Analytica Chimica Acta 888 (2015) 1–9

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Enzyme-assisted extraction and liquid chromatography mass spectrometry for the determination of arsenic species in chicken meat

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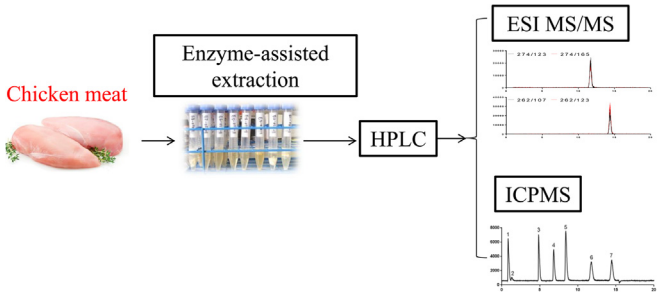
HIGHLIGHTS

- Proteolytic enzymes enhance extraction of arsenic species from chicken meat.
- HPLC with simultaneous ICPMS and ESIMS detection improves arsenic speciation.
- Papain digestion of chicken meat increases the extraction efficiency of arsenic species.
- Ten arsenic species are detected in chicken breast meat at $\mu g\,kg^{-1}$ concentrations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 March 2015
Received in revised form
28 April 2015
Accepted 3 May 2015
Available online 7 May 2015

Keywords:
Arsenic speciation
Chicken
Papain
Roxarsone
High performance liquid chromatography and inductively coupled plasma mass spectrometry
Electrospray ionization mass spectrometry

ABSTRACT

Chicken is the most consumed meat in North America. Concentrations of arsenic in chicken range from $\mu g\,kg^{-1}$ to $mg\,kg^{-1}$. However, little is known about the speciation of arsenic in chicken meat. The objective of this research was to develop a method enabling determination of arsenic species in chicken breast muscle. We report here enzyme-enhanced extraction of arsenic species from chicken meat, separation using anion exchange chromatography (HPLC), and simultaneous detection with both inductively coupled plasma mass spectrometry (ICPMS) and electrospray ionization tandem mass spectrometry (ESIMS). We compared the extraction of arsenic species using several proteolytic enzymes: bromelain, papain, pepsin, proteinase K, and trypsin. With the use of papain-assisted extraction, 10 arsenic species were extracted and detected, as compared to 8 detectable arsenic species in the water/methanol extract. The overall extraction efficiency was also improved using a combination of ultrasonication and papain digestion, as compared to the conventional water/methanol extraction. Detection limits were in the range of 1.0–1.8 μg arsenic per kg chicken breast meat (dry weight) for seven arsenic species: arsenobetaine (AsB), inorganic arsenite (As^{III}), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), inorganic arsenate (As^{V}), 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone), and *N*-acetyl-4-hydroxy-*m*-arsanilic acid (NAHAA). Analysis of breast meat samples from six chickens receiving feed containing Roxarsone showed the presence of (mean ± standard deviation $\mu g\,kg^{-1}$) AsB (107 ± 4), As^{III} (113 ± 7), As^{V} (7 ± 2), MMA (51 ± 5), DMA (64 ± 6), Roxarsone (18 ± 1), and four unidentified arsenic species (approximate concentration 1–10 $\mu g\,kg^{-1}$).

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http://dx.doi.org/10.1016/j.aca.2015.05.001
0003-2670/

1. Introduction

Humans are exposed to arsenic (As) mainly through ingestion of food and water. Chronic exposure to high concentrations of arsenic is associated with a variety of adverse health effects. As a consequence, regulatory agencies around the world have tightened guidelines on arsenic in water. For example, the World Health Organization (WHO) [1], the United States Environmental Protection Agency (EPA) [2], and Health Canada [3] have guidelines for arsenic ($10\ \mu g\ L^{-1}$) in drinking water. However, many arsenic species can be present in food [4–12]. Arsenic in food can range from highly toxic inorganic arsenite to the virtually non-toxic arsenobetaine. Even among the commonly encountered toxic arsenic species, their relative toxicity, e.g., in terms of medium lethal concentration (LC_{50}), varies by 3–4 orders of magnitude [13–17], with arsenite ($LC_{50} = 2.3\ \mu M$) being much more cytotoxic than dimethylarsinic acid ($LC_{50} = 1680\ \mu M$), tested on HL60 cells [14]. Therefore, the determination of total arsenic in food is not sufficient for human health risk assessment; it is necessary to determine the speciation of arsenic in food.

Extensive research has been carried out on the speciation of arsenic in seafood [18–24] for the need to differentiate toxic arsenic species (e.g., inorganic arsenicals) from those of high concentration but little toxicity (e.g., arsenobetaine). Recently, there has been much attention paid to the determination of arsenic in rice and chicken because of the relatively high concentrations of arsenic in these food items [25–30]. The source of arsenic in chickens is mainly due to the use of Roxarsone (Rox), an organoarsenical, as a feed additive to control infection and promote growth of chickens [29]. Chicken is one of the most consumed meats, and it may contribute a considerable amount of arsenic to the total dietary exposure. However, little is known about the arsenic speciation in chicken meat [12,30,31]. The primary objective of this research was to develop a method that enables the determination of arsenic species present in chicken meat at trace concentrations.

Determination of trace concentrations of various arsenic species in solid samples, such as chicken meat, requires appropriate extraction [32–38], followed by efficient separation and sensitive detection [38–44]. Most of the highly sensitive methods for arsenic speciation have used high performance liquid chromatography (HPLC) separation with detection of atomic fluorescence, inductively coupled plasma mass spectrometry (ICPMS), and electrospray ionization mass spectrometry (ESIMS) [21,22,38–44]. For solid samples, such as chicken meat, arsenic species must be extracted into solution amenable for HPLC analysis. The method of extraction must be efficient and must not change the pertinent property of the original arsenic species. Methods such as ultrasound water-bath assisted extraction [45] and microwave-enhanced extraction [46] have been employed to enhance the extraction of arsenic from seafood. But extraction of arsenic species from chicken meat has not been fully tested.

The quantitative and reproducible extraction of arsenic species was considered as the most challenging aspect in the speciation analysis of organoarsenicals relevant to chicken feed [47]. Both organic and inorganic arsenic species are expected to be present in chicken. Harsh digestion conditions could alter arsenic species, whereas mild extraction conditions might not efficiently release the arsenic species that could potentially be bound to proteins. To maintain the integrity of arsenic species, we chose to use mild pH and temperature conditions. To enhance extraction efficiency, we incorporated proteolytic enzymes to digest proteins. Previous research has demonstrated several applications of enzymatic extraction for chemical speciation studies [48–53]. Enzymes such as pronase E [49], amylase [50], and lipase [51] have been used for extraction of arsenic species from seafood, freeze-dried apple, and hair samples, respectively. Enzymatic extraction has not been demonstrated for arsenic speciation in chicken meat.

We chose to test a number of proteolytic enzymes, such as bromelain, papain, pepsin, proteinase K, and trypsin, for extraction of arsenic species from chicken breast meat. Because of their proteolytic activities, some of these proteases have been widely used for meat tenderization. For example, papain can degrade both myofibrillar and collagen proteins. Obtained from plant sources, papain is relatively inexpensive among the various proteolytic enzymes.

The concentration of arsenic in chicken meat has been reported to be on the order of sub-mg kg^{-1} [12,30,31]. The concentrations of individual arsenic species in chicken meat are expected to be on the order of $\mu g\ kg^{-1}$. Therefore, highly sensitive detection and efficient separation approaches are required to enable determination of trace amounts of individual arsenic species in chicken breast meat. We chose HPLC separation because of its demonstrated capability for resolving various arsenic species. To achieve highly-sensitive quantitation and identification of arsenic species, we incorporated HPLC with simultaneous detection by both ICPMS and ESIMS. We report here speciation of arsenic in chicken breast meat using the method of enzyme-assisted extraction, HPLC separation, and mass spectrometry detection. The analytical method should contribute to improving human exposure assessment associated with arsenic intake from chicken meat.

2. Materials and methods

2.1. Instrumentation

A PRP-X110S anion exchange column (7 μm particle size, 100 Å pore size, 4.1 mm internal diameter, and 150 mm in length; Hamilton, Reno, NV), installed with an Agilent 1100 series HPLC system (Agilent Technologies, Germany), was used for separation of arsenic species. An Agilent 7500cs ICPMS system (Agilent Technologies, Japan) and an AB SCIEX 5500 QTRAP ESIMS system (Concord, ON, Canada) were used for detection. The operating conditions for these two mass spectrometers are summarized in Table S1 of Supplementary material. The eluent from the HPLC column was split so that 80% of the flow ($1.6\ mL\ min^{-1}$) was introduced to ICPMS and 20% of the flow ($0.4\ mL\ min^{-1}$) was introduced to ESIMS. This split was achieved by using a 300 series stainless steel tee (Valco Canada, Brockville, ON, Canada). A schematic of the HPLC coupled with ICMPS and ESIMS is shown in Fig. S1 of Supplementary material.

2.2. Reagents and arsenic standards

Stock solution ($10\ mg\ L^{-1}$) of arsenobetaine (AsB), arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), *N*-acetyl-4-hydroxy-*m*-arsanilic acid (NAHAA), and 3-nitro-4-hydroxyphenylarsonic acid (Rox) were prepared from arsenobetaine (98% purity, Tri Chemical Laboratories Inc., Japan), sodium *m*-arsenite (97.0%, Sigma, St. Louis, MO), sodium arsenate (99.4%, Sigma), monosodium acid methane arsonate (99.0%, Chem Service, West Chester, PA), cacodylic acid (98%, Sigma), *N*-acetyl-4-hydroxy-*m*-arsanilic acid (Pfaltz and Bauer Inc.), and 3-nitro-4-hydroxyphenylarsonic acid (98.1%, Sigma–Aldrich, St. Louis, MO), respectively. The concentrations of these arsenic species were calibrated against a primary arsenic standard (Agilent Technologies, Santa Clara, CA) and were determined using ICPMS. Standard solutions of arsenic species (0.1, 0.5, 1, 5, 10, 50, and $100\ \mu g\ L^{-1}$) were freshly prepared daily by serial dilution from the stock solutions. Milli-Q 18.2 MΩ cm deionized water (Millipore Corporation, Billerica, MA) and HPLC grade methanol (Fisher Scientific, Fair Lawn, NJ) were used as solvents. Proteinase K was