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Analytical Methods

Determination of toxic heavy metals and speciation of arsenic in seaweeds from South Korea

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ABSTRACT

This study aimed at determining the levels of toxic heavy metals including As, Pb, Cd, Al, Hg and As species, such as, As-III, As-V, MMA, DMA, AsB, and AsC in various edible species of seaweeds from South Korea. ICP-MS was used for determination of As, Pb and Cd, ICP-OES was used for Al, DMA was used for Hg, and LC-ICP-MS was used for As speciation. The analytical methods were validated by linearity, detection limits, precision, accuracy and recovery experiments, obtaining satisfactory results in all cases. From the results toxic heavy metals were found in the decreasing order of: Al > As > Pb–Cd > Hg. Generally concentrations of all analysed heavy metals and both organic and inorganic species of As were very low compared to PTWIs specified by JECFA and EC. Their contribution to the overall intake by the subject seafoods was found very low and thus would not pose any threat to consumers.

1. Introduction

South Korea is surrounded by the sea on the eastern, western and southern sides. A large variety of industrial complexes and municipal cities are located in coastal areas and around 33% of the national population lives along the entire coast comprising around 2,900 km^2 (Kim, Lee, Oh, & Kahng, 2000). Due to such geographical location, seafoods contribute a significant part to their daily foods (Hsieh & Jiang, 2012). Seaweeds are well known seafoods, most popular in China, Japan and Korea, although they are also used in other Asian countries, and in countries where there are ethnic Asian communities. Seaweeds are proved to be the rich sources of mineral elements than other usual edible plants and therefore often recommended as food supplements to help meet daily intake of essential mineral and trace elements (Rupérez, 2002). According to the Food and Agriculture Organization (FAO), Fisheries and Aquaculture Department statistics for the year 2011, the total world aquaculture production of seaweeds was 21.0 million tones, with 20.80 million tones only from Asia. China was the largest producer with 11.5 million tones of seaweeds followed by Indonesia (3.9 million tones), Philippines (1.8 million tones) and Republic of Korea (444,300 tones) (FAO, 2013). The harvest of cultured seaweeds from coastal waters removes nearly a million tones of proteins, with around 150,000 metric tones of nitrogen annually (Troell et al., 2003). In Korea, seaweeds are eaten raw, cooked or processed. Also many cosmetic and pharmaceutical products contain seaweed polysaccharides namely agars, alginates and carrageenans (Chung, Beardall, Mehta, Sahoo, & Stojkovic, 2011).

The significant alterations of industrial development in the recent past, lead to an increased discharge of chemical effluents into the environment, resulting to damage of aquatic life in many countries around the world. Heavy metals discharged into the marine environment can damage marine species and whole ecosystem, due to their accumulative behaviour and well known toxicity (Sivaperumal, Sankar, & Viswanathan-Nair, 2007). The impacts of toxic elements like, Al, Pb, Hg, Cd and As on human health and the environment are of great interest, especially from aquatic products prospective (Uluozlu, Tuzen, Mendil, & Soylak, 2007; Verstraeten, Aimo, & Oteiza, 2008; Yabanli, Alparslan, & Baygar, 2012). These elements can be very damaging even at low levels when ingested over a long period of time. Even the essential metals are known to produce toxic effects when their intake is excessive to their recommended levels (Celik & Oehlenschlager, 2007).

Aluminium is proposed to be involved in the pathophysiology of neurodegenerative disorders (Parkinsonism dementia, Alzheimer's disease). Pb is a widespread environmental hazard, and the

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neurotoxic effects of Pb are a major public health concern (Verstraeten et al., 2008). Mercury is another dangerous xenobiotic, especially its vapours and water soluble salts. It has the ability to accumulate in the internal organs of living organisms (Boszke, Siepak, & Falandysz, 2003). In the past century, the anthropogenic inputs of mercury into the environment also significantly increased and therefore monitoring these inputs and estimating their many possible adverse impacts is very important, along with other toxic elements across the food chain, especially seafoods (Konieczka, Misztal-Szkudlińska, Namieśnik, & Szefer, 2010). Similarly research studies have indicated that arsenic is abundant in seafoods at concentrations as high as several hundred μg/g. Therefore, it is essential to know the concentrations of individual arsenic species, along with total arsenic, to realise the level of toxicity in countries, such as China, Japan, Taiwan, and Korea, where food from marine sources constitutes a major part of the diet (Hsieh & Jiang, 2012).

This study was designed to monitor the exposure of the Korean population to arsenic, cadmium, mercury, aluminium and lead from seaweeds, due to increasing concern about the intake of toxic contaminant elements in foods, by determining their levels in all edible species, commercially available to consumers all around South Korea. Furthermore, the speciation of the important organic arsenics were considered including monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC) and inorganic species such as arsenite (As-III), and arsenate (As-V), in order to know about their contamination levels if any in the food subjects. The provisional tolerable weekly intakes (PTWIs) recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Commission (EC) was used for all analytes to compare and determine any toxicity from seaweeds to the consuming population.

2. Materials and methods

2.1. Instrumentation

A microwave system (Multi-wave 3000, Anton Paar, Graz, Austria) was used to digest the samples. It was programmable for time and power between 600 and 1400 W, and equipped with 16 high pressure PTFE (polytetrafluoroethylene) vessels (MF 100), digestion tubes.

The quadrupole, inductively coupled plasma mass spectrometer (ICP-MS) used for determination of As, Cd and Pb, was Elan DRC II (Perkin-Elmer SCIEX, Norwalk, CT, USA). It had a high efficiency sample introduction desolvating system equipped with a quartz cyclonic spray chamber and an additional mixing peristaltic pump (APEX-IR, Omaha, NE, USA). The operating conditions were: forward power 1.35 kW, argon gas flow rate 16.00 L/min (plasma); 1–1.3 L/min (auxiliary), 1–1.07 L/min (nebulizer). The argon gas utilised was of spectral purity (99.9998%). Before each experiment, the instrument was tuned for daily performance, using Elan 6100 DRC Sensitivity Detection Limit Solution, PerkinElmer Pure (N8125034) USA.

A Varian Model 730-ES simultaneous CCD, inductively coupled plasma-optical emission spectrometer (ICP-OES), (Wyndmoor, PA, USA), was used for determination of Al in the samples. This spectrometer had a SeaSpray concentric nebulizer (Glass Expansion, Pocasset, MA) and cyclonic spray chamber. Operating conditions for ICP-OES instrument were: forward power 1.3 kW, argon gas flow rate 16.00 L/min (plasma); 1.5 L/min (auxiliary), 0.94 L/mi, (nebulizer), wavelength 396.153 nm. The background correction wavelength was selected manually at appropriate background position for the analyte peak, after scanning a blank, a standard solution and a sample solution in the programmed wavelength range.

Mercury analyser (MA-2000; NIC, Japan) was used to determine the content of total mercury in the samples. It was an automatic direct mercury analyser, with sample preheating furnace, decomposing furnace, mercury-gold amalgamation chamber and Silicon UV photodetector. The instrument was also attached with auto sample changer (NIC BC-1).

A Shimadzu High Performance Liquid Chromatography System (HPLC, LC-VP series, Kyoto, Japan), was coupled to ICP-MS for quantification of six As species. The quadrupole mass analyser was operated in the single ion monitoring mode (m/z 91) for detecting arsenic and dynamic reaction cell (DRC) mode using oxygen. The instrumental specification and various operating conditions followed for LC-ICP-MS were as mentioned in Table 1.

2.2. Reagents

The analytical reagent grade concentrated HNO_3 (70%) was obtained from Dong Woo Fine-Chem Co., Ltd. Iksan, Korea. For calibration curves of metals, standard stock solutions of 100 mg/L, for all analytes were purchased from AnApure KRIAT Co, Ltd. Daejeon, Korea. The certified reference material (NMIJ CRM 7405-a), Hijiki Seaweed, was obtained from National Metrology Institute of Japan/National Institute of Advanced Industrial Science and Technology (NMIJ/AIST), Ibraki, Japan. Sodium (meta) arsenite [$NaAsO_2$; As(III); 99.0%] and sodium arsenate dibasic heptahydrate [$Na_2HAsO_4.7H_2O$; As(V); 99.9%] were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dimethylarsinic acid [$(CH_3)_2AsO(OH)$; DMA; 98%], monosodium acid methane arsonate sesquihydrate [$(CH_3)AsO(OH)_2$; MMA, 99.0%], arsenobetaine [$[(CH_3)_3As]^+CH_2$-COO^-; AsB, 97%], and arsenocholine [$[(CH_3)_3As]^+CH_2CH_2OH$, Br^-; AsC, 95%] were purchased from Strem Chemicals (Newburyport, MA, USA), Chem Service (West Chester, PA, USA), Fluka (Buchs, Switzerland), and Wako Pure Chemical Industries (Osaka, Japan), respectively. Ultrapure deionised water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA).

2.3. Apparatus

All containers were thoroughly cleaned with a detergent solution, rinsed with metal free water, and soaked for overnight, or longer in a covered acid bath containing 10% HNO_3 (v/v) solution. These were rinsed several times with de-ionised metal free water and dried in drying oven (HB-502M, Hanbaek Co., Ltd. Korea). All plastic containers, polypropylene flasks, pipette tips, PTFE vessels digestion tubes and reagents that came into contact with samples or standards were checked for contamination.

2.4. Samples preparation and digestion

Samples (198) belonging to five species of edible seaweeds commercially available to consumers were purchased from super markets all around South Korea. These species included laver (*Porphyra tenera*) (53), seatangle (*Laminaria japonica*) (45), sea mustard (*Undaria pinnatifida*) (58), hijiki (*Hizikia fusiforme*) (27) and gulf weed (*Sargassum fulvellum*) (15). All the samples were purchased at different time intervals during April to October 2012, in triplicate thus making a total of 594 samples analysed. In each variety, the samples belonged to different packaging and brands. These were rinsed with tap water followed by deionised distilled water and air dried in a clean fume hood. For determination of moisture, each sample was dried at 105 °C in oven (HB-502M, Han Back, Korea), until constant weight was achieved. These were powdered in a blender (MR 350CA, Braun, Spain), properly labelled and stored in polyethylene bags in refrigerator (MICOM CFD-0622, Samsung, Korea) at −20 °C until analysis.