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High intensity microwave mineralization of commercial canned tuna for the subsequent determination of total mercury by cold vapor atomic absorption spectrometry

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Abstract

The presence of mercury (Hg) in aquatic food chains is worth concern. These circumstances demand reliable analytical procedures to quantify Hg in highly-consumed commercial canned seafoods. such as tuna. Cold vapor atomic absorption spectrometry (CVAAS) is preferentially selected for Hg evaluation (1). In this work we present the successful mineralization of tuna material (Eveba, Propisca SA, Carúpano, Venezuela), based upon the use of high intensity (ca. 950 W of full power) microwave irradiation and closed reactors provided with fiber optic probes for pressure and temperature control, Approximately 200 mg of lyophilized tuna and 15 mL of concentrated nitric acid were placed in the sample vessel and capped with a cover that employs a pressure assisted type seal for leak-free operation (e.g., as internal vapor pressure rises from microwave heating of the liquid, the seal is energized, forming a progressively tighter seal with increasing pressure). The vessel was put into the microwave-transparent body of the bomb and closed. The system was placed into the high intensity microwave oven and irradiated for 300s at 100 % power (equivalent to 950 W and 2450 Hz); the optimized temperature and pressure were 190°C and 190 psi, respectively. Two positive circumstances for the analyst were found when using the high intensity microwave system. Firstly, temperature and pressure conditions inside the sample vessel were monitored and controlled adequately, guaranteeing a much safer mineralization than those reported previously, avoiding chances for explosion of the reactor. Secondly, sample vessels were able to deal with a large organic sample size (ca. < 0.5 g), which favored the adjustment of the detection limit of the CVAAS analysis. Test portions of lyophilized tuna were also mineralized in accordance with a reported method. In both cases, digestion solutions were analized by CVAAS to quantify total Hg. No significant differences were observed between the proposed decomposition procedure (1.47± 0.01 μg/g of Hg) and that reported (1.45 ± 0.10 μg/g of Hg). The average precision was better than 3,5 % (RSD). The commercial tuna analyzed presented a higher mercury content than the upper mercury level recommended for fish 10.5 μg/g) by the World Health Organization. Additionally, the Action Level in fisb of 1 μg/g (wet weight), established by the U.S. Food and Drug Administration, was also exceeded. It is concluded that mineralization was efficiently achieved and the analyte element was freed from chemical bonding, allowing the posterior quantification of total Hg by CVAAS.

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Key words: Cold vapor atomic absorption spectrometry; high intensity microwave; total mercury.

Mineralización de atún comercial enlatado con microondas de alta intensidad para la determinación posterior del mercurio total por espectrometría de absorción atómica con vapor frío

Resumen

El mercurio (Hg) es un metal extremadamente tóxico . Su determinación espectrométrica requiere de una previa mineralización de la muestra para destruir la materia orgánica, garantizando la liberación del analito sin pérdidas por volatilización. Se presenta un procedimiento para la rápida mineralización de atún comercial (Eveba, Propisca SA, Carúpano, Venezuela), utilizando calentamiento por microondas de alta intensidad y control de presión y temperatura. Posteriormente, se determinó el contenido de mercurio total por espectrometría de absorción atómica con vapor frío (CVAAS). Se pesaron alícuotas de aproximadamente 200 mg de atún liofilizado, adicionándose 15 mL de ácido nítrico concentrado. Las muestras se mineralizaron en reactores cerrados provistos de sensores de presión y temperatura, irradiándose en un horno de microondas a 950 W de potencia (e.g., la potencia convencional es de 600 W). Las condiciones óptimas de temperatura, presión y tiempo de mineralización fueron 190°C, 190 psi y 5 minutos, respectivamente. Se encontraron dos circunstancias significativas cuando se usó el sistema con microooondas de alta intensidad. En primer lugar, las condiciones de temperatura y presión dentro del recipiente fueron monitoreadas y controladas adecuadamente, garantizando de esta manera la seguridad del procedimiento, al evitar la explosión de los reactores. En segundo lugar, el recipiente permitió un tamaño considerable de muestra favoreciendo así el ajuste del límite de detección para el análisis por CVAAS. Tambien se mineralizaron alícuotas del atún utilizando un método reportado. No se observaron diferencias estadísticamente significativas entre la concentración de mercurio total encontrada en el atún mineralizado por el procedimiento propuesto (1,47± 0,01μg/g de Hg) y el valor certificado para la misma muestra utilizando la metodología reportada (1,45± 0,10 μg/g de Hg). La precisión hallada (< 3,5 % DSR) fue adecuada para este tipo de análisis. El contenido mercurial encontrado fue superior al permitido por la Organización Mundial de la Salud (0,5 μg/g). En conclusión, la mineralización fue rápida, obteniéndose un digerido adecuado para la subsiguiente determinación cuantitativa del mercurio por CVAAS.

Palabras claves: Espectrometría de absorción atómica con vapor frío; mercurio total; microonda de alta intensidad.

Introduction

Mercury (Hg) is among the most dangerous elements on earth because the potential hazards that it creates are related to its irreversible toxic effects. Mercury is accumulated throughout the food chain and can, finally, reach the human being. The

presence of mercury in aquatic environments is worth concern. Marine Hg contamination can severely damage the ecosystem, affecting populations of frequently consumed marine foodstuffs (1). In the body, mercury concentrates in the inner organs (i.e., brain, kidney, liver, etc.) because of its strong affinity for the S-H groups present in these organs (2,3). When in the metallic state, mercury can be transformed into several organic species by bacteria-governed methylation processes in the presence of sunlight (3). Methylmercury is more soluble and bioavailable and, therefore, is easily absorbed by humans, increasing its toxicity. All these circumstances demand reliable analytical procedures to quantify Hg in highly-consumed commercial seafoods, namely canned tuna.

As analytical sample, seafoods are complex matrices. Most methods of analysis require prior mineralization of these materials (4). Several decomposition procedures have been recommended for fish samples, but many of these techniques are time-consuming (4-6). Another problem in the sample digestion for the determination of mercury is the volatility and mobility of this element (7). Many procedures have been reported for the mineralization of biological samples containing mercury (7-11). Recently, there has been great interest in using microwave to accelerate the decomposition of a variety of solid samples (8,12,13). This is the result of sample superheating by interaction of molecular dipoles and ions with the rapidly oscillating electromagnetic field of the microwaves. On the contrary, traditional heating methods use energy transmitted to the samples via the container primarily by conduction and advection (14),extending thus the mineralization time. The merits of pressurized acid digestions in closed PTFE vessels using microwave heating are widely recognized, particularly the increased speed and reduced losses of volatile elements, (15,16).

Several techniques are currently available for the determination of mercury in biological materials (11,17-20). These include electrothermal atomization atomic absorption spectrometry (18,21,22), inductively coupled plasma mass spectrometry (19,23), nuclear magnetic resonance (24), capillary column gas chromatography (20), and cold vapor atomic absorption spectrometry (CVAAS) (11,12,19,25,26,27). However, the latter is preferentially employed because of its extremely high sensitivity, the absence of background attenuation-type spectral interferences and the relatively low operating costs (11,28,29). Measurement of Hg by CVAAS requires sufficient oxidation of the organic matter present in the sample to liberate the analyte element from its chemical bonding; hence, a single labile mercury species (e.g., Hg^{II}) is produced, being afterwards reduced quantitatively to Hg^{0} for spectrometry evaluation (assuming that volatilization losses of mercury are eliminated) (17).

Tahán *et al.* (17) recently reported the mineralization of biological materials lasting for 70s, using conventional microwave heating. (600 W of full power). A microwave digestion system that utilized a non-invasive infrared probe assembly to monitor digestion vessel temperature was recently described; the output of the probe assembly fed a data acquisition system and a digital computer was programmed to control the operation of the microwave oven magnetron (30).

In this paper we present the successful mineralization of commercial canned tuna material based upon the use of high intensity (ca. 950 W) microwaves and closed reactors provided with fiber optic probes for pressure and temperature controls. Posteriorly, Hg was determined by cold vapor atomic absorption spectrometry.

Materials and Methods

Apparatus

A Perkin-Elmer Model 460 atomic absorption spectrometer (Norwalk, CT, USA), equipped with a mercury hollow cathode lamp operated at 6 mA (spectral bandpass 0.7 nm) and at a resonance wavelength of 253.6 nm, was used throughout this work. A Perkin-Elmer Model MHS-10 mercury/hydride system was attached to the spectrometer to generate the mercury vapors. Nitrogen (inlet pressure of 255 kPa) was the purge gas. Instrumental conditions are shown in Table 1. The commercial tuna was freeze-dried in a Virtis Unitrapp Model II lyophilizer (Gardiner, NY, USA), kept at -60°C for 24 h. Mineralizations were performed with closed reactors provided with fiber optic probes for pressure and temperature controls (CEM Corporation, Matthews, NC, USA), irradiated in a CEM Model MDS-2100 laboratory microwave oven (950 W for 100% power).

Reagents

All chemicals were of analytical-reagent grade. The sodium tetrahydroborate solution (3% m/v) was prepared by dissolving sodium tetrahydroborate powder (Riedel-de Haën, Hannover, Germany) in appropriate amounts of grade I [as established by the American Society for Testing and Materials (ASTM), electrical resistivity 16.6 MΩ/cm at 25°C] (31) triply-distilled and de-ionized water, then stabilized with 1% m/v sodium hydroxide (J. T. Baker, Phillipsburg, NJ, USA). This solution was prepared daily before use. Concentrated nitric acid (Riedel-de Haën) was used during the digestion procedures. The stock solution (1,000 mg/L of Hg) was prepared from Titrisol (Merck) concentrates. Standard solutions were freshly prepared by serial dilution of the stock with 0.01 mol/L nitric acid.

For accuracy evaluation, the following standard reference materials were used: Albacore Tuna RM 50 and Oyster Tissue SRM 1566a from the National Institute of Standards and Technology (US Department of Commerce, Gaithersburg, MD, USA); and Pond Sediment (NIES No. 2) from the National Institute for Environmental Studies (Ibaraki, Japan).

Samples

Tuna samples were obtained by pooling 40 cans of a commercial brand (Eveba, Propisca SA, Carúpano, Venezuela), lyophil-

Table 1
Instrumental conditions for the CVAAS determination of total mercury in commercial canned tuna

Wavelength/nm	253.6
Hollow cathode lamp current/mA	6
Slit-width/nm	0.7
Absorbance measured	Peak height
Measurement time/s	45
Nitrogen carrier gas pressure/KPa	255
Signal scale expansion	None
Flow rate of reducing agent/mL/min	17

ized, ground, mixed and kept in polyethylene bags at 4°C until analysis.

Mineralization Procedure

Approximately 200 mg of lyophilized tuna and 15 mL of concentrated nitric acid were placed in the sample vessel and capped with a cover that employs pressure assisted type seal for leak-free operation (e.g., as internal vapor pressure rises from microwave heating of the liquid, the seal is energized, forming a progressively tighter seal with increasing pressure). The vessel was put into the microwave-transparent body of the bomb and closed. The system was placed into the high intensity microwave oven and irradiated for 300 s at 100% power (equivalent to 950 W and 2450 MHz); the optimized conditions for temperature and pressure were 190°C and 190 psi, respectively. After cooling to ambient temperature, the final mineralization solution was transferred into a 25-mL calibrated flask and diluted to volume with grade I ASTM triply-distilled, de-ionized water. Blanks were prepared with the same reagents, without the samples, undergoing a similar digestion treatment. Mineralization were done in triplicate.

Mercury determination by CVAAS

An aliquot of the sample digestion solution (volume ranged between 2 and 5 mL depending on mercury concentration) was placed into the generator vessel of the mercury/hydride system. Nitric acid solution (1.5% v/v) was added to obtain a final volume of 20 mL. Sodium tetrahydroborate solution was added and the mercury vapors generated were directed to the optical cell. The absorbance reading was taken at the maximum value reached.

Working curves were obtained by adding 10, 20 y 50 µL of a 1.0 mg/L Hg solution to the reaction flask of the mercury/hydride system, which represented 10, 20 y 50 ng of Hg, respectively.

Results and Discussion

As reported before (11,17), the lyophilization procedure resulted in mercury losses (by mass) lower than 1% and a loss of water of 66%; the mercury losses were determined from recoveries of Hg spikes added to test portions undergoing lyophilization. Mercury concentrations found for samples collected and stored in plastic containers were not significantly different ($p>0.001$) from those of samples manipulated with glass materials (11,17). During sample pre-treatment, mercury losses by volatilization were eliminated by carrying out the acid mineralization in sealed vessels. Mercury standards were prepared daily in order to avoid losses due to metal adsorption on the container walls and volatilization. The determination of total mercury by CVAAS from undigested samples was not feasible, as the presence of organic matter is a source of interferences (29,32). A mineralization procedure was developed in order to remove concomitant substances and produce suitable digestion solutions for CVAAS analysis.

Addition of 15 mL of concentrated nitric acid was sufficient to mineralize all organic matter contained in about 200 mg of commercial tuna. The temperature and pressure conditions inside the sample vessel were monitored and controlled adequately (Figure 1), guaranteeing a much safer mineralization than those reported previously, avoiding chances for explosion of the reactor system. Moreover, sample vessels were able to deal with a larger organic sample size (ca.<0.5 g), which favored the adjustment of the detection limit of the CVAAS analysis. The mineralization procedure using Teflon PFA vessels of 120 mL, heated by irradiation with microwaves of high intensity, required a total time of 300 s, without cooling intervals.

Accuracy was tested by analysis of the standard reference materials Albacore Tuna, Pond Sediment and Oyster Tissue. These materials were decomposed by using

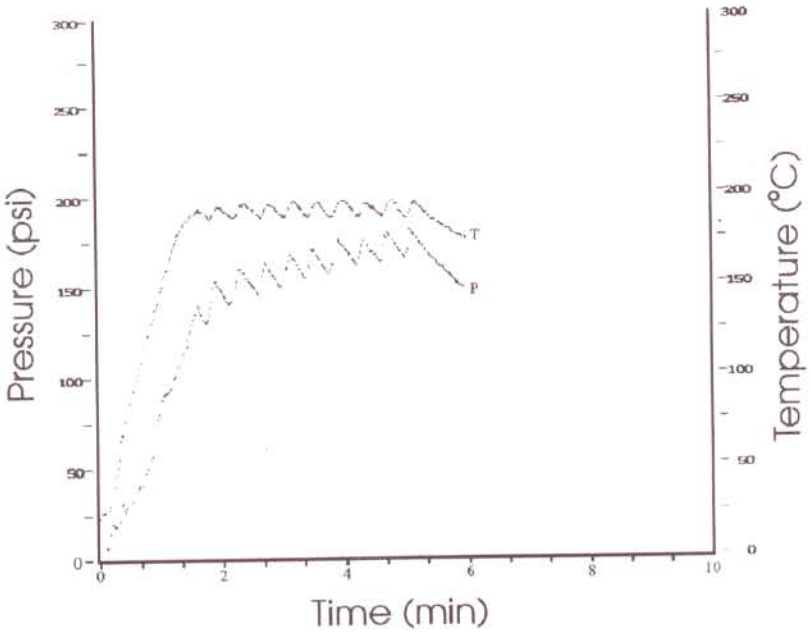

Figure 1. Monitoring temperature and pressure in closed reactors heated by microwave irradiation in the MDS-2100 microwave oven.

Table 2
Accuracy of the determination of mercury using the proposed mineralization procedure and CVAAS

Reference material[a]	Concentration of Hg±1 SD/μg/g	
	Certified	Experimental
Oyster Tissue[b]	0.0642±0.0067	0.0670±0.007
Pond Sediment[c]	1.3[d]	1.3 ±0.02
Lyophilized commercial Tuna	1.45±0.10[e]	1.42±0.04
Albacore Tuna[f]	0.95±0.10	0.96±0.08

[a] Seven aliquots of each sample mineralized; three determinations per aliquot perfomed
[b] NIST SRM 1566a from the National Institute of Standards and Technology (USA)
[c] NIES No. 2 from the National Institute for Environmental Studies (JAPAN)
[d] Provisional value
[e] Reported value from reference 17
[f] NIST RM 50

Table 3
Recovery of mercury added to freeze-dried commercial tuna mineralized by high intensity microwave heating.
Triplicate analyses of each sample were performed with three runs each

Sample	Mass of Hg/ng			
	Added	Expected	Found	Recovery (%)
Tuna	20	36	37	103
	40	56	54	96
	60	74	76	97
			Average	99±4

Table 4
Within- and between-run precision study for mercury in different real samples mineralized by using microwave heating and analyzed by CVAAS

Undiluted samples	Mean Hg concentration (μg/g)	Within-run[a]		Between-runs[b]	
		SD (μg/g)	RSD (%)	SD (μg/g)	RSD (%)
Freeze-dried tuna	1.42	0.04	2.8	0.05	3.5

[a] Triplicate samples; three runs each
[b] Triplicate samples per analysis; three runs each

the proposed mineralization procedure. Under these conditions, the total mercury concentrations found by CVAAS for the standard reference materials were statistically indistinguishable ($p>0.001$) from the certified values. Results are shown in Table 2 and attest to the excellence of the analytical method. Test portions of lyophilized tuna were also mineralized by the reported method of Tahán *et al.* (17). In both cases, digestion solutions were analyzed by CVAAS to quantify total Hg. No significant differences ($p>0.001$) were observed between the proposed decomposition procedure (1.42±0.04 μg/g of Hg) and that reported (1.45±0.10 μg/g of Hg) (see Table 2). The reliability of the mineralization was further assessed through a recovery study. This was done by performing triplicate determinations of mercury in different mercury-spiked aliquots of the freeze-dried commercial tuna (Table 3). The study of non-spectral interferences was carried out by comparing the slopes of the working curves with those obtained by the method of standard additions. The slope of the standard additions graphs were identical to those of the aqueous standard calibration graphs. This implied the absence of non-spectral interferences in the CVAAS determination of mercury in these types of samples (canned tuna and standard reference materials) under the proposed analytical conditions, and permitted the use of either the calibration graphs or standard additions method for quantification. The average precision was better than 3.5% (RSD), for both the within- and between-run analysis (Table 4).

Working graphs with a linear range of 10-200 ng of Hg were obtained. Most of the

samples analyzed were within this linear range. Peak height absorbance readings (A_p) increased linearly in relation to the mass (in ng) of mercury (C) present according to the equation $A_p = 0.0010\ C$ (correlation coefficient 0.9999). The amount of Hg required to give a 1% absorption was 4.4 ng (11,17). The detection limit, defined as twice the standard deviation of the blanks, was 53 ng/L which corresponds to 159 pg of mercury for 3 mL of solution undergoing analysis (11,17). These results can be considered adequate for this kind of analysis.

The commercial tuna analyzed presented a higher mercury content than the upper mercury level recommended for fish (0.5 μg/g) by the World Health Organization (33). Additionally, the Action Level in fish of 1μg/g (wet weight) established by the U.S. Food and Drug Administration (34), was also exceeded. A tuna can weights approximately 140 g, representing a mercury intake of about 0.2 mg. Hence, an individual may incorporated 8.4 mg of Hg, in about one year, considering an intake of five cans monthly. At the present time, the consumption of canned tuna has increased to compensate for the high cost of other foods, representing a real health threat.

Results show that no mercury losses by volatilization or adsorption were observed with the proposed mineralizations. In conclusion, the use of high intensity microwave systems is an attractive alternative to overcome problems related to sample size and safety for acid mineralizations carried out in closed vessels.

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