoroughly by tap water and then rinsed three times by deionized water to remove dust. After dried at 80 °C until constant weight, the samples were grounded with an agate mortar and passed through a 0.20 mm sieve to obtain uniform size. All homogenized sample powders were stored in sealed polyethylene bags and preserved in a desiccator before analysis.

2.3. Comparison of extraction methods

Three common extraction methods, shaking, sonication and microwave, were introduced to assist As extraction in this study. Three common extractants including ultrapure water, ammonium phosphate buffer and diluted nitric acid were compared in extracting As species from CRM GBW10049. The extraction efficiency was calculated by the ratio between extracted As concentration and digested total As concentration.

2.3.1. Shaking extraction

Approximately 0.5 g of sample powders were weighed into a 50 mL polyethylene centrifuge tube with addition of 20 mL extractant. In current study, ultrapure water, ammonium phosphate buffer (PB, 80 mM, pH = 7.0) and 1% HNO_3 have been employed as extractants in the shaking method. The covered tube was shaken in water bath, and then centrifuged at 9000 rpm for 30 min. The collected supernatant was filtered through a 0.22 μm cellulose acetate membrane and stored at 4 °C until analysis.

2.3.2. Ultrasonic extraction

Approximately 0.5 g of sample powders were weighed into a 50 mL polyethylene centrifuge tube and 20 mL of 1% HNO_3 was added to extract As species under sonication at room temperature (25 °C) for 2 h. After centrifuged at 9000 rpm for 30 min, the supernatant was filtered through a 0.22 μm cellulose acetate membrane and then stored at 4 °C until analysis.

2.3.3. Microwave assisted extraction

Approximately 0.5 g of sample powders were weighed into a polytetrafluoroethylene (PTFE) vessel and 20 mL of extractant (ultrapure water, PB or 1% HNO_3) was added for the microwave