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Analytical Methods

Sequential extraction combined with HPLC–ICP-MS for As speciation in dry seafood products

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ABSTRACT

A sequential extraction procedure followed by HPLC–ICP-MS analysis was developed for the speciation and quantization of arsenic species in dry seafood products (DSPs). The extraction process involved three major steps, which produced respectively three As fractions: non-polar ($As_{nonpolar}$), polar (As_{polar}) and inorganic arsenic species ($As_{inorganic}$). The extraction efficiency (EE%) is in the range of 87–115%. $As_{nonpolar}$ is 0.6–10.2% of total arsenic in DSPs, which is served as the index of level of arsenolipid. For As_{polar} and $As_{inorganic}$, a hyphenated HPLC–ICP-MS technique was established for the separation and quantification of six arsenic species including arsenobetaine (AsB), arsenocholine (AsC), arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The results indicate that AsB and AsV are the two dominate species in DSPs, while all other species are present in relatively low concentrations. The recovery efficiency of 77–02.8% could be obtained with spike recovery test in this two-steps extraction.

1. Introduction

Dry seafood products (DSPs) are very popular in China and some Asian countries. The production of DSPs in the region is huge, and their manufacturing involves a widely diverse processes including braise, baking, drying, flavoring, etc. Just as fresh seafood, DSPs are also an important source of proteins, polyunsaturated fatty acids, and a wide range of vitamins (B, D, and A) and minerals (calcium, phosphorus, iron, etc.) in this part of the world. (FAO., 1998). In fact, compared with the fresh ones, DSPs are more convenient to store and cook, and are often sold commercially in instant food formulations. Both DSPs and the fresh seafood are sometimes contaminated with trace toxic elements such as arsenic. (Sikorski & Kolakowska, 1994). For safety evaluation, the speciation of these trace elements is just as important as the determination of their total concentration levels since toxicity is critically dependent on their exact chemical forms (Andreae, 1986; Penrose, 1974). For example, the LD50 values (50% lethal dose; mg/kg) of different arsenic species vary widely as: arsine 3, arsenite 14, arsenate 20, monomethylarsonic acid 700–1800, dimethylarsinic acid 700–2600, arsenocholine 6500 arsenolipids >8000, and arsenobetaine >10,000 (Nriagu, 1994).

Unfortunately, same as their fresh seafood counterparts, in most countries, there is yet no well-defined, species-specific, legislative control of DSPs (Buchet & Pauwels, 1994; Francesconi & Edmonds, 1994; Phillips, 1994). One of the best cited As standards for arsenic is for drinking water and its value in China is 50 mg/l. The setting of this limit is largely based on toxicity data for inorganic arsenite and arsenate. If this same limit were to applied to DSPs, most of them would probably be banned from the market since the arsenic content in seafood typically exceeds that of the drinking water by a factor of 1000 (Cullen & Reimer, 1989; National Academy of Sciences, 1997). In these cases, it is obvious that the setting of limits based solely on total arsenic concentrations is scientifically unfounded, and instead, the identification and quantification of individual arsenic species in the sample would be a more reasonable approach. In this regard, the absence of effective analytical method has been the main obstacles. The subject of arsenic speciation analysis has been extensively reported in the literatures but those related to applications in DSPs are very scarce (Gomez-Ariza & Sanchez-Rodas, 2000; Larsen & Pritzi, 1993; Larsen & Quetel, 1997). In the present work, a sequential solvent extraction procedure has been developed for the fractionation of different arsenic species. These fractions were then analyzed by ICP-MS or hyphenated HPLC–ICP-MS for total As and arsenic speciation analysis, respectively.

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2. Experimental

2.1. Standards and reagents

Stock solutions of individual arsenic compounds were prepared using the following standard: arsenite triiodide (AsIII), and arsenate oxide (AsV) from Alfa Aesar (Ward Hill, MA), and dimethylarsinic acid (DMA) from Acros Organics (NJ, USA). Arsenobetaine (AsB), arsenocholine (AsC), and monomethylarsinic acid (MMA) were prepared by Tsinghua University (Beijing, China). All the stock solutions were stored in a dark room at 4 °C, and the mixed standard solutions containing all arsenic species was prepared from the above single standard solutions.

Milli-Q ultra pure deionized (DI) water (Millipore, USA) was used throughout the experiment. The solvents acetone, methanol and hydrochloric acid, used for extraction were of high purity grade purchased from Merck (Darmstadt, Germany), Strong oxidants included 65% nitric acid (Merck) and 30% hydrogen peroxide (Merck) were used for DSP samples digestion. HPLC grade ammonium carbonate from Fischer Scientific (Fairlawn, NJ, USA), was used to prepare the mobile phase used for the chromatographic separations.

2.2. Instrumentation

A lyophilizer (Labconco, USA) was used to freeze dry the samples to a constant weight. A variable speed reciprocal shaker (Apparatyus, China) was used for sample homogenization. A rotary evaporator (EYELA, Japan) was used to evaporate the organic solvents and water used in the extraction process. Centrifugation (HERMLE, Germany) was used to separate the extract. The Accelerated solvent extraction (ASE) instrumentation used for sample extraction was an ASE 100 from Dionex Corporation (Sunnyvale, CA, USA).

An Agilent 1100 series high performance liquid chromatography (HPLC) system consisting of a binary pump, a vacuum degasser, and a manual-injector with a 20 μl sampling loop was used in HPLC analysis. A PRP-X100 anion exchange column (Hamilton, Reno, NV, USA) was used for the separation of arsenic species.

The Arsenic determination was performed by an Agilent 7500a ICP-MS (Agilent Technologies, USA), equipped with a Babington nebulizer, a glass double path spray chamber and a standard quartz torch, and HPLC operating conditions are listed in Table 1.

2.3. Sample and sample treatment

Reference material TORT-2 (lobster hepatopancreas) was purchased from National Research Council of Canada (Ottawa, Ontario, Canada). Eight of the more popular dried seafood products in China were selected for real samples in this work. The samples were collected in April 2006 from some large supermarkets in Qingdao. Detail information of the samples was summarized in Table 2. All samples were rinsed three times with deionized water to remove the salt on the surface, and then frozen dry a constant weight. They were grinded into powders and then (particle size: <2 mm) stored in the dark at −70 °C before analysis.

3. Determination of total Arsenic

Dried seafood (0.2 g) and 5 ml of concentrated HNO_3 were added together into a PTFE bomb. After 10 min the bombs were sealed off and put into an oven at 80 °C for 4 h. Afterwards the bombs were cooled down and the gas was released to discharge the high pressure in the bombs. The bombs were then placed back in the oven at 170 °C for 4 h. After cooling down the bomb, 1 ml H_2O_2 was added onto the residue inside the bomb to form a clear solution. This solution was diluted to a final mass of 25 g with deionized water for ICP-MS analysis.

Table 1
Instrumental parameters for total As determination and As speciation analysis

Total arsenic			
ICP-MS			Agilent 7500a
RF power			1350 W
RF matching			1.6 V
Carrier gas flow rate			1.10 L/min
Peristaltic pump flow rate			0.1 rps
Arsenic speciation			
HPLC	Agilent 1100 Series		
Analytical column	Hamilton PRP-X100 (250 × 4.1 mm; 10 μm)		
Flow rate	1.5 mL/min		
Injection volume	25 μL		
Mobile phase A	H_2O		
Mobile phase B	50 mM $(NH_4)_2CO_3$ (pH = 9.5)		
Gradient program	Time (min)	A (%)	B (%)
	0	100	0
	15	0	100
	30	100	0
ICP-MS	Agilent 7500a		
RF power	1350 W		
RF matching	1.6 V		
Carrier gas flow rate	1.10 L/min		
Peristaltic pump flow rate	0.5 rps		
Monitored signals	^{75}As, ^{72}Ge, ^{35}Cl, ^{77}Se and ^{83}Kr		

The total arsenic content in the dry seafood samples was measured before and after extraction using inductively coupled plasma mass spectrometry (ICP-MS). The rare earth element ^{89}Y was used as an internal standard. The interference correction equations were chosen from the library for EPA200.8 to correct $^{75}ArCl^+$ interference.

4. Sequential extraction procedures

4.1. Step I: non-polar arsenic compounds extraction

The DSPs were first extracted by acetone to extract the relatively non-polar arsenic compounds. In the procedure, 0.2 g of powdered samples were accurately weighed into 10 ml centrifugal tubes followed by the addition of 4 ml of acetone. After closing the lids securely, the sample powders and acetone solvent were mixed thoroughly by reciprocal shaker for 5 min. The samples were then sonicated for 20 min, and the resulting mixtures were centrifuged at the rate of 5000 rpm for 10 min at 4 °C. The bottom residue was then re-dispersed in the extractant solvent by shaking, and sonicated for another 10 min. The supernatant and the residue were again separated by centrifugation, and this samples extraction procedure was repeated for three times. The three supernatants were combined and filtered through a 0.22 μm membrane filter. The solvent in the filtrate was removed by rotary evaporation, and the final concentrate was digested by the procedure as described in EPA method 200.3 (Wahlen & McSheehy, 2004). The arsenic concentration in this fraction, denoted as $As_{nonpolar}$, was determined by ICP-MS. (McKiernan & Creed, 1999).

4.2. Step II: extraction of polar arsenic compounds

The solid residue from step I was dried in an oven at 50 °C to drive away the residue acetone. It was then extracted by 4 ml of a mixture of 50% methanol in deionized water (DI). The extraction procedure was the same as those described in step I. After extraction, the solutions were filtered and then placed in an oven at 65 °C to remove the solvent mixture. The drying process proceeded slowly to a final volume of about 3–5 ml. During the process DI