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Arsenic speciation in edible alga samples by microwave-assisted extraction and high performance liquid chromatography coupled to atomic fluorescence spectrometry

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ABSTRACT

Twelve commercially available edible marine algae from France, Japan and Spain and the certified reference material (CRM) NIES No. 9 *Sargassum fulvellum* were analyzed for total arsenic and arsenic species. Total arsenic concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after microwave digestion and ranged from 23 to 126 $\mu g\,g^{-1}$. Arsenic species in alga samples were extracted with deionized water by microwave-assisted extraction and showed extraction efficiencies from 49 to 98%, in terms of total arsenic. The presence of eleven arsenic species was studied by high performance liquid chromatography–ultraviolet photo-oxidation–hydride generation atomic–fluorescence spectrometry (HPLC–(UV)–HG–AFS) developed methods, using both anion and cation exchange chromatography. Glycerol and phosphate sugars were found in all alga samples analyzed, at concentrations between 0.11 and 22 $\mu g\,g^{-1}$, whereas sulfonate and sulfate sugars were only detected in three of them (0.6-7.2 $\mu g\,g^{-1}$). Regarding arsenic toxic species, low concentration levels of dimethylarsinic acid (DMA) (<0.9 $\mu g\,g^{-1}$) and generally high arsenate (As(V)) concentrations (up to 77 $\mu g\,g^{-1}$) were found in most of the algae studied. The results obtained are of interest to highlight the need to perform speciation analysis and to introduce appropriate legislation to limit toxic arsenic species content in these food products.

1. Introduction

Arsenic occurs in seawater mainly as inorganic arsenic (arsenite, As(III), and arsenate, As(V)) at low $\mu g\,L^{-1}$ level [1]. However, some marine organisms accumulate arsenic; thus, they often have much higher arsenic concentrations than in the surrounding environment, typically in the range of 10–100 $\mu g\,g^{-1}$ [2], even though arsenic has been reported to be mostly biotransformed into less toxic organic arsenic compounds [3]. Arsenobetaine (AsB) has been the major arsenic species found in marine fauna, while in algae the most frequently occurring arsenic species are arsenosugars, especially the derivatives of dimethylarsonylribosides commonly named glycerol, phosphate, sulfonate and sulfate sugars [4–8], whose structures are shown in Fig. 1. Besides these species, others like monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), tetramethylarsonium ion (TETRA), arsenocholine (AsC) and some inorganic arsenic (As(III) and As(V)) can be found in marine organisms as well [9–12].

Toxicity studies of arsenic have shown that different forms exhibit different toxicities, thus inorganic arsenic species are more toxic than organic compounds and toxicity generally decreases with increasing degree of methylation, with the exception of TETRA species [13]. Arsenosugars are assumed to be much less toxic than inorganic arsenic species [14], although their toxicity has not been well assessed [15]. However, studies based on the presence of arsenic metabolites in humans point to indirect toxicity resulting from the ingestion of algae containing arsenosugars, due to their biotransformation in toxic arsenicals [16]. Since arsenic species have different toxicity, information about the distribution of the species within algae is necessary for assessing the risk associated with the entry of arsenic into the wildlife and human food chains. Therefore, there is considerable interest in developing analytical methodologies for arsenic speciation in food products. This interest is accentuated in the case of algae, because it is well known that they contribute substantial amounts of arsenic to the human diet, and their consumption is nowadays increasing due to their properties as food additives, nutritional values (high contents of iodine, minerals and vitamins) and suggested medical applications [17].

Detailed information concerning analytical methods for arsenic speciation can be found in several reviews [17–20]. High performance liquid chromatography–inductively coupled plasma

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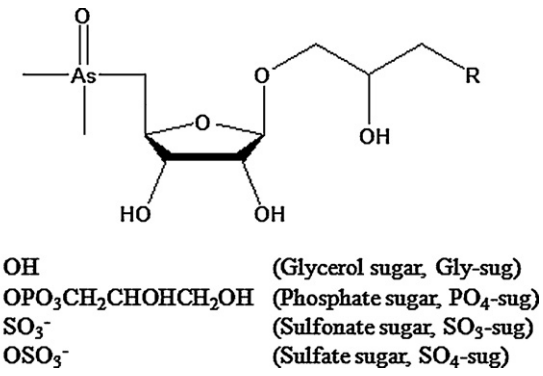

Fig. 1. Structures of the four most common arsenosugars found in algae.

mass spectrometry (HPLC–ICP-MS) is the most frequently used hyphenated technique for arsenic speciation [5–7,11,12,21]. However, the hydride generation–atomic fluorescence spectrometry (HG–AFS) coupled to HPLC represents a suitable alternative to this technique [22,23]. HG–AFS has been reported to be similar to inductively coupled plasma mass spectrometry (ICP-MS) regarding sensitivity and linear calibration range, although it has some advantages for arsenic speciation analysis, such as simplicity, lower acquisition and running costs [24–26]. However, the destruction of the organic part of arsenic species is required to determine them by HG–AFS, as they do not generate volatile hydrides or because the generation efficiency is very low [27]. Photo-oxidation to convert these species into inorganic arsenic is achieved after chromatographic separation by using a strong oxidant ($K_2S_2O_8$) in basic media and ultraviolet (UV) radiation emitted from a low pressure mercury lamp. Regarding arsenic species separation, ion-exchange chromatography is preferred for coupling to HG–AFS detection, as the separation process is more reproducible and less prone to sample matrix interferences than ion-paring chromatography [22]. On the other hand, both anion and cation exchange chromatography is needed for arsenic speciation in biological samples, due to the different ionic character of aqueous soluble arsenic species. The mobile phases commonly used are phosphate and pyridine solutions, for anion and cation exchange, respectively. HPLC–(UV)–HG–AFS have been successfully applied to a great variety of samples. For example, Šlejkovec et al. [28] analyzed six CRMs of biological origin (dogfish muscle and liver, lobster hepatopancreas, oyster tissue, brown algae and scallop) by HPLC–(UV)–HG–AFS, using both anion and cation exchange chromatography methods. They identified AsB, DMA, TETRA, AsC, TMAO, As(V), MMA and two arsenosugars, together with four unidentifiable compounds (one in brown algae, one in dogfish muscle and liver and lobster hepatopancreas, and two in scallop). In 2002, Sánchez-Rodas et al. [29] applied this same hyphenated technique for the analysis of aqueous extracts of oysters. These authors concluded that it is an alternative technique to liquid chromatography–electrospray-mass spectrometry (LC–ES-MS) for the detection of arsenosugars in crude extracts, since signal response in HPLC–(UV)–HG–AFS is little influenced by the sample matrix, whereas LC–ES-MS analysis usually requires previous sample clean-up steps. Schaeffer et al. [30] determined twelve arsenic compounds in mussels, anchovies, seabreams, sea bass and sardines by HPLC–(UV)–HG–AFS, using both anion and cation exchange methods. AsB was detected as the major compound in all the samples, with trace amounts of As(III), DMA and AsC, whereas arsenosugars were detected only in mussel samples. Šlejkovec et al. [31] applied it for the determination of arsenic species in ten different marine algae (red, green and brown) from the littoral zone along the Adriatic Sea coast of Slovenia. They found that arsenosugars were the most abundant arsenicals in most of the analyzed algae, although they also found AsB, As(III), As(V) and DMA, As(V) being the major species in three alga samples (*Ceranium* sp., *Polisyphonia* sp., *Cystoseira barbata*). In 2009, Geng et al. [32] compared the performance of the combination of solvent extraction and HPLC–(UV)–HG–AFS with the combination of alkaline digestion and cryogenic trap–hydride generation–atomic absorption spectrometry (CT–HG–AAS), for arsenic speciation in marine product samples. They analyzed six CRMs of marine animal samples and four seaweed samples, and concluded that results obtained by both methodologies are comparable, because the concentrations of arsenobetaine obtained in CRMs from the extraction–HPLC method were very consistent with those of trimethylated arsenic species measured by the digestion–CT method. In the case of seaweed samples, the amounts of dimethylated arsenic species measured by the digestion–CT method were approximately equal to the sum of the amounts of DMA and three arsenosugars determined by the extraction–HPLC method.

The aims of this work consisted on: (1) the development of arsenic speciation analysis methods based on the coupling of high performance liquid chromatography (anion and cation exchange) with ultraviolet photo-oxidation–hydride generation–atomic fluorescence spectrometry (HPLC–(UV)–HG–AFS); (2) the application of the developed methods to carry out the identification and quantification of arsenic species present in the water extracts, obtained by microwave-assisted extraction, of commercially available edible alga samples.

2. Materials and methods

2.1. Instrumentation

Microwave assisted extraction was conducted using a MARS 5 microwave oven (CEM, Matthews, NC, USA), with Pyrex extraction vessels. The temperature was monitored in a control vessel by an armored fiber-optic temperature control probe.

An ETHOS 1 closed vessel microwave digestion system (Milestone S.r.l., Sorisole, BG, Italy), with built-in ATC-400-CE automatic temperature control and employing TFM-Teflon microwave vessels, was used for alga samples digestion.

Total arsenic determination was performed with an inductively coupled plasma atomic emission spectrometer (ICP-AES) model Liberty Series II Axial Sequential (Varian Australia, Mulgrave, Australia).

For arsenic speciation analysis, the HPLC–(UV)–HG–AFS system used consists of a Jasco PU-2089 plus quaternary gradient pump (Jasco, Tokyo, Japan) and a Rheodyne 7725 six-port sample injection valve, fitted with a 100 μl sample loop (Rheodyne, CA, USA). Chromatographic separations were carried out in a Hamilton PRP-X100 (250 mm × 4.1 mm, 10 μm) anion exchange column and in a Hamilton PRP-X200 (250 mm × 4.1 mm, 10 μm) cation exchange column (Phenomenex, Torrance, CA, USA). The corresponding guard columns (25 mm × 2.3 mm, 12–20 μm) (Phenomenex) were used in order to preserve the analytical columns. The column outlet was coupled to a 10.570 UV Cracker (PS Analytical), used for the destruction of the organic part of the arsenic compounds, followed by a PSA 10004 continuous-flow vapor system (PS Analytical, Sevenoaks, Kent, UK), equipped with a B-type gas–liquid separator and a Perma Pure drier tube (PS Analytical). The hydrides generated were transported through a stream of argon to a PS Analytical Millennium Excalibur atomic fluorescence spectrometer PSA 10.055, equipped with a boosted discharge hollow cathode lamp (BDHCL) (Superlamp, Photon, Victoria, Australia) as an AFS excitation source, a hydrogen diffusion flame as the atomization cell and specific filters (multireflectance filters) to achieve isolation and reduction of flame emission.