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Analysis of Lead in Evaporated Milk by Flameless Atomic Absorption Spectroscopy

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A procedure for determining lead in canned evaporated milk is evaluated by flame atomic absorption and the flameless atomic absorption technique using the tantalum ribbon and carbon-cup atomization devices. A fairly rapid pre-ashing procedure to eliminate the mechanical loss of lead encountered during the dry-ash sequence of the flameless method is described. A least-squares fit of the data taken using the method of standard additions is used to determine the Pb in each sample. The results from the analyses of actual samples taken from supermarket shelves fall within the federal guideline of <0.5 ppm.

In order to establish a maximum allowable content of heavy metals in canned foods it is necessary to have a rapid and reliable means of analyzing for these metals. Evaporated milk is widely used in preparing baby formulas and in various recipes prepared by the housewife. It is known to contain some amount of lead as a contaminant, presumably arising from the canning process which is discussed by Shea (1973). Recent reports by the U. S. Food and Drug Administration (FDA) and Consumers Union (AP and UPI releases, 1973; *Consumer Reports*, 1973; Fiorino *et al.*, 1973) indicate lead levels ranging from 0.02 to 0.37 ppm with an average of 0.12 ppm and in the range of 0.56–0.84 ppm with an average of 0.70 ppm. The FDA guideline for lead content in evaporated milk is <0.5 ppm (AP release, 1973).

The purposes of this study are: (1) to evaluate the lead content in canned milk using the recently developed technique of flameless atomic absorption spectrometry (AAS) and to compare the lead content of samples taken randomly from supermarket shelves with the federal guideline, and (2) to compare the carbon-cup and tantalum ribbon flameless techniques with the more cumbersome and time-consuming flame technique.

Amos (1972), in a review of nonflame atomization in AAS, describes the various designs of filament and furnace atomizers presently in use and includes a detailed description of the carbon-cup atomizer used for this study. Kurz *et al.* (1973) describe the operation of the carbon rod atomizer and the determination of its optimum settings. Additional descriptions of the carbon rod atomizer are given by Brodie and Matousek (1971) and Matousek (1971). Manning (1973) describes a single-point calibration determination of lead in milk using a Perkin-Elmer graphite furnace without any sample pretreatment; however, neither data nor statistical analyses are given for real samples. Hwang *et al.* (1971, 1972), Donega and Burgess (1970), and Takeuchi *et al.* (1972), describe the use of the tantalum ribbon in flameless AAS. Absolute sensitivities and absolute detection limits for the flameless methods are in the picogram range with the tantalum ribbon

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being slightly less sensitive than the carbon rod atomizer. Amos (1972) reports that the absolute sensitivity and detection limit of the Varian Techtron M-63 carbon rod atomizer for lead are 2×10^{-12} and 5×10^{-12} g. Matousek (1971) reports a sensitivity for lead at 217.0 nm of 6.8×10^{-12} g using the carbon rod atomizer. Hwang *et al.* (1971) found that the absolute sensitivity and detection limit for lead of the tantalum ribbon flameless atomizer are 3×10^{-10} and 7×10^{-11} g. Hwang *et al.* (1972) later determined the sensitivity to be 3×10^{-11} g and the detection limit to be 1×10^{-11} g at the 217.0-nm lead line using the tantalum ribbon.

EXPERIMENTAL SECTION

Apparatus. Measurements were made with a Jarrell-Ash 82-500 MVAA spectrophotometer equipped with a triflame laminar-flow burner assembly, a Glomax Model ETA-2 tantalum ribbon electrothermal atomizer (Barnes Engineering Co.), and a Varian Techtron Model 63 water-cooled carbon rod atomizer complete with power supply and gas control unit, the details of which are given elsewhere (Matousek and Stevens, 1971). Argon (flow rate 6.5 SCFH) was used as the inert gas for the flameless determinations. The settings (voltage–time) for the carbon rod atomizer were: dry, 4–20; ash, 5–20; and atomize, 7–2. Slight variations might be observed for different instruments. The carbon rod atomizer power supply was used for the tantalum ribbon electrothermal atomizer and required reduced voltage–time settings: dry, 1.5–60; ash, 0.5–20; and atomize, 2–1. Peak heights were recorded with a Sargent-Welch Model SRG recorder which has a full scale response time of less than 1 sec. All readings were made using minimum damping, minimum amplifier gain, and an unexpanded scale. A Jarrell-Ash hollow cathode lamp was operated at 75% of the maximum recommended current with determinations made at the sensitive lead resonance line, 217.0 nm (Slavin, 1968). A 5-μl Autopipette precision pipetting device (1% accuracy in the volume delivered) with "nonwetting" polypropylene tips was used to inject the samples into the flameless atomization devices. Entrance and exit slits for the 0.5-m Ebert grating monochromator were fixed at 100 and 150 μ, respectively. A wide response photomultiplier tube which was supplied by Jarrell-Ash with the instrument and which had a useful range of 1970–7800 Å was mounted at the exit slit.

Reagents. Baker Analyzed reagent lead nitrate, which was oven dried at 120° and stored in a desiccator over anhydrous magnesium perchlorate, was used to prepare the 1000-ppm stock solution from which spike solutions for the standard additions method were prepared. Distilled water was passed through a Crystalab Deeminizer (Cole-Parmer) demineralizer immediately prior to preparing solutions or rinsing glassware. All glassware used in this study was acid soaked a minimum of 12 hr in a 1:1 nitric acid bath prior to use. ACS certified magnesium nitrate was used as the ashing aid in the pre-ashing of samples. Major brand canned evaporated milk samples were purchased at random from the shelves of local supermarkets and shaken a minimum of 5 min using an Adams Utility Shaker (275–285 oscillations/min) prior to sampling.

Procedure. Two procedures were used to analyze the samples. The first procedure utilizes the flameless capabilities of the instrument as follows. Five-microliter aliquots of undiluted and tenfold diluted evaporated milk samples are injected into the carbon-cup and the programmed sequence for drying, ashing, and atomizing is implemented. Samples spiked with 0.2 and 0.4 ppm of aqueous lead nitrate standard solutions are also analyzed using the same instrument parameters. A minimum of three measurements at each concentration is made on each sample. Aqueous lead nitrate standard solutions are used to establish the working curve.

The second procedure involves a pre-ashing prior to injection and subsequent analysis. The method is described as follows. Following 5 min of automatic shaking, 10 ml of the milk sample is pipetted into each of three thoroughly clean and acid-rinsed platinum crucibles. Approximately 0.5 g of reagent grade magnesium nitrate is added to each crucible and appropriate samples are spiked from a freshly prepared lead nitrate solution. Each crucible is slowly dried using a microburner. The crucibles are covered with platinum lids and partial ashing of the organic matter is accomplished with a low flame. Care is taken not to ignite the samples. Final ashing to a white residue involves the addition and careful repetitive evaporation of small quantities of concentrated nitric acid using a Meker burner. Upon cooling to room temperature the residue from the respective crucibles is taken up quantitatively with a 1:4 nitric acid solution and diluted in 10-ml volumetric flasks. This ashing procedure requires approximately 1.5 hr per sample. This solution can then be directly aspirated into the flame for atomic absorption measurements or appropriately diluted and directly injected onto the tantalum ribbon or carbon-cup for the flameless AA determinations. At least three measurements were made on the unspiked sample, the sample with a 0.2-ppm lead nitrate spike, and the sample with a 0.4-ppm lead nitrate spike. At the dilutions used, there is no detectable lead in an unspiked sample of magnesium nitrate taken through the ashing procedure. A check of the 280.2-nm nonabsorbing lead line gave less than 1% absorption and thus no corrections for nonatomic absorption were made.

Triplicate recovery studies of lead standards at the spike solution concentrations used in the analysis of the milk samples and taken through the ashing procedure gave 96.8 ± 10.1 and 99.1 ± 8.4% (mean ± standard deviation) recoveries. The average recovery was found to be 98 ± 13.1 in the range studied. Kurz *et al.* (1973) observed an average recovery of 97.5% for zinc in serum and 97.6% for zinc in urine using flameless AA. The standard deviation of the recovery in this study is comparable to their value of 94.6 ± 11.0% (mean ± standard deviation) for zinc in serum.

RESULTS AND DISCUSSION

In the flameless techniques the sample is placed in a graphite cup or tantalum ribbon above or through which the radiation from the hollow cathode lamp passes. An appropriate electric current is passed through a resistive load causing heating sufficient to dry, ash, or atomize the sample, depending on the amount of current passed. The atomizer is sheathed by a continuous flow of argon to protect it from atmospheric oxidation. This flameless procedure involves a minimum of sample handling and sample pretreatment which requires the use of reagents and can result in loss and/or contamination. Once the light beam is focused on the atomizer and the sample is injected, the dry–ash–atomize sequence is initiated. Moisture or solvent is evaporated during the dry step and the organic matrix is pyrolyzed during the ash step, leaving analyte atomization to occur during the final step. For a sample containing a large amount of organic matter a nonatomic absorption peak usually occurs during the dry and ash steps. Voltage settings regulating the current flow are determined such that there is no splattering during the dry step and the recorder trace returns to the base line following the dry and ash sequences. Diluted samples generally require slightly altered drying and ashing settings.

Using the method of standard additions described by Christian (1969), it was discovered that lead was lost during the dry and ash steps, even for milk samples that had been diluted prior to attempting the analysis. The amount of lead loss in the analysis of 1:10 diluted evaporated milk samples without pre-ashing was found to be not reproducible. However, in an effort to quantify the lead loss, evap-

Table I. Amounts Found (ppm)

Sample	Flame[a]	R^b	Carbon-cup[a]	R^b	Tantalum ribbon[a]	R^b
A	0.34 ± 0.07	0.97	0.46 ± 0.08	0.94	0.42 ± 0.06	0.97
	0.40 ± 0.09	0.93	0.44 ± 0.05	0.98	0.44 ± 0.13	0.92
B	0.43 ± 0.06	0.96	0.42 ± 0.07	0.95	0.46 ± 0.08	0.96
C	0.50 ± 0.08	0.95	0.34 ± 0.06	0.95	0.31 ± 0.03	0.98
D	0.42 ± 0.05	0.97	0.27 ± 0.09	0.82	0.29 ± 0.06	0.92
E	0.42 ± 0.11	0.96	0.41 ± 0.08	0.93	0.54 ± 0.07	0.97
	0.40 ± 0.0006	1	0.40 ± 0.08	0.94	0.43 ± 0.03	1
F			0.22 ± 0.04	0.93	0.24 ± 0.03	0.94

[a] The ± values reported represent the standard deviation of the least-squares intercept determined by the method of standard additions. The technique for determining the standard deviation of an intercept is found in Youden (1955). The carbon rod was employed in a subsequent study involving a total of 38 analyses of 7 different samples for which standard deviations of the analyses had a range of 0.011–0.034 and an average standard deviation of 0.024. [b] R is the correlation coefficient.

orated milk samples were diluted 1:10 with deionized water and spiked with 0.1, 0.3, and 0.5 ng of lead and a range and average per cent absorption for six injections was determined with each concentration. The same aqueous concentrations were found to be reproducible and averages were determined. Per cent recoveries calculated on this basis varied from 34 to 66% with an average recovery of 45%. Thus, the average lead loss in the analysis without pre-ashing is 55% compared with an average recovery of 98% using pre-ashing. This indicated that a pre-ashing would be necessary before attempting the flameless AA analysis and resulted in the second experimental procedure described previously. Since aqueous standards of the lead nitrate stock solution showed no such loss at these particular voltage and time settings, it is believed that the lead in the milk samples is lost mechanically during the destruction and volatilization of the organic matrix in the ash step. It should also be noted that Manning (1973) does not report any such difficulty in his study. This perhaps is a bit surprising as the advertising literature for these devices implies that volatilization losses for samples such as lead or mercury may be troublesome during the ashing step. Our experiments with lead nitrate solutions indicate this is not a problem. The loss apparently occurs as the organic matrix is destroyed during the ashing step by mechanically carrying the analyte away. One of the features which make the nonflame devices highly attractive is the potential ability for ashing *in situ;* therefore, loss of analyte by mechanical means may be a serious drawback in that such losses would presumably occur for all analytes, not just the volatile ones. Other investigators have pretreated samples prior to flameless AA analysis of organic samples. Using the tantalum strip atomizer, Hwang *et al.* (1971) found it necessary to perform a solvent extraction to eliminate matrix interference on the metal absorption before determining lead in blood by flameless AA. Yanagisawa *et al.* (1973) had a similar experience in the determination of antimony in metallurgical samples. Munns and Holland (1971) found it necessary to digest fish muscle with a nitric–sulfuric–perchloric acid mixture before their flameless determination of mercury. Similarly, Hoover *et al.* (1971) used a nitric acid digestion before a flameless AA analysis of mercury in foods.

The pre-ashing used in this study utilizes magnesium nitrate as an ashing aid (Analytical Methods Committee, 1954) in a procedure developed by Taphorn (1973) (details of this particular ashing technique have not yet been published by Taphorn). This pre-ashing eliminated the nonatomic absorption peaks and subsequently gave reproducible lead atomization peaks in the standard additions method. Even though pre-ashing of evaporated milk samples is required prior to a flameless AA analysis, it is believed that this digestion, combined with the method of standard additions, is preferable to the one-point calibration procedure described by Manning (1973). The ashing procedure used in this investigation also is more rapid and involves fewer reagents and manipulations than that employed by Fiorino *et al.* (1973) in a collaborative study to establish a procedure for determining lead in evaporated milk by AAS and anodic stripping voltammetry.

Gajan and Larry (1972) observed that AA flame measurements are biased at very low lead levels, but that this bias disappears as known amounts of lead are added to the sample. Our observations indicate that we are at the lower limits for quantitative lead determination when the flame is used as the atomization source; however, a 10-fold dilution was required for the tantalum ribbon atomizer and a 100-fold dilution was needed for the carbon-cup atomizer, indicating the greater sensitivity of these flameless atomization sources.

Since the per cent absorption was observed to be linear up to 2 ng (and slightly curved beyond) when aqueous lead nitrate standard solutions were used, per cent absorption was used instead of absorbance in a linear least-squares determination of the intercept in the standard additions method (Christian, 1969). Dilutions and standard additions were such that analyses were performed in the linear region. Standard deviations of the parameters of the least-squares equation used to establish the intercept were used to calculate the standard deviation of the intercept. Results and the correlation coefficients are given in Table I.

From Table I it is seen that all the samples studied fall within the federal guideline of <0.5 ppm for lead in evaporated milk. Although the standard deviations are somewhat higher than might be desired, this is probably a result of the small number of data points collected for each sample. The proximity of the data points to a linear fit is indicated by the fact that the correlation coefficient is close to unity. Results of duplicate determinations of separate aliquots of samples A and E indicate that the results are reproducible within experimental error. Samples C and D give higher values for the flame analysis compared to the nonflame analyses, probably reflecting the sample bias indicated by Gajan and Larry (1972). Solvent extractions to further concentrate the lead would be necessary to eliminate this effect. Although an analyte peak was observed for the flame analysis of sample F, a quantitative determination could not be made, even using only the spiked solution data. The average value for all of the nonflame analyses of all the samples in this study is 0.36 ppm of lead.

The results of this study reinforce those of the FDA (UPI release, 1973). Although the lead content of the samples employed in this study approached the 0.5-ppm guideline, it was not found to exceed that value in any single sample. The results also do not illustrate the wide range of values assimilated by Shea (1973). The flameless techniques employed in this study are quite sensitive and even though a pre-ashing of the samples is required to

eliminate matrix interferences, the analysis is fairly rapid and reliable, at least 4–5 times more rapid than the accepted atomic absorption method of the AOAC (1973).

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Volatile Constituents of Pressure Cooked Pork Liver

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The volatile constituents of pressure cooked pork liver were isolated by simultaneous steam distillation and continuous solvent extraction. Analysis by gas chromatography and coupled gc–mass spectrometry led to the identification of 179 compounds. The mass spectral identifications were confirmed by matching retention indices. Pyrazines were the largest group of compounds found.

As a food, liver is an important source of protein, fat, and vitamins, particularly vitamins A, D, E, and vitamin B complex. A search of the literature, however, has revealed that no previous work has been reported on the volatile constituents of cooked liver. Pork liver was chosen for this study because its odor is typical of liver, and it is considerably stronger than calf or beef liver.

EXPERIMENTAL SECTION

Locally procured pork liver (42.5 lb) was sliced into small pieces and passed through a Fitz Mill Model D Comminuter (knives forward, no screen). The sliced liver was transferred to a 20-gal, steam jacketed, doubly stirred, stainless steel, pressure reaction vessel (Groen Div., Dover Corporation, Elk Grove, Ill.) and then slurried with 27 l. of distilled water. The vessel was sealed, heated to 325°F, and held at this temperature for 15 min. The maximum head pressure attained was 98 psi. At the end of the cooking period the vessel was cooled by passing water through the jacket, and the contents, which had a typical cooked liver aroma, were filtered through cheese cloth and a wire basket funnel into 5-gal polyethylene containers and stored at −20° until used.

The filtrate was atmospherically steam distilled, and the distillate continously extracted with distilled diethyl ether in a scaled-up (22 l.) model of William's apparatus (Williams, 1969). The distillation-extraction was carried out over a 48-hr period for each of the two batches.

The extracts were combined, dried over anhydrous sodium sulfate, and initially concentrated to about 80 ml by careful distillation in a 1-l. Kuderna-Danish concentrator (Kontes Glass Co., Vineland, N. J.) equipped with a 508 mm × 25.4 mm i.d. reflux column packed with 6 mm × 6 mm Raschig rings. The extract was further concentrated to about 15 ml in a 100-ml round-bottomed flask with a 35 mm × 10 mm o.d. test tube sealed to the bottom and equipped with a 300 mm × 13 mm i.d. Vigreux reflux column. The open container was then allowed to stand at room temperature until the sample had concentrated to a final volume of about 10 ml. The concentrated extract possessed a typical cooked liver aroma.

The concentrate was analyzed on a Hitachi Model RMU-6E mass spectrometer coupled with a Hewlett-Packard Model 5750 gas chromatograph using a Watson–Biemann helium separator (Watson and Biemann, 1965). The chromatographic columns used were 1000 ft × 0.03 in. i.d. stainless steel open tubular columns coated with SF-96 and Carbowax 20M. Further analysis was preceded by area trapping in Varian 1-ml collection bottles cooled with Dry Ice–isopropyl alcohol. The instrument used was a Varian Series 712 preparative gas chromatograph with a flame ionization detector employing a 12 ft × ⅜ in. stainless steel column packed with 20% SE-52 on 45–60 mesh acid-washed, DMCS-treated Chromosorb W. The oven temperature was programmed from 80 to 225° at 4°/min after a 5-min post-injection hold. The injector and detector temperatures were 230° and the helium flow rate was approximately 300 ml/min. Six traps were collected at arbitrary intervals and analyzed in the gc–mass spectrometry system. Traps 1–4 were analyzed on 1000 ft × 0.03 in. stainless steel open tubular SF-96 and Carbowax 20M columns. Trap 5 was analyzed on a 500 ft × 0.03 in. stainless steel open tubular SF-96 column and an 8 ft × ⅛ in. stainless steel column packed with 10% Carbowax 20M on 80–100 mesh acid-washed, DMCS-treated Chromosorb W,

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