✂Determination of Heavy Metals in a Menhaden Oil after Refining and Hydrogenation Using Several Analytical Methods[1]

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ABSTRACT

A series of menhaden oils collected at various stages of processing have been analyzed for zinc, cadmium, lead, copper and arsenic by wet digestion and electrothermal atomization-atomic absorption spectrophotometry (aas). The results are compared, for some metals, with 2 other methods of oil treatment: extraction with nitric acid and dilution with methyl isobutyl ketone. Both the extraction and dilution procedures appeared to measure only the loosely bound, inorganic portion of the metals: determination of the total metal content including organometallics required wet digestion. The crude oil contained the largest metal burden but successive refining steps reduced the metal content to a level which met the FAO/WHO Codex standards. Hydrogenation did not significantly alter the metal concentration in the oils.

INTRODUCTION

Heavy metals in oils and fats are of concern to both the producer and consumer. A level of 0.03 ppm copper has been reported to alter the flavor stability of soybean oil (1) and lead, cadmium, zinc and copper are thought to be linked either directly or indirectly via cholesterol levels to coronary heart disease (2,3).

As an extension of our earlier work on trace metals in rapeseed and herring oils (4,5), we have reviewed several methods of solubilizing the total trace metal content of oils. Methods that could be coupled to flameless atomic absorption spectrometry (aas) included burning followed by ashing (6), acid and acid-EDTA extractions (7-9), char-ashing (10), dilution with methyl isobutyl ketone (MIBK) plus flame aas (1), and dilution with iso-amyl acetate plus flameless aas under an oxygen atmosphere (11). Among the simpler methods requiring minimal manipulation, the lowest probability of sample contamination, and the smallest sample weight, was wet digestion in a sealed Teflon vessel. We have compared this method with simple dilution for copper and lead, and with acid extraction for zinc, copper and lead, and find it superior. The samples were 8 menhaden oils collected at different stages of pilot-plant refining and partial hydrogenation to give margarine stock. Values for the levels of arsenic and cadmium are also reported.

EXPERIMENTAL

Reagents

All solutions were prepared from reagent-grade chemicals in glass-distilled water. Glassware and Teflon vessels were cleaned with hot 1:1 HNO_3. The nitric acid used for sample preparations was ultrahigh purity grade (Ultrex, J.T. Baker Co.).

Standard solutions of Cu, Pb, Zn and Cd were prepared by diluting aas standard solutions (Fisher). Arsenic standard solutions were prepared from weighed amounts of dried As_2O_3.

Sample Preparation

Approximately 380 lb of crude menhaden oil (iodine value = 159, free fatty acids = 1.75%, phosphatide content <1%) was degummed with 0.2% phosphoric acid at room temperature and then alkali-refined with 6.6% (w/w) sodium hydroxide at 55 C. After settling overnight and removing the foots (<5 lb), the oil was washed with water to carry off residual soaps. To bleach the oil, 1% activated clay (Filtrol) was mixed with the oil at 104 C for 30 min followed by filter press clarification. The refined and bleached oil was hydrogenated with 0.2% Calsicat nickel catalyst (Mallinckrodt) and deodorized by heating to 250 C for 1.5 hr followed by cooling to 50 C over a further 30-min period with steam sparging at 1% weight of oil/hr at a pressure of 5 mm Hg.

Methods of Analysis

The oils were stored in a refrigerator, melted in a water bath and mixed thoroughly before sampling.

Digestion. 0.20-0.25 g oil was weighed into a 30-mL Teflon digestion vessel fitted with screw-on lid. Three mL of HNO_3 was then added and the closed vessel was heated at 70 C for 2-3 hr. Heating was continued at 140 C overnight. One mL HNO_3 was added and the sample was again heated overnight at 140 C. Care must be taken during this digestion procedure! The sample was diluted to 5 mL and 10-μL aliquots were introduced into the electrothermal atomizer. The metal concentration was established using the method of standard additions.

TABLE I

Temperature and Timing Parameters of Electrothermal Atomizer

Program	Cu (λ=324.7 nm)	Cd (λ=228.8 nm)	Pb (λ=283.3 nm)	As (λ=193.7 nm)
Dry	150 C; 25 sec	150 C; 25 sec	150 C; 25 sec	150 C; 25 sec
Char	800 C; 25 sec	200 C; 25 sec	800 C; 25 sec	450 C; 25 sec
Atomize	2500 C; 4 sec	2000 C; 5 sec	2300 C; 4 sec	2300 C; 7 sec

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Extraction. Oil (2.5 g) was dissolved in 50 mL methyl isobutyl ketone (MIBK) and extracted for 15 min with 10 mL of 0.1 M HNO_3 at room temperature on a wrist-action shaker. Phases were allowed to separate in a refrigerator for 1 hr. Portions of aqueous phase (10 μL) were analyzed by aas and the method of standard additions was employed.

Dilution. Oil (0.5 g) was dissolved in 10 ml MIBK (or a multiple of this ratio) and 10-μL aliquots were pipetted into a graphite furnace. The concentration of the metals was determined by an addition procedure in which 10 μL of standard aqueous sample was injected into the graphite furnace and dried at 150 C for 20 sec prior to the injection of the diluted oil sample.

Atomic Absorption Spectrophotometry (aas)

The spectrometer (Model 403, Perkin Elmer Ltd.), was equipped with a deuterium background corrector, an electrothermal atomizer (Model HGA-2100, Perkin Elmer Ltd.) and the appropriate lamps (electrodeless discharge lamps were used for Pb and As). A slit width of 0.7 mm was used throughout. Pyrolytically-coated graphite tubes were used for Cu and As analyses and the nitrogen gas flow (50 mL min^{-1}) was interrupted during atomization stage. Zinc (λ = 213.9 mm) was determined using a 4-in. single slot burner and an air-acetylene flame (flow rates: 6 L min^{-1} C_2H_4 and 24 L min^{-1} air). The temperature programs are summarized in Table I and were used for both aqueous and MIBK matrices.

RESULTS

The concentration of the metals in the different oils as determined by the 3 analytical methods are given in Table II. The error limits represent the standard deviation of at least 3 determinations. A signal equal to twice the baseline noise was used to calculate the detection limit along with typical dilution factors and sample weights. Only the 3 least refined oils, which had the heaviest metal content, were extracted with HNO_3. Cadmium was not determined by this method because of its low and relatively constant concentration. Attempts to extract arsenic using either water or 0.2 M NaOH produced very stable emulsions which did not separate, even upon acidification.

To analyze the oils directly, following dilution with MIBK, required a charring temperature of 800 C to burn off all organic matter. Arsenic, therefore, could not be determined, even with the addition of nickel nitrate, since the oil inhibited the formation of a stable nickel-arsenic compound during the drying cycle; attempts to measure cadmium were also unsuccessful, even in the presence of $(NH_4)_2SO_4$ (1 mg SO_4^{2-}/mL). The detection limit for cadmium was 20-30 ppb which was close to the actual levels in the oils. Zinc was not analyzed after dilution, since the linear working range was very limited (0-6 ppb).

DISCUSSION

The relatively large values of the standard deviations associated with the concentration of the metals in the 3 least refined oils, as compared to those of earlier work (4,5), were in part, due to sample inhomogeneity. All 3 samples contained particulate matter and degummed oil plus gums had physically obvious gums present. Once the oil had been alkali-refined, the samples became more uniform.

The metal levels in the crude menhaden oil were of the same order of magnitude as those found in other crude fish oils (5), with the exception of the zinc concentration,

TABLE II

Metal Content of Menhaden Oil at Different Stages of Processing (μg/g)

Oil	Wet digestion					Extraction			Dilution	
	Cd	Pb	Cu	As	Zn	Pb	Cu	Zn	Pb	Cu
Crude	0.04 ± 0.01	0.32 ± 0.05	0.23 ± 0.03	10.4 ± 0.7	64.9 ± 13.3	0.16 ± 0.03	0.25 ± 0.02	50.1 ± 11.7	0.11 ± 0.01	0.20 ± 0.01
Degummed	0.04 ± 0.01	0.08 ± 0.04	0.10 ± 0.04	1.8 ± 0.1	8.3 ± 1.1	0.02 ± 0.01	0.02 ± 0.01	8.4 ± 2.9	0.03 ± 0.01	0.04 ± 0.01
Degummed plus gums	0.02 ± 0.01	0.06 ± 0.02	0.13 ± 0.02	2.0 ± 0.9	9.0 ± 1.1	0.04 ± 0.01	0.04 ± 0.02	8.9 ± 2.7	0.03 ± 0.01	0.04 ± 0.01
Refined	0.02 ± 0.01	0.09 ± 0.03	0.08 ± 0.04	Bdl*	2.7 ± 0.4					
Refined and bleached	0.01 ± 0.00	0.04 ± 0.03	0.04 ± 0.03	Bdl	Bdl					
Hydrogenated and filtered	0.02 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	Bdl	Bdl					
Hydrogenated, filtered and bleached	Bdl	0.06 ± 0.02	0.04 ± 0.01	Bdl	Bdl					
Hydrogenated, filtered, bleached and deodorized	Bdl	0.05 ± 0.02	0.06 ± 0.01	Bdl	Bdl					

*Bdl = below detection limit (0.005 μg/g Cd; 0.7 μg/g As; 0.8 μg/g Zn; 0.02 μg/g Pb).

which was 13 times higher. Refining the crude oil lowered the concentration of all metals and once the oil had been alkali-refined it satisfied the FAO/WHO Codex Alimentarius Commission's standards of 0.1 μg/g for copper and lead (12). The refined oil presumably satisfied the Codex standard of 0.1 μg As/g since refined herring oil (5) and fish oil (12) have been reported to meet this requirement.

The 2 most effective refining steps for removing trace metals were degumming and alkali refining. The phosphoric acid wash employed to degum the oil is comparable to our nitric acid extraction which was found to remove certain metals efficiently (vide infra). During alkali refining, the phospholipids are extracted from the oil, and since phospholipids are reported to carry metals into oils (13), all metals complexed by phospholipids were also removed.

Nitric acid extraction of the crude, degummed, and degummed plus gums oils confirmed that the crude oil contained higher metal levels than the other 2 samples. The extraction was very efficient for zinc for all 3 samples but, with the exception of copper in the crude oil, generally less than 50% of the copper or lead was extracted. Nevertheless, the acid extraction procedure was adequate to confirm the wet digestion results, indicating that degumming would be the most important step in lowering the trace metal content. Since zinc was extracted relatively easily, it was assumed that zinc existed in a loosely bound, inorganic form in fish oils. Nickel has also been reported to be quantitatively extracted with dilute nitric acid (9). The lower efficiencies in extracting copper and lead suggested that a portion of these metals was either tightly bound to the constituents of the oils, or else existed in a form, such as an organometallic compound, which has a high affinity for the oil phase. Copper, unlike lead, does not generally form organometallic complexes; hence, under sufficiently vigorous conditions, such as refluxing in the presence of an HCl-EDTA mixture, copper has been quantitatively recovered from crude oils (7).

The lead and copper levels in the 3 least refined samples, as determined by the dilution method, were in good agreement with the results of the extraction method, but lower than the values obtained by wet digestion. Assuming that the method of standard additions, which involved overlaying an aliquot of diluted oil on a dried portion of an aqueous standard solution, did not introduce a serious error, then we suggest that the dilution method, like the extraction procedure, measures only the level of the nonvolatile, inorganic, forms of copper and lead. Volatile compounds such as tetraalkyl leads with boiling points near 200 C will be lost during the charring step in the graphite furnace and could only be measured following wet digestion. Sirota and Uthe (14) have reported that 39 and 81% of the lead in a mackerel muscle homogenate and a lobster digestive gland homogenate, respectively, were in the tetraalkyl form. The results of the present work implied that ca. 40% of the lead in the crude, degummed, and degummed plus gum oils might be in an organometallic form. Furthermore, metals that are tightly bound to oil constituents and organometallic compounds are unlikely to be removed by bleaching, deodorizing, or hydrogenation, which explains the relatively constant level of lead and copper in the more highly refined and processed (hydrogenated) oils.

Although method simplification is always a desirable objective, there does not yet appear to be any alternative, with the possible exception of low temperature ashing (15), to the simple wet digestion of oils with nitric acid if a complete and accurate analysis is required. The simpler and more rapid method of extraction with dilute nitric acid offers some potential for development if its limitations are realized. The dilution method suffers from matrix effects which require that high charring temperatures be employed.

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