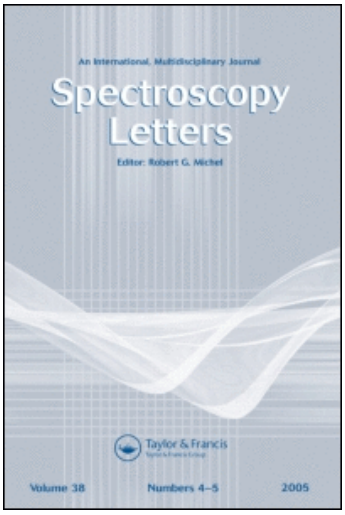

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Preliminary Investigations of Correlations Between Total Mercury in Tuna and Quality Control, and Mercury Recoveries Using Microwave Digestion

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Preliminary Investigations of Correlations Between Total Mercury in Tuna and Quality Control, and Mercury Recoveries Using Microwave Digestion

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ABSTRACT Mercury levels in commercially available tuna are of particular concern, as mercury found in tuna exists mostly as highly toxic methylmercury compounds that are readily absorbed by the gastrointestinal tract in humans. For five brands of canned tuna purchased locally and analyzed in quadruplicate for Hg via microwave digestion and cold vapor atomic absorption spectrometry (CVAAS), Hg levels ranged from 0.19-(±0.07)-μg Hg/g to 3.60-(±0.17)-μg Hg/g. Statistical analysis of the results suggested that three of the brands of tuna studied were statistically comparable and that two of the brands were significantly different, at the 95% confidence interval. Mercury recoveries for external calibration standards and known amounts of mercury added to tuna samples indicated minimal or no loss of Hg during microwave digestion.

KEYWORDS calibration, cold vapor atomic absorption spectrometry (CVAAS), mercury, microwave digestion, recovery, sample inhomogeneity, tuna

INTRODUCTION

Among the many species of tuna are the large bluefin, the smaller and more popular albacore, and the yellowfin.[1] Tuna usually remain in the upper portion, or "mixed layer", of the ocean, in which warm air and sunlight are more abundant.[1] Mercury is a naturally occurring element that is released into the air by industrial processes as well as naturally via geothermal vents, mainly as inorganic Hg(II).[2–4] Mercury released into the air via industrial sources is eventually deposited on land or in water, in which bacteria and other microorganisms can chemically methylate the mercury to form highly toxic methylmercury, CH_3Hg, and other organomercury compounds.[3–5] Of mercury accumulated in tuna, 80–90% exists as methylmercury,[2–6] which is easily absorbed into blood and muscle tissue. The predatory, long-lived tuna can accumulate significant concentrations of methylmercury by feeding on small fish that have accumulated methylmercury within their tissues.[2–4,6]

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Mercury entering muscle tissue in tuna binds to sulfhydryl groups in proteins in tuna.[7] The estimated half-life of methylmercury in fish is between 0.5 and 2 years.[8] Studies have shown no detectable emission of methylmercury for tuna, possibly due to the amount of methylmercury absorbed surpassing that excreted from muscle tissue.[8] Skinning, trimming, or cooking tuna or other fish does not appear to decrease mercury concentrations; instead, cooking tuna draws out moisture and increases overall mercury concentration.[9]

Tuna, which figures prominently in the diets of various cultures worldwide, has been shown to be a primary source of dietary methylmercury contamination in humans.[9,10] Methylmercury, with an estimated half-life of 44–80 days,[8] is almost entirely absorbed by the gastrointestinal tract[11] due to its lipophilicity.[11–13] This toxic property of methylmercury poses a significant threat to pregnant women and unborn children;[11–13] disrupts the central nervous, cardiovascular, and gastrointestinal systems; damages kidneys; and may potentially lead to attention-deficit and other neurological disorders in children and memory loss and heart disease in adults.[14–17] The FDA-mandated limit of human consumption for mercury is presently set at 1 microgram Hg per g of tuna.[18]

This paper will present preliminary results of our work. Additionally, this paper will attempt to address a concern of the investigators that microwave digestion, employed for decomposition of tuna samples, may lead to loss of mercury.

MATERIALS AND METHODS

Apparatus, Reagents, and Solutions

Spectral measurements were made using a Bacharach Model MAS-50 mercury analyzer (Bacharach, Inc., Pittsburgh, PA, U.S.A.) equipped with a BOD bottle reaction vessel plus impinger, and a Drierite scrubber to remove moisture, and operated according to manufacturer suggestions. A conventional microwave oven, with variable power settings, was used for digestion of the tuna samples. Microwave digestions were performed in 60-mL Teflon containers with screw-cap Teflon lids (Savillex Co., Minnetonka, MN, U.S.A.). Tin(II) chloride dihydrate, $SnCl_2 \cdot 2H_2O$, was purchased from Fisher Scientific, Inc. (Pittsburgh, PA, U.S.A.). NIST-traceable stock mercury standard solution, 1000-μg Hg mL^{-1}, was purchased from GFS Chemicals, Inc. (Powell, OH, U.S.A.). Concentrated nitric acid, HNO_3, and concentrated hydrochloric acid, HCl, were purchased from Fisher and used for digestion of tuna and preparation of tin(II) chloride solution, respectively.

A 10% (w/v) solution of tin(II) chloride was prepared by dissolution of 20.0 g of reagent-grade tin(II) chloride dihydrate in an equivolume mixture (100 mL each) of 12-M HCl and deionized water, and subsequently stored in a clean plastic bottle. A 10.0-μg Hg mL^{-1} working standard solution was prepared by accurate one-hundred-fold dilution of an accurately measured aliquot of the 1000-μg Hg mL^{-1} stock standard solution to the appropriate volume.

Experimental Procedure

Microwave Digestion of Tuna Samples

For each brand of tuna, we drained the tuna of excess liquid, took four 1–2-g samples of tuna directly from the can, and weighed each sample. Each weighed sample was transferred into a microwave digestion vessel, along with 5.0-mL concentrated HNO_3. The vessel was sealed and irradiated for 20s at a power level of 5 (approximately 50% power) and then allowed to cool for 60–120s. Successive 20-s irradiation intervals, at the same power level and cool-down time as initially performed, were repeated as needed until the tuna was completely digested. After final cooling, the digest was transferred, with deionized water rinsing, to a 25-mL volumetric flask and subsequently diluted to volume with deionized water and mixed well. The digest was then transferred to a clean, labeled plastic container for storage.

Determination of Mercury in Tuna by CVAAS

Mercury was determined by a modified version of NIOSH method 6009 (determination of Hg by CVAAS).[19] For all blanks, standards, and samples, 75 mL of deionized water were added to the BOD bottle reaction vessel, followed by the appropriate aliquot of sample digest or 10.0-μg Hg mL^{-1} working

standard. For blanks, 5.0 mL of deionized water were used in place of a standard or sample.

Mercury Recovery Check

Aliquots of the 10.0-μg Hg mL^{-1} working standard corresponding to 0.0-, 0.10-, 0.20-, 0.45-, 0.80-, and 1.00-μg Hg were added to 5.0-mL aliquots of concentrated HNO_3 in microwave digestion vessels and irradiated according to the procedure described in the section *Microwave Digestion of Tuna Samples*. The micrograms of Hg recovered from each microwaved Hg standard were determined as described in the section *Determination of Mercury in Tuna by CVAAS*, and the percentage of Hg recovered was calculated as follows:

$$\%\ \text{recovery of Hg} = (\mu\text{g Hg recovered}/\mu\text{g Hg added}) \times 100.$$

RESULTS AND DISCUSSION

Limits of Detection

Limits of detection for the determination of Hg by CVAAS were determined to be 0.01-μg Hg/g, based on eight replicate blanks and a 1-g sample mass. Using an average standard deviation of the intercept from calibration curves[20] and a 1-g sample mass, we determined an average limit of detection to be 0.03-μg Hg/g. These detection limits indicate that our method for determination of Hg in tuna is well suited for our investigations.

Accuracy and Precision Studies

To address suspected loss of mercury from tuna during microwave digestion, an external calibration curve was constructed from Hg standards that were added to the requisite amount of concentrated HNO_3 and subjected to microwave irradiation in the same manner as the tuna samples. The calibration data obtained for the microwaved Hg standards (0.0-, 0.10-, 0.20-, 0.45-, 0.80-, and 1.00-μg Hg) were then compared with the calibration data from the non-microwaved Hg standards (0.0-, 0.10-, 0.20-, 0.45-, 0.80-, and 1.00-μg Hg). A regression of the corrected absorbances of the microwaved Hg standards vs. the corrected absorbances of the non-microwaved Hg standards was performed and plotted (Fig. 1). As observed from Fig. 1, the two types of Hg calibration data compare quite favorably ($R^2 = 0.9952$, standard error of estimate = 0.0121), suggesting minimal or no loss of Hg during the microwave digestion process. Recoveries for the microwaved Hg standards were determined to be 111%, 96.1%, 95.7%, 94.7%, and 101% for 0.10-, 0.20-, 0.45-, 0.80-, and 1.00-μg Hg, respectively. Further studies of the effect of microwave digestion on loss of Hg lend support to the results given in Fig. 1.

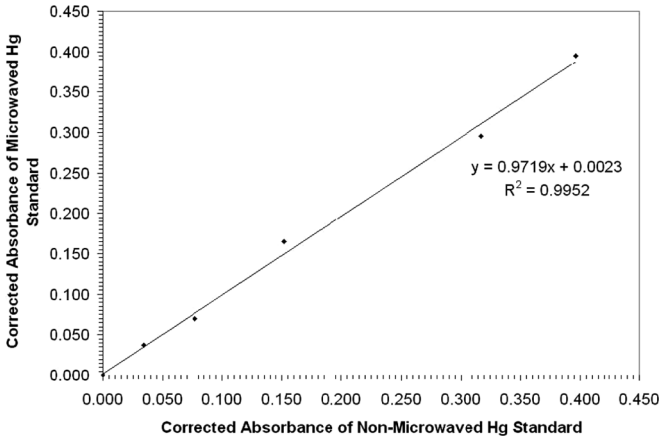

FIGURE 1 **Comparison of calibration data for determination of Hg in tuna by CVAAS, using microwaved and non-microwaved Hg external standards.**

The issue of sample inhomogeneity becomes apparent in the precision obtained from quadruplicate Hg determinations in each of the five brands of tuna. The standard deviations and corresponding percent relative standard deviations (%RSD) (Table 1) suggest that the distribution of total mercury in the tuna is inhomogeneous. Even for Brands 3 and 5, which exhibit the lowest %RSD values, the precision is still wider than desired. A more recent

TABLE 1 **Results for Determination of Mercury in Tuna by CVAAS[a]**

Brand	Mean Hg conc. (μg Hg/g tuna)	Std. Dev. (μg Hg/g)	% RSD	95% M.E. (μg Hg/g)	Cost (2007)
1	0.92	0.43	46.9	0.42	$1.99
2	0.19	0.07	36.6	0.07	$1.59
3	3.60	0.17	4.8	0.17	$0.49
4	1.25	0.32	25.8	0.31	$0.49
5	1.17	0.07	6.1	0.07	$0.99

[a]Four replicate determinations were performed for each brand of tuna.

study of precision for Hg in tuna carried out in our group gave %RSD values (27.9 and 30.4, respectively) for two different brands of tuna with average Hg concentrations of 0.09-μg Hg/g each. These additional results hint further at sample inhomogeneity for tuna. Other contributors to the wide precision of the Hg results may be the limited number of replicates and no homogenization of the contents of an entire can of tuna, performing the sample digestion and subsequent Hg determination on separate days, and the possibility that a can of tuna may contain meat from more than one tuna. Possible experiments toward further exploration of this issue of sample homogeneity of canned tuna include using tuna from two or more cans of the same lot, with prior homogenization of one can's contents and direct sampling from another can, increasing the number of replicates from a single can, using different types of tuna, and using one type of tuna from different producers.

Determination of Mercury in Tuna Samples

Table 1 displays the mean Hg concentrations (μg Hg/g) for each brand with standard deviations, relative standard deviations (%RSD), 95% margins of error (M.E.), and the cost (2007) of each brand of tuna.

An analysis of variance (ANOVA) was performed to compare the mean Hg concentrations (μg Hg/g) for each brand. The overall F-test ($F_{4,15} = 100.80$, $p < .01$) revealed a mean difference between brands, so pair-wise differences were investigated to determine which brands differed. Pair-wise comparisons were conducted using the least significant difference (LSD) criteria[21] to adjust for multiple comparisons (Table 2). Brands 1, 4, and 5 did not differ from one another. Brands 2 and 3 differed from the other brands. Brand 2 had significantly lower Hg concentration than the other brands, whereas Brand 3 had significantly higher Hg concentration than the other brands.

Regarding an initial hypothesis that Hg concentration in canned tuna is associated with its cost, the brands of tuna and their costs are listed in Table 1, along with the Hg concentrations. Brand 2, the second most expensive brand, had a significantly lower concentration of Hg than the other brands. Brand 3, the least expensive (along with Brand 4) brand, had a significantly higher concentration of Hg than the other brands. Because of the limited sample size in this study, this hypothesis is not supported unequivocally. A larger sample size would be needed to provide stronger support of this hypothesis. Possible experiments for future study include expanding the number of brands of tuna, and the number of replicates from each brand, toward obtaining a clearer idea of the relationship between the cost of canned tuna and Hg concentration.

TABLE 2 Results of Significance Testing of the Mean μg Hg/g for the Five Brands of Tuna

Brands compared	Mean difference	LSD adjusted *p*-value
1,2	.730	.001
1,3	−2.680	<.0005
1,4	−.329	.089
1,5	−.254	.182
2,3	−3.410	<.0005
2,4	−1.058	<.0005
2,5	−.983	<.0005
3,4	2.351	<.0005
3,5	2.427	<.0005
4,5	.075	.683

The determination of mercury in a sample such as canned tuna has great potential for use as a one-session experiment or a multisession project in an undergraduate instrumental analysis course. Along with learning about sample preparation techniques, method calibration, instrument operation, and data analysis, students could learn much about the importance of homogenization of samples such as canned tuna, and the effects of sample inhomogeneity on precision of the analytical results.

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