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Analytical Methods

Speciation analysis of six arsenic species in marketed shellfish: Extraction optimization and health risk assessment

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ABSTRACT

A comparative study between microwave assisted and sonication methods was carried out to extract arsenic (As) species in shellfish samples using different extractants. Six As species including arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC) were simultaneously separated and determined by the HPLC–ICP-MS method. The microwave assisted method exhibited higher efficiency than sonication, especially using diluted HNO_3 as extractant. By compromising extraction efficiency, pretreatment time and stability of As species, the microwave assisted method using 1% HNO_3 at 100 °C for 1.5 h was selected to extract As from real samples. The proposed method has been applied to extract and determine As species in shellfish samples. The result of correlation analysis indicated that the proportion of AsB in the shellfish samples was decreased with total As concentration increasing due to the biotransformation threshold from inorganic As to AsB.

1. Introduction

Arsenic (As) is a ubiquitous toxic element present in soils, water, plants and living organisms. This element is originated from two main sources: natural processes and anthropogenic activities (Escudero, Martinis, Olsina, & Wuilloud, 2013). In the last decades, the environment of China has been severely polluted by As contaminations with rapid urbanization and industrialization (Jia, Kong, Yang, & Wang, 2016; Ma, Yang, Li, & Wang, 2016). There were many studies concerning about As contamination in marine organisms, especially in shellfish due to their ability to accumulate As from surrounding environment up to considerable level (Whaley-Martin, Koch, & Reimer, 2013; Zhang, Wang, & Zhang, 2013). The As contamination can be bioaccumulated and bio-magnified through food chain from low trophic level to higher one, causing significant effects on the aquatic ecosystem. Moreover, as part of food consumption for humans, the contaminated seafood may increase the ingestion of As and pose potential health risk to the consumers. Therefore, exposure to As from seafood consumption attracts more and more attention recently.

The biotoxicity and bioavailability of As are depended on its chemical species. More than 50 species of As have been identified in marine organisms (Nischwitz & Pergantis, 2006; Zmozinski, Llorente-Mirandes, Lopez-Sanchez, & da Silva, 2015). Among them, inorganic species including arsenite [As(III)] and arsenate [As(V)] are considered to be the most toxic forms, which are categorized to a class 1 non-threshold carcinogen (Tchounwou, Centeno, & Patlolla, 2004). Compared to inorganic As (iAs), the organic species such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC) are far less toxic to marine organisms and humans. In uncontaminated aquatic environments, the predominant As species observed in marine organisms is AsB, one of the non-toxic arsenicals (Mato-Fernandez et al., 2007). Other organic As such as AsC, MMA and DMA were detected in minor concentrations (Whaley-Martin et al., 2013; Zmozinski et al., 2015). However, the concentrations of iAs found in seafood spread in a wider range (de la Calle et al., 2012). Normally, higher level of iAs was observed in the contaminated marine organisms (Lorenzana, Yeow, Colman, Chappell, & Choudhury, 2009). Therefore, extraction and identification of As species in marine organisms are important for further risk assessment (Chen, Gu, Yang, Sun, & Wang, 2013).

Extraction of As species from seafood without altering their chemical forms is critical for As speciation. The extraction efficiency depends on various parameters including sample matrix, solvent type, species to be extracted, extraction time, and temperature (Santos et al., 2013). Although the As extraction method from seafood has been investigated widely, there is still no consensus between authors about the

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most available method. Due to As species of different polarity, various extraction solvents including diluted acids, water, methanol and methanol-water mixture have been employed for As extraction from biological samples using ultrasonic or microwave-assisted method (Moreda-Pineiro et al., 2011; Petursdottir, Gunnlaugsdottir, Krupp, & Feldmann, 2014; Santos et al., 2013). Since most of the As species present in biological tissues are soluble in water, they can be extracted with water alone or with a mixture of water and methanol. However, the extraction efficiency decreased when inorganic As increased in the samples collected from contaminated sites (Whaley-Martin, Koch, Moriarty, & Reimer, 2012; Whaley-Martin et al., 2013). Low concentrated acid or base was introduced to improve extraction efficiency of inorganic As, where diluted acid exhibited higher extracted As concentration than base (Entwisle & Hearn, 2006). Moreover, a two-step method with combination of water-methanol mixture and diluted nitric acid was developed to extract As from contaminated marine snails (Foster, Maher, Krikowa, & Apte, 2007; Whaley-Martin, Koch, & Reimer, 2012). Nevertheless, the extraction procedure was complicated and time-consuming which is not suitable for pretreatment of large amount of environmental samples.

Dietary seafood and related products are considered to be the most important source of human exposure to As contamination. Shellfish which have close correlation with sediments can accumulate more As and heavy metal contents than other marine organisms. The Chinese maximum contaminant levels (CMCLs) of inorganic As in shellfish (fresh weight) is regulated to 500 μg/kg by China Food Standard Agency (China Food Standard Agency, 2012). In order to evaluate As exposure risk from shellfish consumption, the actual As species present in shellfish samples should be determined accurately. The goal of this work was to establish a simple and economic method for extraction and identification of As species in shellfish. Three common extractants, diluted nitric acid, water and water-methanol mixture, were investigated in this study due to their capacity to elute As species from various biological samples (Foster et al., 2007; Petursdottir et al., 2014). The parameters including assisted method, temperature and time involved on the pretreatment procedure were optimized through a univariate approach. The As speciation was performed by the HPLC-ICP-MS method to evaluate the extraction efficiency and species integrity. Furthermore, the proposed method was applied in different cultivars of shellfish collected from several markets. The chronic health risk from human exposure to iAs in the shellfish was also estimated.

2. Materials and methods

2.1. Chemical and standards

Ultrapure water (18.2 MΩ cm, Direct-Q 3, Millipore SAS, France) was used in preparation of standards, mobile phase and extraction solutions. Nitric acid (HNO_3, 70%, ≥99.999% metals basis), ammonium hydroxide ($NH_3{\cdot}H_2O$, ≥99.999% metals basis), ammonium bicarbonate (NH_4HCO_3, ≥99.995% metals basis) and hydrogen peroxide (H_2O_2, 30%, guaranteed reagent grade) were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). HPLC grade methanol (MeOH, ≥99.9%) was delivered by Sigma-Aldrich Corp. (USA). Stock solutions of As(III) and As(V) (1000 mg/L As) were purchased from O2SI smart solutions (Charleston, USA). Stock solutions of MMA and DMA were prepared from methylarsonic acid mono sodium salthydrate and cacodylic acid sodium salt (Dr. Ehrenstorfer, Ausberg, Germany), respectively. Stock solutions of AsB and AsC were diluted from concentrated corresponding solutions provided by CRM/RM information center (Beijing, China). The secondary stock solutions and working solutions for further analysis were daily prepared.

2.2. Sample collection and preparation

Four shellfish species including hard clam (*Meretrix meretrix L.*), undulating venus (*Paphia undulate*), manila clam (*Venerupis philippinarum*) and razor clam (*Sinonovacula constrzcta*) were collected from six supermarkets in Changsha City, Southern China. After transported to the laboratory, the samples were washed by tap water. Then the edible parts of shellfish were removed from shells, rinsed by tap water and followed with ultrapure water several times. One composite sample was merged from 8 to 10 subsamples. All the composite samples were lyophilized and then homogenized by grinding with an agate mortar. The fine powders were store at −20 °C until further preparation and analysis.

2.3. Extraction of As species

Three kinds of extractants including water, methanol-water mixture and diluted nitric acid were compared in extracting As species using sonication or microwave assisted method. The detailed information of extractant and method was summarized in Table 1. Certified reference material (CRM) GBW10024 scallop (CRM/RM information center, Beijing, China) was employed to evaluate the extraction efficiency. The reference value of total As in GBW10024 is 3.6 ± 0.6 mg/kg.

2.3.1. Sonication extraction

Approximately 0.2 g of sample powders were weighed into a 50 mL polyethylene centrifuge tube with addition of 12 mL extraction solution. After sealing with a lid, the tube was placed on a vortex apparatus for 1 min to mix the extractant and sample powders thoroughly. The sealed tube was then sonicated at room temperature for 2 h. Methanol was removed by evaporation at 60 °C for 4 h. After centrifuged at 10,000 rpm for 10 min, the supernatant was decanted into a 25 mL volumetric flask and diluted by ultrapure water. The extraction solutions were stored at 4 °C. Prior to analysis, the solutions were filtered through a 0.22 μm cellulose acetate membrane.

2.3.2. Microwave assisted extraction

Approximately 0.2 g of sample powders were weighed into a polytetrafluoroethylene (PTFE) vessel with addition of 12 mL extraction solution. The extraction was performed following the procedure using a microwave digestion system (MDS-6G, Sineo Microwave Chemistry Technology Co. Ltd, Shanghai, China): 5 min to 80 °C, and 1.5 h at 80 °C. After cooled down, the extracted solutions were transferred into a centrifuge tube. Further pretreatment was same as the sonication method.

The extraction efficiency was calculated as the ratio between the sum of extracted As species (sAs) concentrations and the certified tAs value. The microwave assisted method using diluted nitric as extractant exhibited higher extraction efficiency than other combinations. Therefore, further optimization was performed to determine the best extraction conditions such as extraction time, temperature and concentration of HNO_3 in the selected method.

2.4. Arsenic speciation by HPLC-ICP-MS

The determination of As species in the extracted solutions were performed by the HPLC-ICP-MS method developed in our previous study. The detailed operating parameters for HPLC (Agilent 1260, Tokyo, Japan) and ICP-MS (Agilent 7700x, Tokyo, Japan) are listed in Table S1. Six As species including As(III), As(V), MMA, DMA, AsB and AsC were simultaneously separated in one single column run. The polyatomic interference (e.g. $^{40}Ar^{35}Cl^+$ at m/z 75) was eliminated by a collision/reaction cell (Sun, Yang, Lee, & Wang, 2015).

2.5. Digestion and determination of total arsenic

Approximately 0.5 g of sample powders were weighed and transferred into a PTFE vessel. The mixture of HNO_3 and H_2O_2 (5:1, v/v) was employed to dissolve shellfish samples through microwave digestion.