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Fractionation of inorganic arsenic by adjusting hydrogen ion concentration

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ABSTRACT

The inorganic fraction of arsenic species, iAs = ∑[As(III) + As(V)] present in fish samples can be quantified in the presence of other arsenic species also found in fishes, such as: monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AsB). The toxic arsenic fraction was selected taking into account the dissociation constants of these arsenic species in different hydrogen ions concentration leading to the arsine formation from iAs compounds detected as As(III) by HG AAS. For thus, a microwave assisted extraction was carried out using HCl 1 mol L^{-1} in order to maintain the integrity of the arsenic species in this mild extraction media. Recovery experiments were done for iAs fraction, in the presence of other arsenic species. The recovery values obtained for iAs fraction added were quantitative about 87–107% (for N = 3, RSD ⩽ 3%). The limit of detection (LOD), and the limit of quantification (LOQ), were 5 μg kg^{-1} and 16 μg kg^{-1} respectively.

1. Introduction

Nowadays is well known that the element arsenic (As) is prejudicial to the environment and health, and unfortunately it is found in soils, groundwater, surface water, air and also in food items. In the twentieth century, arsenic was utilized in livestock dips and feed supplements, semi-conductors, wood preservatives, and medicines (Henke, 2009). However, in the end of that century, toxicologists and other scientists began to recognize widespread arsenic poisoning in Bangladesh, West Bengal (India) and elsewhere (Tsuji, Perez, Garry, & Alexander, 2014).

Exposure to arsenic can result in a variety of healthy problems in humans, including various forms of cancer (e.g. skin, lung and bladder), cardiovascular and peripheral vascular disease, and diabetes (Bhowmick et al., 2014).

In the marine environment, more than 50 different naturally occurring compounds containing As have been identified, comprising both organic and inorganic forms. In fish and other seafood, As is bioaccumulated and is presented predominantly as the organoarsenical arsenobetaine (AsB), which does not present toxic characteristics to the human health. However, methylated and inorganic forms of arsenic are also found in the marine environment becoming a toxicological concern. It is well known that the methylated arsenic species, like monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenocholine (AC) and tetramethylarsonium ion (TMA^+), are found as minor constituents in marine samples and present the minor toxicological concerns. Arsenosugars and arsenolipids are also found but their toxicological studies are still limited, eventhough the arsenosugars studies indicate a low human toxicity (Moreda-Piñeiro et al., 2012). Inorganic arsenic species [As(III) + As(V)] are the more toxic forms, and the content of inorganic arsenic (iAs) in seafood is generally low, especially in fish, where the concentrations below detectable levels (<0.001 mg kg^{-1}) are reported. However it is important to mention that iAs level in shellfish, (iAs > 0.5 mg kg^{-1}) have been reported in bivalves (Sloth & Julshamn, 2008). The toxicological characteristic of iAs has been proposed to bind to thiol groups of biologically active proteins, inhibiting the function of various metabolic enzymes. The iAs fraction was also determined in rice and samples of marine origin (Musil et al., 2013), employing hydride generation coupled with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS), as an arsenic detector. In this study a chromatography separation step was not necessary due to the arsenic species separation (iAs target species) is given by adjusting of hydrogen concentration variation. The instrumental conditions were also studied and optimized and no statistical differences in the iAs con-

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centrations were found between the proposed method employing HG-ICP-MS and the conventional method using the HPLC-ICP-MS.

In spite of the iAs found in minor levels in fish, the monitoring and background data for future risk assessment analysis is urgently necessary. The European Food Safety Authority (EFSA) (EFSA, 2009) and FAO/WHO Joint Expert Committee on Food Additives (JECFA) (Codex Standard, 1995) have been made several assumptions on the basis of the total As content in the foodstuffs due to limited specific data on the iAs content in food. Despite the importance of this subject there is no official control of total and inorganic arsenic levels in foodstuffs in Brazil, especially concerning marine food that generally contains higher levels of arsenic and is considered the main source of arsenic intake to the human organism (Brazil, Ministry of Agriculture, Livestock and Food Supply, 2009). Thus, it is important to know the concentration levels of iAs present in fish consumed and to estimate the limit values for the iAs level in fish. The need for developing validated methods of analysis for specific determination of iAs has also been emphasized by EFSA (2009) and (Codex Standard, 1995) in their evaluations.

Several methods of sample preparation have been proposed in the literature for the iAs extraction, employing the hyphenated analytical technique, such as, High Performance Liquid Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry, (HPLC – ICP – MS) (Nearing, Koch, & Reimer, 2014), or employing atomic absorption technique, e.g. Hydride Generation Atomic Absorption Spectrometry, HG AAS, as currently reported in the literature (Anderson, Thompson, and Culbard (1986); Rasmussen, Hedegaard, Larsen, and Sloth (2012)). Extraction procedures for arsenic speciation analysis in marine food including the use of methanol or water or even the mixture of both are largely used (Choi, Park, Kim, & Kim, 2011). Other methods include enzymatic (Wang, Geisel, & Chris Le, 2012) and ionic extractants (Styblo, Hughes, & Thomas, 1996), in order to improve the extraction of iAs by breaking bonds of thiol groups in the proteins, which have affinity to As(III). These extraction procedures generally employ microwave assisted extraction, sonochemistry method (ultrasonic extraction) and heating conventional method. Chen, Corns, Stockwell, and Huang (2014) evaluated the proposed method employing HG-AFS technique with the currently technique HG-ICP-MS, in order to assess the accuracy of both proposed methods. In this work, the total arsenic and iAs fraction were quantified in rice grains. High hydrogen concentration is need to separate the different arsenic species when the chromatography is not required, and in this case, possible interferences caused by $^{40}As^{35}Cl$ on 75 m/z of As, using the quadrupole instrument has been reported. The performs using the HG-AFS was better than HG-ICP-MS, due to lack of Cl interferences and the As(III) signal is more sensitive than As (V) signal detected by HG-ICP-MS.

In this present study, an analytical method was developed to determine the toxicological relevant fraction of arsenic, iAs [(AsIII) + As(V)], in fish samples based on the reduction of iAs compounds with sodium tetrahydroborate (Howard, 1997), taking into account the pH dependence and the pKa values of the individual arsenic compounds in acidic aqueous medium (Henke, 2009). The microwave assisted extraction was employed for the extraction of iAs in fish samples followed by AAS detection.

2. Material and methods

2.1. Chemical and reagents

Ultrapure water (18 MΩ cm) obtained with a Milli-Q water system (Millipore Inc., Bedford, USA) was used for the preparation of reagents and standards.

Concentrated HCl (36% w/w) (Merck, Darmstadt, Germany) was used for the iAs microwave assisted extraction in fish samples prior to analysis. External calibration standards were prepared daily by dilution of a certified 1000 mg L^{-1} As(III) stock solution (SPEX CertiPrep, Assurance, Metuchen, USA).

Standard solutions (1000 mg L^{-1}) of the other arsenic species were prepared by dissolving appropriate amounts in water of MMA (Sigma-Aldrich, St. Louis, USA); DMA (Sigma-Aldrich, St. Louis, USA) and AsB (Sigma-Aldrich, St. Louis, USA). The As(V) solution was obtained by appropriate dilutions of 1000 mg L^{-1} certified arsenic(V) stock solution (SPEX CertiPrep, Assurance, Metuchen, USA). A pre-reducing solution for samples treatment before inorganic arsenic quantification was used, consisting in a fresh mixture containing 5% w/v KI (Sigma-Aldrich, St. Louis, USA) and 5% w/v ascorbic acid (Sigma-Aldrich, St. Louis, USA). As reducing solution for hydride generation, 0.20% w/v sodium tetrahydroborate was prepared by dissolving appropriate amounts of $NaBH_4$ (Sigma-Aldrich, St. Louis, USA) in 0.05% w/v NaOH (Sigma-Aldrich, St. Louis, USA) solution. Fresh $NaBH_4$ solution was prepared daily. All glassware was treated with 10% v/v HNO_3 for 24 h, and then rinsed three times with Milli-Q water before being used.

DORM-3 (Dogfish muscle) certified material for total As at 6.88 ± 0.30 mg kg^{-1}, was obtained from National Research Council Canada (NRCC), Ontario, Canada.

2.2. Samples, sample preparation and extraction procedure

The inorganic arsenic method was developed and optimized employing white salmon fish sample, a kind of fish largely consumed by Brazilian people. The white salmon was used for the development of the method because this matrix contains arsenic total content lower than the limit of detection, LOD, of the official method (Díaz et al., 2004) for determination of total arsenic adopted by the National Agricultural Laboratory, also called as LANAGRO, which is the official Brazilian laboratory for food monitoring.

Other fish species and crustaceans were obtained directly from producers or sales point specialized in seafood. Ministry of Agriculture, Livestock and Food Supply (MAPA) has monitoring the arsenic concentration levels in these samples, through the Brazilian National Plan of Residues and Contaminants Control (Brazil, Ministry of Agriculture, Livestock and Food Supply, 2009), which constitutes a tool of risk management in order to ensure the quality of production system of animal and vegetal foods along the supply chains.

Lyophilized white salmon and Flathead grey mullet fish as well as seafood samples like shrimp and blue mussels were crushed and homogenized to a fine powder (250 mesh particle size) in a laboratory mill (Retsch, Haan, Germany). The resulting powder was stored in previously decontaminated falcon flasks and kept in the freezer until analysis. The samples were submitted to a solubilizing process of arsenic species using closed vessels in a microwave oven system according to the heating procedure reported in Table 1.

About 0.3000 g of fish and seafood samples were accurately weighted into PTFE vessels and 7.30 mL of water was added before the addition of 0.70 mL of 36% (w/w) HCl, to obtain 1.00 mol L^{-1} HCl as final concentration. The slurry formed was slightly stirred and the closed vessels were placed inside the microwave oven

Table 1
Microwave oven heating program for fish and seafood samples decomposition.

Step	Power/W	Ramp/min	Hold/min	Fan
1	400	5	30	1
2 (cooling)	0	0	20	3