Food Control 82 (2017) 266–273

Contents lists available at ScienceDirect

Food Control

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

Mercury speciation in seafood using non-chromatographic chemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry method – Evaluation of methylmercury exposure

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ARTICLE INFO

Article history:
Received 28 April 2017
Received in revised form
4 July 2017
Accepted 5 July 2017
Available online 6 July 2017

Keywords:
Mercury speciation
Methylmercury exposure
Seafood
Photo-induced cold vapor generation
Optical emission spectrometry
Plasma microtorch

Chemical compounds:
Methylmercury (1+) (CH_3Hg^+) (PubChem CID:6860)
Mercury ion (Hg^{2+}) (PubChem CID:26623)

ABSTRACT

A non-chromatographic method was developed for Hg speciation as CH_3Hg^+ and Hg^{2+} using simple instrumentation based on optical emission spectrometry in a low power and low Ar consumption capacitively coupled plasma microtorch and detection with a low resolution microspectrometer. The method is based on (i) determination of total Hg after sample digestion in nitric acid-hydrogen peroxide mixture, derivatization to cold vapor with 20% $SnCl_2$ in 15% HCl medium; (ii) determination of CH_3Hg^+ separated by double liquid-liquid extraction as recommended in JRC Technical Report of European Commission, followed by photochemical vapor generation in 0.6 mol l^{-1} formic acid and (iii) calculation of the inorganic (Hg^{2+}) species as difference. The calibration curve was generated with external Hg^{2+} standards in 5% (v/v) HCl for total Hg determination and 0.6 mol l^{-1} HCOOH medium for CH_3Hg^+, respectively. The method was validated against certified reference materials and successfully applied for Hg speciation in most consumed fish species. The limits of detection of the method were 2 and 3 μg kg^{-1} CH_3Hg^+ and total Hg respectively, which made possible quantification of 6/9 μg kg^{-1} organic/total Hg. The method proved to be accurate with recovery of 101 ± 10% (total Hg), 100 ± 8% (CH_3Hg^+) and 102 ± 13% (Hg^{2+}), and precision in the range 2.4–7.8% (total Hg), 2.4–11.9% (CH_3Hg^+) and 3.8–14.0% (Hg^{2+}). The speciation method allowed accurate and precise determination of CH_3Hg^+ and Hg^{2+} even when the organic species represented more than 98% of total Hg. Total Hg in fish depended on variety but in all cases it was below the maximum admitted level (0.5 mg kg^{-1}). Methylmercury represented 22.3–93.3% of total Hg, with higher weights in salmon, carp, mackerel and hake and lower in cod, trout and pangasius irrespective of total Hg. Evaluation of risk exposure to CH_3Hg^+ from occasional fish consumption based on Provisional Tolerable Weekly Intake seems to be much more informative than the concentration of total or organic Hg species. It has been concluded from our study that there is no health risk from exposure to methylmercury for 300–1000/60-200 g weekly fish consumption by adult/child when fish comes from reliable sources.

1. Introduction

Fish meat is considered an important source of essential nutrients for the human body, such as polyunsaturated fatty acids (Omega 6), acting against hypertension, coronary heart diseases and cancer, but also the main source of exposure to mercury species, mostly methylmercury (CH_3Hg^+) recognized for its toxicity and high bioaccumulation factor in fish tissue and further transfer in the food chain (Ariya et al., 2015; Caroli, 2007; Cornelis, Caruso, Crews, & Heumann, 2005; EFSA, 2012; Sioen, Henauw, Verdomck, Thuyne, & Camp, 2007). The reason for the high bioaccumulation factor is the solubility of organic Hg species in lipids

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http://dx.doi.org/10.1016/j.foodcont.2017.07.006
0956-7135/

and their low elimination rate from the organism (Horvat, 2001; Mason & Benoit, 2003). Maximum levels for total mercury in foodstuffs are given in the European Legislation, namely 0.5 and 1 mg kg^{-1} for different seafood, but no maximum level for CH_3Hg^+ is set till now in Commission Regulation (2006/1881/EC). European Food Safety Authority (EFSA, 2012) established a Provisional Tolerable Weekly Intake (PTWI) of 1.3 μg kg^{-1} CH_3Hg^+ and 4 μg kg^{-1} Hg^{2+} body weight (b.w.). The Joint World Health Organization Expert Committee on Food Additives (JECFA, 2006, pp. 53–59; WHO, 1989) stipulated the maximum levels of 1 mg kg^{-1} in predatory fish and 0.5 mg kg^{-1} of CH_3Hg^+ for all other fish and proposed a PTWI for CH_3Hg^+ of 1.6 μg kg^{-1} b.w. According to the Scientific Opinion of EFSA (2012) on the risk for public health related to the presence of mercury and methylmercury in food following a study in 20 European countries during the period 2004–2011, dietary exposure to CH_3Hg^+ may result in neurodevelopmental, neurological and locomotion deficits, cardiovascular diseases and carcinogenicity. The same document reports a dietary exposure to methylmercury of 0.06–1.57 μg kg^{-1} b.w., most of which coming from fish consumption. However for high fish consumers PTWI can reach 7.48 μg kg^{-1} b.w. exceeding six times the set value. For infants, the exposure to CH_3Hg^+ via breast milk was found to be 0.09–0.94 μg kg^{-1} b.w. Unlike CH_3Hg^+, exposure to inorganic Hg coming mostly from no-fish food did not exceed PTWI (4 μg kg^{-1} b.w.) as it was in the range 0.13–4.06 μg kg^{-1} b.w.

Evaluation of total mercury/methylmercury levels in fish/fishery products on human risk and evaluation of exposure through intake of different types of fish have been the subject of several studies (Galimberti et al., 2016; Mieiro et al., 2016; de Paiva, Alves, Milani, Boer, Quintaes, & Morgano, 2016; Cardoso, Afonso, Lourenco, & Nunes, 2013; EFSA, 2012). For the determination of extremely low levels of Hg species in food, analytical techniques of high sensitivity, accuracy and precision are required. The classical methods mostly reported for the determination of total mercury are thermal decomposition atomic absorption spectrometry (TD-AAS) on solid matrix (Mieiro et al., 2016), inductively coupled plasma mass spectrometry (ICP-MS) after acidic attack of sample (Galimberti et al., 2016) and cold vapor generation atomic absorption spectrometry (CV-AAS) using common derivatization reagents ($SnCl_2$ or $NaBH_4$) (Fernandez et al., 2015; Shah et al., 2010). The non-chromatographic methods for CH_3Hg^+ determination in seafood involve sample hydrolysis with HBr followed by extraction with toluene, further separation of CH_3Hg^+ with L-cysteine and analysis of the extract by TD-AAS (de Paiva et al., 2016; Cardoso et al., 2013). The non-chromatographic approaches for the speciation as inorganic (Hg^{2+}) and organic (CH_3Hg^+) forms are based on extraction under non-oxidative conditions (HCl or a mixture of HCl and NaCl/KCl), separation of CH_3Hg^+ and Hg^{2+} species based on the difference in their covalent/ionic properties followed by cold vapor generation and detection by electrothermal atomic absorption spectrometry (CV-ETAAS) (Duarte, Bizzi, Antes, Dressler, & de Moraes Flores, 2009). The speciation can be also performed by taking advantage of the difference in reactivity of the Hg^{2+} and CH_3Hg^+ species to $SnCl_2$ and $NaBH_4$ in cold vapor generation and subsequent spectral detection by CV-AAS or cold vapor inductively coupled plasma atomic emission spectrometry (CV-ICP-AES) (Kaercher, Goldschmidt, Paniz, de Moraes Flores, & Dressler, 2005; Serafimovski, Karadjova, Stafilov, & Cvetkovic, 2008).

Greener derivatization approaches using less harmful reagents (low molecular weight organic acid such as formic acid) and environmentally friendly energies (Bendicho, Lavilla, Pena-Pereira, & Romero, 2012; Lavilla, Romero, Costas, & Bendicho, 2014; Yin, Liu, & Jiang, 2011) were successfully applied for the determination of total Hg and its speciation as Hg^{2+} and CH_3Hg^+ using non-chromatographic UV photochemical vapor generation atomic fluorescence spectrometry (UV-PVG-AFS), sono-induced cold vapor generation atomic fluorescence spectrometry (SI-CVG-AFS) (Zhang, Peng, Zheng, Xu, & Hou, 2016) and UV photochemical vapor generation atomic absorption spectrometry (UV-PVG-AAS) (Lopez-Rouco, Stanisz, Matusiewicz, Lavilla, & Bendicho, 2008; Vieira, Ribeiro, Curtius, & Sturgeon, 2007).

In our laboratory, analytical methods were developed for the determination of total Hg in water and food after $SnCl_2$-assisted or sono-induced cold vapor generation in formic acid and detection using a simple, miniaturized instrumentation based on optical emission spectrometry in a capacitively coupled plasma microtorch ($SnCl_2$-CV-μCCP-OES, SI-CV- μCCP-OES) (Frentiu et al., 2015a; Frentiu et al., 2015b; Frentiu et al., 2012).

Several hyphenated techniques such as liquid chromatography-cold vapor generation atomic fluorescence spectrometry (HPLC-CV-AFS) (Wang et al., 2010) and liquid chromatography-on-line UV irradiation-cold vapor–atomic fluorescence spectrometry (LC-UV-CV-AFS) (Li, Xu, Yang, & Wang, 2015; Zmozinski et al., 2014) and gas chromatography-mass spectrometry (GC-MS) (Berzas Nevado et al., 2011) were reported in literature for mercury speciation as CH_3Hg^+ and Hg^{2+} in seafood.

Reviews of state-of-the-art methods of sample preparation and chemical analysis for the control of contaminants in foodstuffs and food raw materials were recently published (Amelin & Lavrukhina, 2017; Ferreira et al., 2015; Gao et al., 2012; Taylor et al., 2017). Performance of laboratories in Hg speciation analysis as CH_3Hg^+ in seafood was assessed by the European Commission (Baer et al., 2011). Speciation of Hg should be taken into consideration as it provides information of the level of CH_3Hg^+ in fish, which will improve exposure assessments although maximum limits for this species has not yet been set in European legislation (Baer et al., 2011). From the results collected by EFSA (2012) on mercury in food including fishery products, most were for total mercury (98.2%) and only 1.8% for methylmercury. This is probably because methylmercury is considered a rather problematic species in terms of sample preparation and measurement and has been generally thought that its quantification in food requests sophisticated instrumentation (Baer et al., 2011; Berzas Nevado et al., 2011).

The aim of this work was the development of a simple, non-chromatographic method for mercury speciation as CH_3Hg^+ and Hg^{2+} in fish and evaluation of risk exposure to CH_3Hg^+ through occasional consumption of fish. Only fish was taken into consideration as it is consumed all over the world (average 20 kg/capita per year), (FAO, 2016) and represents the main source of human exposure to CH_3Hg^+. The non-chromatographic method developed on a miniaturized instrumentation was based on low power and low Ar consumption capacitively coupled plasma microtorch optical emission spectrometry (μCCP-OES) using a low resolution microspectrometer. The developed method involved: (i) determination of total Hg after sample digestion in nitric acid-hydrogen peroxide mixture and on-line derivatization to cold vapor using the $SnCl_2$-HCl system ($SnCl_2$-CV-μCCP-OES); (ii) determination of CH_3Hg^+ species after selective extraction and on-line UV-photochemical vapor generation in formic acid medium (formic acid-UV-PVG-μCCP-OES) and (iii) calculation of inorganic (Hg^{2+}) species as difference. The figures of merit (limit of detection and quantification, precision and accuracy) for the determination of total Hg and CH_3Hg^+ species by the proposed method were assessed. Accuracy and applicability of the method were demonstrated by analyzing certified reference materials and fish test samples.