Journal of Food Composition and Analysis 38 (2015) 7–12

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Research Article

Evaluation of different digestion systems for determination of trace mercury in seaweeds by cold vapour atomic fluorescence spectrometry

Rodolfo Fernández-Martínez [a], Isabel Rucandio [a,*], Isabel Gómez-Pinilla [b], Fernando Borlaf [a], Fernando García [a], María Teresa Larrea [c]

[a] Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Av. Complutense, 40, 28040 Madrid, Spain
[b] CAI de Técnicas Geológicas, Universidad Complutense de Madrid, C/ José Antonio Novais, 2, 28040 Madrid, Spain
[c] Centro Nacional de Investigaciones Metalúrgicas (CSIC), Avda. Gregorio del Amo, 8, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 6 September 2013
Received in revised form 19 September 2014
Accepted 2 October 2014
Available online 4 November 2014

Keywords:
Mercury
Seaweeds
Food analysis
Digestion procedure
Cold vapour atomic fluorescence spectrometry
Sample pretreatment
Food safety
Heavy metal contamination of seafood
Food composition

ABSTRACT

Four methods for acid digestion of seaweeds were compared in 10 commercially available seaweeds: (i) in stainless steel-Teflon®PTFE-bombs at high pressure and temperature, (ii) in closed-Teflon®PFA-vessels at high pressure and temperature, (iii) in open-polypropylene-tubes with reflux caps in a graphite heating block at high temperature and (iv) in closed-TFM™PTFE-vessels with microwave-assisted controlled pressure and temperature. Hg was determined in all digests by cold vapour atomic fluorescence spectrometry (CV-AFS). Assessment of digestion methods was performed by comparison with the results obtained for total mercury determination by the Method EPA 7473, based on direct mercury analysis in the solid samples, and with a reference material BCR-279. The open vessel digestion system with reflux in a graphite heating block at high temperature constitutes the best choice since it was found to give the better Hg extraction (83–103%) as well as the lowest variability, being RSD $< 10\%$ for most of the studied seaweeds. A previous freeze-drying and intensive grinding was the best pre-treatment. Similar results were obtained with and without the presence of oxidizing agents ($KMnO_4$, $K_2Cr_2O_7$) and with different tube-materials (borosilicate glass, PTFE and polypropylene).

1. Introduction

In recent decades, there has been an incredible increase in direct consumption of seaweed as food in Western countries, partly because of the nutritional and therapeutic benefits these products provide. Seaweeds, as processed and unprocessed food, have a commercial value of several billion dollars annually. Approximately 500 species are eaten by humans, and some 160 are commercially important. In addition to the use of algal extracts in prepared foods, seaweeds are eaten directly in many parts of the world. The ability of marine seaweeds to effectively retain mercury as well as other heavy metals is well known (Ródenas de la Rocha et al., 2009). It has been suggested that seaweeds could absorb mercury from seawater and even from the atmosphere. Hence, seaweed consumption is of concern since it represents a source of mercury for human beings (MacArtain et al., 2007; Løvstad Holdt and Kraan, 2011).

Published data on Hg content in seaweeds are scarce because of this element usually occurs at very low concentration levels. Hence, Hg determination usually requires a preconcentration step (Fernández-Fernández et al., 2007) and/or very sensitive analytical techniques. Quantification of total mercury in food products can be performed by cold vapour atomic fluorescence spectrometry (CV-AFS) (Almela et al., 2002; Josef et al., 2008; Liu et al., 2008; Fu et al., 2011). Its inherent sensitivity offers very low detection levels and wide lineal dynamic range. This makes CV-AFS a powerful analytical tool clearly advantageous over atomic absorption techniques. In addition, the required instrumentation is greatly more cost effective and simpler than that required for mass spectrometry techniques (Morita et al., 1995; Brahma et al., 1997; Leermakers et al., 2005).

Prior to quantification by the CV-AFS instrument, mercury must be extracted from seaweeds products using a digestion method. The literature about sample digestion for total analysis is extensive

* Corresponding author. Tel.: +34 913466570.
E-mail address: isabel.rucandio@ciemat.es (I. Rucandio).

http://dx.doi.org/10.1016/j.jfca.2014.10.003
0889-1575/

due to the numerous combinations of acid reagents (De Oliveira, 2003; Sneddon et al., 2006; Capar et al., 2007; Gao et al., 2012). Losses due to volatilization must be avoided in order to select a suitable digestion method for determining elements that can be present as volatile compounds as occur with Hg (De Oliveira, 2003). Hence, the selected digestion method ideally must be capable of removing the total mercury content with no losses. A number of digestion methods for mercury analysis in seafood have been previously described (Phaneuf et al., 1999; Almela et al., 2002; Muniz-Naviero et al., 2004; Kelly et al., 2006; Misheer et al., 2006; Besada et al., 2009; Reyes et al., 2009; Clemens et al., 2011). Among them, traditional methods based on closed vessels combined with conventional heating are still frequently reported (Phaneuf et al., 1999; Kelly et al., 2006; Besada et al., 2009).

However, most methods are based on microwave acid digestions (Almela et al., 2002; Muniz-Naviero et al., 2004; Environmental Monitoring Division, 2005; Misheer et al., 2006; Reyes et al., 2009). Low digestion time and solvent consumption are the main advantages of this technique over traditional methods. Although open vessel digestion methods are traditionally not recommended for mercury analysis due to the high risk of losing volatile compounds, in the last years several graphite block digestion systems have been successfully applied to mercury determination in seafood (Environmental Monitoring Division, 2005; Clemens et al., 2011).

The overall aim of this work was the evaluation of different digestion methods for determining the total Hg content in seaweeds. For this purpose, 10 algae food products commercially available as well as a certified reference material were digested with four different methods and then mercury was determined by CV-AFS. The four digestion methods evaluated were: (i) digestion in stainless steel-Teflon®PTFE bombs at high pressure and temperature (PA), (ii) digestion in closed vessels at high pressure and temperature in Teflon®PFA vessels (SV), (iii) digestion assisted by microwave in closed TFM™PTFE vessels with controlled pressure and temperature (MW) and (iv) reflux digestion in open vessels by means of a graphite heating block with controlled temperature in polypropylene tubes (DP). Several parameters have been considered for the analysis optimization such as grinding, homogenization, sample to solution ratio, oxidizing reagents and the vessel materials, among others. Assessment of digestion methods was performed by comparison with the results obtained by the Method EPA 7473, based on direct mercury analysis using thermal decomposition, amalgamation and atomic absorption spectrometry. This method has been validated for determination of Hg in solid materials (EPA, 2007).

2. Materials and methods

2.1. Instrumentation

Total mercury contents in digested seaweeds were determined by CV-AFS using a PS Analytical Millenium Merlin Instrument equipped with a mercury hollow cathode lamp and a Perma pure drying membrane (Perma Pure Products, Farmingdale, NJ, USA) for drying the generated mercury vapour. Direct mercury analysis in solid samples was performed by thermal decomposition, catalytic conversion, amalgamation and atomic absorption spectrophotometry using a DMA-80 instrument (Milestone, Sorisole, Italy).

Microwave digestions were performed by an Ethos One microwave system (Milestone, Sorisole, Italy) equipped with sensors for temperature and pressure control in all vessels and direct temperature and pressure control in a single reference TFM™PTFE vessel. Screw top Teflon®PFA vessels (Savillex) and stainless steel-Teflon®PTFE Parr Acid Digestion Bombs (Parr Instrument Company, Moline, IL, USA) were used for digestions at high pressure with conventional heating. Finally, open polypropylene tubes digestions were carried out with a Digiprep Jr block digester (SCP Science, Montreal, Canada) equipped with a temperature–time programmable controller.

Other equipment included a drying thermostatized oven (Proeti S.A.) with a maximum adjustable temperature of 200 °C and a bench-top planetary automatic ball mill (Retsch ball mill PM100).

2.2. Materials and reagents

All glass and PTFE material used for digestion methods was exhaustively washed with a common laboratory detergent, thoroughly rinsed with tap water and dried in an oven at 105 °C. Then, PTFE and glassware were soaked in a 25% clean nitric acid bath overnight following the recommendations of Da Silva et al. (2010). Afterwards, all the material was rinsed three times with ultrapure water, dried in an oven at 105 °C and stored until analysis. Regarding Hg determination by CV-AFS, glassware used for preparing, reducing and carrier solutions as well as Hg standard solutions were cleaned with a 0.002 M KBr and 0.002 M $KBrO_3$ solution overnight and rinsed with ultrapure water.

All reagents were purchased from Merck (Darmstadt, Germany). The reagents $K_2Cr_2O_7$ (Hg < 0.000001%), $KMnO_4$ (Hg < 0.000005%), $SnCl_2$ (Hg < 0.000001%) and $KBrO_3$ were of reagent grade while KBr, 65% HNO_3 and 37% HCl were of suprapur quality. Ultrapure water (resistivity ≥18.2 MΩ cm) was obtained by a Milli-Q Element A10 (Millipore, Bedford, MA, USA). A Hg stock standard solution of 1000 mg L^{-1} in 5% HNO_3 (Merck, Darmstadt, Germany) was used to prepare the Hg standard solutions. Argon gas of purity higher than 99.999% was used as carrier gas in CV-AFS instrument. Dry air was used as dryer gas.

The certified reference material BCR-279 (sea lettuce, *Ulva lactuca*) (Community Bureau of Reference, Belgium) with a recommended Hg concentration of 47.6 ± 4.2 μg kg^{-1} was used as reference material for comparative purposes.

2.3. Samples and sample pretreatments

In this study, 10 products commercially available from green algae (Chlorella), blue-green algae (Spirulina), red algae (Chondrus, Dulse, Nori) and brown algae (Hiziki, Wakame, Arame, Agar and Kombu) were bought as dried material or pellets from local supermarkets in the city of Madrid (Spain). Approximately 20 g of each product were dried in a thermostatized oven at 60 °C for 24 h and then divided into two fractions for subsequent pre-treatments. Two different pre-treatment methods were tested in all samples: (i) manual grinding of about 10 g of sample in an agate mortar and (ii) freezing at −18 °C of about 10 g of sample followed by grinding in an automatic ball mill.

2.4. Digestion procedures

All the samples considered were previously subjected to manual grinding in an agate mortar. From this mass, 0.5 g of sample were accurately weighed for the digestion procedure.

Experiences for the selection of the most appropriate digestion method were conducted subjecting samples to manual grinding in an agate mortar. Then, 0.5 g of sample were accurately weighed. Subsequent experiments for the optimization of the selected method and for the study of oxidizing reagents and vessel material were conducted subjecting samples to freezing and automatic grinding with 0.25 g sample weight.

All digestions were conducted in triplicate with 6 mL of concentrated HNO_3. In the case of microwave digestions the appropriate temperature control requires a minimum volume in the vessels of 8 mL. Then, 2 mL of water were added to the