An Improved Ashing Procedure for the Determination of Heavy Metals in Edible Oils

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ABSTRACT

An accurate, reliable, and simple method was described for the determination of Pb, Cu, Cd, Mn, Zn, and Fe in edible oils by atomic absorption spectrophotometry following burning of the sample in a restricted air supply. Recoveries for the six heavy metals by the proposed methods were about 100%. The relative standard deviation of the analysis for the lead at the level of 0.074 ppm was 6%, and the copper at the levels of 0.064 ppm and 0.158 ppm were 7% and 2%, respectively. The recovery of lead was found to be suppressed by the presence of a large quantity of iron in the oil, but the suppression was alleviated by adding a $Mg(NO_3)_2$ solution to the charred residue before ashing.

INTRODUCTION

Apart from the consideration of the quality of edible oil, as heavy metals apparently have some detrimental effects on the stability of edible oils (1), heavy metals themselves in the products are of concern to the consumer's health. Tolerable limits for various heavy metals have thus been set by the regulations of governments. In Taiwan, the limits of 0.1 ppm for lead and copper, and 0.05 ppm for mercury were established. The need for simple, accurate, and reliable methods for routine analysis had been expressed in conjunction with a report on the method developed in this laboratory for determining mercury in the order of ppb in edible oils (2). As to the determination of other heavy metals, though there are some informative methods described either for the preparation of sample or for the determination of heavy metals in edible oils, such as wet digestion with sulfuric acid and hydrogen peroxide (3), charring and ashing (4), the employment of carbon rod atomizer (5), dilution with methyl isobutyl ketone (MIBK) (6), extraction with nitric acid (7), and mixing with starch and ashing at low temperature (8), none of these methods seemed wholly ideal for routine analysis. Some comparative studies had been made with the methods mentioned along with our preliminary development (9) of the method described in detail in this report. Principally, a known amount of sample was burned with the aid of a wick in a crucible under controlled air supply. Then the residue after sulfuric acid treatment was charred and ashed and dissolved in hydrochloric acid or nitric acid for atomic absorption analysis. A burning method without air control was also employed recently by Hirayama et al. (10) for iron determination. This method permits considerable improvement in the recovery and reproducibility of results in the examples presented here.

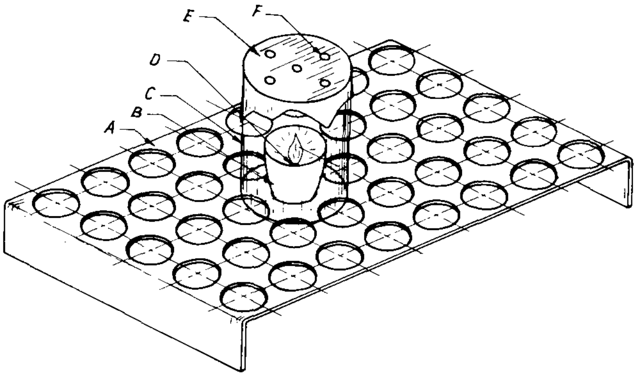

FIG. 1. Oil burning system: A. honeycombed stainless steel plate; B. crucible place in the hole or center of holes; C. glass cylinder diameter 7.0 cm, height 8.5 cm; D. wick; E. aluminum foil cover; F. the holes with 0.5 cm diameter.

EXPERIMENTAL PROCEDURES

Reagents

(a) Ammonium citrate buffer (40%), pH 7.4, was prepared by dissolving 400 g of diammonium hydrogen citrate, adjusting the pH with 25% ammonium water, and making up the solution to 1000 ml with deionized distilled water. Diammonium hydrogen citrate as well as other chemicals used in this study were reagent grade and unless otherwise indicated were obtained from E. Merck.

(b) Sodium diethyldithiocarbamate (DDC), a 5% solution, was prepared by dissolving 5 g of DDC in 100 ml of deionized distilled water and filtered. The filter paper used was Whatman No. 1 which had been washed with 3 N HCl and throughly rinsed with deionized distilled water.

(c) Water-saturated methyl isobutyl ketone (MIBK) was made by thorough mixing of MIBK (from Wako) with an adequate amount of deionized distilled water.

(d) Bromothymol blue (BTB) 0.1% indicator was prepared by dissolving 0.1 g of BTB in 100 ml of deionized distilled water and filtering it through the washed Whatman No. 1 filter paper.

(e) Stock solutions of Pb, Cu, Cd, Mn, Zn, and Fe were obtained from E. Merck (1000 µg/ml) for atomic absorption spectrophotometric analysis, and each working solution was prepared immediately before use by 100-fold

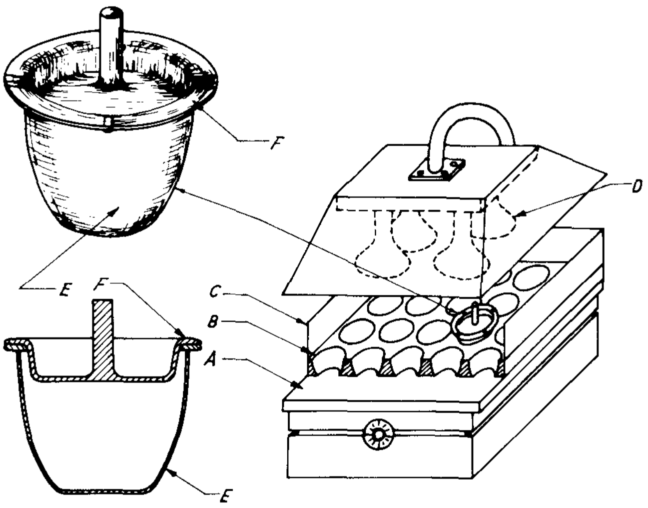

FIG. 2. Charring system for residue oil and wick: A. hot plate with maximum temperature of 350 C; B. heating block, thickness 2.7 cm with holes slightly bigger than crucible; C. stainless steel plate 10 cm high; D. infrared light; E. crucible; F. Pyrex crucible cover.

TABLE I

Recoveries of Lead Added to Soybean Oil

Lead Compound	Lead nitrate				Lead acetate			Cyclohexanebutyric acid lead salt		
Pb added ($\mu g/g$)	0	0.050	0.100	0.200	0	0.100	0.200	0	0.100	0.200
Pb found ($\mu g/g$)	0.009	0.054	0.107	0.204	0.028	0.131	0.236	0.018	0.120	0.217
	ND[a]	0.056	0.104	0.204	0.024	0.135	0.232	0.018	0.117	0.213
	0.009	0.054	0.107	0.204	0.031	0.127	0.232	0.020	0.114	0.215
	ND[a]	0.059	0.110	---	0.031	0.127	0.224	0.018	0.122	0.226
	0.009	0.056	0.104	---	0.028	0.124	---	0.015	0.120	0.209
Mean ($\mu g/g$)	0.005	0.056	0.106	0.204	0.028	0.128	0.231	0.018	0.119	0.216
Corrected for blank		0.051	0.101	0.199		0.100	0.203		0.101	0.198
Recovery (%)		102	101	100		100	102		101	99

[a]ND: nondetectable.

TABLE II

Recoveries of Copper Added to Soybean Oil

Copper compound	Cupric chloride				Cupric acetate			Cyclohexanebutyric acid copper salt			
Cu added ($\mu g/g$)	0	0.050	0.100	0.200	0	0.100	0.200	0	0.050	0.100	0.200
Cu found ($\mu g/g$)	0.019	0.066	0.113	0.215	0.061	0.160	0.271	0.057	0.105	0.156	0.254
	0.010	0.062	0.110	0.216	0.063	0.158	0.260	0.055	0.108	0.156	0.247
	0.019	0.067	0.122	0.214	0.053	0.158	0.266	0.057	0.105	0.153	0.253
	0.016	0.071	0.111	0.214	0.071	0.162	---	0.053	0.102	0.153	0.260
	0.016	0.061	0.120	0.215	0.074	0.168	---	0.055	0.106	0.156	0.252
Mean ($\mu g/g$)	0.016	0.065	0.115	0.215	0.064	0.161	0.266	0.055	0.105	0.155	0.253
Corrected for blank		0.049	0.099	0.199		0.097	0.202		0.050	0.100	0.198
Recovery (%)		98	99	100		97	101		100	100	99

dilution of the stock solution with 3 N HCl.

(f) Lead and copper solutions for recovery studies, in terms of the metals at about 10 $\mu g/ml$, were made. Those of inorganic forms were prepared by diluting the foregoing standard stock solutions, $Pb(NO_3)_2$ and $CuCl_2$, at 1000 $\mu g/ml$ with acetone 100-fold. Those of organic forms were prepared by dissolving lead acetate (15.7 mg) and cupric acetate (28.6 mg) respectively, in 1000 ml of acetone, and also the oil soluble standards, cyclohexanebutyric acid lead salt (26.7 mg), and cyclohexanebutyric acid copper salt (64.1 mg), in 1000 ml of xylene, respectively. The accurate concentrations of the heavy metals concerned in the solutions with organic solvents were standardized against the aqueous standard solutions of corresponding metals and rechecked every time they were used.

(g) Cadmium, maganese, zinc, and iron solutions for recovery studies were all prepared from the standard stock solutions mentioned in (e) by appropriate dilution with acetone to about 10 to 50 $\mu g/ml$ in terms of the metal concerned.

Apparatus

The combustion system as shown in Figure 1, consisted of a wick placed in a 50 ml crucible which was seated on a hole of a honeycombed stainless steel plate and surrounded by a protecting glass cylinder on top of which an aluminum foil with cut-open windows was placed. The wick was a roll (0.7 cm diameter and 3.0 cm height) made of a small sheet (3.0 x 5.5 cm) of Whatman No. 1 filter paper with one end fixed into a small glass ring of the same diameter and of 0.2 cm height which was filled loosely with absorbent cotton to two-thirds of its volume. Before use, the wick was consecutively soaked in 5% (w/v) nitric acid twice for no more than 30 min, washed thoroughly with deionized distilled water and acetone, and then dried in a vacuum oven at 50 C. Also to avoid contamination, the glassware and crucible were soaked overnight in 35% nitric acid, rinsed thoroughly with deionized distilled water and dried. Ignition was started with a lighter made of platinum filament as described (2). For charring the burned sample, the crucible containing the sample was provided with a Pyrex glass cover and heated by a system consisting of infrared lamps and a heating block surrounded with a stainless steel wall (Fig. 2). A muffle furnace heated at 480 C was used for ashing the sample after it was charred. For quantitative determinations, two atomic absorption spectrophotometers were used: Perkins-Elmer Model 603 for Pb and Cu and Varian Techtron Model AA-4 for Cd, Mn, Zn, and Fe following the instrument parameters and wavelengths specified in the manufacturer's guide. An electrodeless discharging lamp was used for Pb and hollow cathode lamps for Cu, Cd, Mn, Zn, and Fe. Intensity reading of the Model 603 was taken from the digital readout system, and that of the Model AA-4 was taken from the Varian A-25 recorder.

Procedure

Precisely 20.00 g of edible oil was weighed into an etch-free, clean crucible. A wick was placed in its center and ignited with the platinum lighter described. The shielding, consisting of a glass cylinder and its aluminum cover with cut-open windows, was installed with the central hole positioned right on the top of the flame. After 3 to 4 hr of burning, 20 drops of conc. H_2SO_4 was added to the oil and wick residue, a Pyrex glass lid was installed, and the sample was charred with the heating system described (Fig. 2). Under a fumehood, the sample was first heated with IR lamps for no less than 30 min, and then the heating was raised with the heating block provided for about 1-2 hr at 250-300 C until smoking ceased. The sample thus charred was moved into a muffle furnace and ashed at 480 C until carbon-free; usually requiring 2 to 4 hr. After cooling, the inside of the Pyrex glass cover and the side walls of the crucible were rinsed with 10 ml of 35% nitric acid into the crucible. Then, evaporation was carried out on a heating block at about 100 C just to dryness. For Pb or Cu determination, the residue was dissolved with 15 ml of 3 N HCl by heating the sample to boiling on a heating block with the Pyrex glass cover in place. The contents and the 15 ml of hot deionized distilled water used for rinsing the crucible and its cover were carefully transferred into a 50 ml

TABLE III

Reproducibilities of Copper and Lead Analyses

Metal added	Cu I	Cu II	Pb I
Cu or Pb found (μg/g)	0.059	0.155	0.071
	0.065	0.159	0.067
	0.064	0.159	0.079
	0.064	0.157	0.065
	0.073	0.155	0.083
	0.062	0.162	0.073
	0.071	0.161	0.075
	0.062	0.155	0.067
	0.058		0.079
	0.059		0.085
Mean ± RSD	0.064 ± 7%	0.158 ± 2%	0.074 ± 6%

TABLE IV

Interference of Fe in the Determinations of Lead and Copper

Iron added[a] (μg/g)	Pb found (μg/g)	Cu found (μg/g)
0	0.064[b] ± 0.008	0.117[b] ± 0.003
2.5	0.055[b] ± 0.008	0.111[b] ± 0.006

[a] Acetone solution of ferric chloride used.
[b] Average of six replicates.

TABLE V

Interference of Zinc in the Determinations of Lead and Copper

Zinc added[a] (μg/g)	Pb found (μg/g)	Cu found (μg/g)
0	0.064[b] ± 0.008	0.117[b] ± 0.003
2.5	0.066[b] ± 0.007	0.118[b] ± 0.007

[a] Acetone solution of zinc chloride was used.
[b] Average of six replicates.

volumetric flask. After adding 2 drops of 0.1% BTB indicator and 5 ml of ammonium citrate buffer, the pH of the contents was adjusted with 25% ammonia water until a light blue color appeared. Then 2 ml of 5% DDC solution was added with vigorous shaking for 1 min; likewise, 3 ml of MIBK was added. After vigorous shaking, the solution was allowed to separate into two layers. Deionized distilled water was then added to elevate the MIBK layer up to the neck of the flask for convenient sampling.

For the Cd, Mn, and Zn determinations, the residue was dissolved in 5 ml to 10 ml of 5% HNO_3 for atomic absorption spectrophotometric determination. For the iron determination, the residue was dissolved in 7 ml of 5% HNO_3 by heating the sample to boiling on a heating block with the Pyrex glass cover in place. The contents and the 3 ml of 5% HNO_3 used for washing the crucible were carefully transferred into a 10 ml volumetric flask and made up to volume with 5% HNO_3, and the iron was determined.

For the recovery studies, known amounts of the heavy metal in solution were placed in a crucible, 20.00 g of refined soybean oil was added, and the foregoing procedure was followed. For the Pb and Cu reproducibility studies, known amounts of cyclohexanebutyric acid lead or copper salts were premixed with the oil, and then several 20.00 g samples were taken for simultaneous analysis.

RESULTS AND DISCUSSION

The results of recovery studies made with known amounts of various lead compounds added to refined soybean oil and determined by the proposed method are shown in Table I. Recoveries of lead nitrate added at levels of 0.05, 0.100, and 0.200 μg/g of oil were 102, 101, and 100%; those of lead acetate added at levels of 0.100 and 0.200 μg/g of oil were 100 and 102%; and those of lead cyclohexanebutyrate added at levels of 0.100 and 0.200 μg/g of oils were 101 and 99%, respectively. Similar studies with the addition of known amounts of copper compounds (Table II) showed that the recovery of copper with copper chloride added at the levels of 0.050, 0.100, and 0.200 μg/g of oil were 98, 99, and 100%; that with copper acetate added at the levels of 0.100 and 0.200 μg/g of oil, recoveries were 97% and 101%; and that with cyclohexanebutyric acid copper salt added at the levels of 0.05, 0.100, and 0.200 μg/g of oil, recoveries were 100, 100, and 99%, respectively. The reproducibilities of the analyses (Table III) showed a relative standard deviation of 6% for ten repeated lead analyses at levels of 0.074 μg/g. Table III also showed the reproducibilities of copper analysis by the method proposed. The relative standard deviations were 7% and 2% for the contents of copper at the levels of 0.064 μg/g and 0.158 μg/g, respectively. Though the relative standard deviation was comparatively higher for the determination of lead and copper at lower concentrations than that at higher concentrations, nevertheless, all the values were within the usually acceptable range. Besides, the recovery studies on the lead and copper present at various levels did indicate satisfactory quantitative results.

The possible effects of iron and zinc on the determinations of lead and copper were studied in view of the fact that sometimes crude oils were found to be contaminated with considerable amounts of iron and zinc. It was found (Table IV) that the presence of iron at 2.5 ppm did suppress the analytical value of lead to 86% of the control while the same amount of zinc exerted no significant effect (Table V). The effect of iron on the recovery of lead was found mainly to be in the step of ashing, possibly by the iron-mediated interaction of lead in the form of lead sulfate with the crucible at the high temperature employed in the process. Similar difficulties had been observed in determining cobalt content, and Saltzman reported (11) that some improvement was made by the addition of sodium sulfate. Following this approach, after burning and charring the sample, magnesium nitrate or sodium sulfate solutions were added and dried prior to ashing and the eventual determination of lead content. It was found that the effect of iron on the reduction of lead recovery was greatly alleviated by the treatment with magnesium nitrate but not with sodium sulfate (Table VI).

TABLE VI

Effects of Magnesium Nitrate or Sodium Sulfate on the Interference of Iron in the Determination of Lead in the Oil

	Control	2.5 μg/ml Fe added: Without treatment	2.5 μg/ml Fe added: $Mg(NO_3)_2$ treated	2.5 μg/ml Fe added: Na_2SO_4 treatment
Pb found (μg/g)	0.108[a] ± 0.009	0.082[a] ± 0.005	0.099[a] ± 0.016	0.077[a] ± 0.013
% of control	100	76	92	71

[a] Average of six replicates.

TABLE VII

Recoveries of Cadmium, Maganese, Zinc, and Iron Added to Soybean Oil

Metal test	Cd			Mn			Zn			Fe		
Metals added (μg/g)	0	0.025	0.050	0	0.250	0.500	0	1.25	2.50	0	1.25	2.50
Metals found	ND[a]	0.021	0.048	ND[a]	0.250	0.475	ND[a]	1.29	2.50	0.46	1.60	2.37
	ND[a]	0.024	0.049	ND[a]	0.245	0.475	ND[a]	1.27	2.56	0.41	1.60	2.42
	ND[a]	0.025	0.046	ND[a]	0.265	0.510	ND[a]	1.28	2.40	0.46	1.64	2.52
	ND[a]	0.024	0.050	ND[a]	0.260	0.503	ND[a]	1.28	2.44	0.39	1.72	2.52
		0.029	0.050	ND[a]	0.250	0.483	ND[a]	1.28	2.46	0.42	1.65	2.64
		0.025	0.050	ND[a]	0.245	---	ND[a]	1.32	2.50	0.45	1.68	2.37
Mean (μg/g)	ND[a]	0.025	0.049	ND[a]	0.255	0.487	ND[a]	1.29	2.48	0.43	1.65	2.47
Corrected for blank		0.025	0.049		0.255	0.487		1.29	2.45		1.22	2.04
Recovery (%)		100	98		102	97		103	99		98	82

[a] ND: nondetectable.

The recovery studies on cadmium, manganese, and zinc by the method described also gave satisfactory results. As shown in Table VII, the recoveries of cadmium added at the levels of 0.025 and 0.050 μg/g were 100 and 98%; those of manganese added at the levels of 0.25 and 0.50 μg/g of oil were 102 and 97%; and those of zinc added at the levels of 1.25 and 2.50 μg/g of oil were 103 and 99%, respectively. The recovery of iron, however, was lower if a larger quantity of iron was added to the edible oil. Results (Table VII) showed that the recoveries of iron added at the levels of 1.25 and 2.50 μg/g of oil were 98% and 82%, respectively. The accurate determination of iron in edible oil present at the level of 1.25 μg/g of oil was largely attributable to the complete dissolution of the ashed sample by boiling it with 5% (w/v) nitric acid. The cause of the lower recovery in the case of higher iron content is not yet known for certain. However, as it had been reported (10) that the use of a quartz crucible gave a better result for iron analysis, it is expected that further improvement possibly can be made by a quartz crucible.

Some other details noted in the proposed method are especially crucial for securing satisfactory results. It was found essential to treat the burned residual oil and wick with sulfuric acid prior to charring for quantitative recovery. For example, without the treatment lead recoveries as low as 50-70% were usually observed. The control of air supply by the aluminum foil with cut-open windows is also of paramount importance without which the recoveries of added lead chloride and lead cyclohexanebutyrate were found to be in the ranges of 60 to 80% and 45 to 60%, respectively.

Except for serious soot formation, the flame size is not that critical. It is advisable to burn up the residual oil as much as possible with the wick in place. Contamination inadvertently occurring during the charring and ashing of the samples is prevented by use of the Pyrex glass cover. Efficient heating for the evaporation of the excess sulfuric acid and the prevention of its condensation beneath the glass cover during the sample charring is provided by the heating system of the heating block and the draft protecting stainless steel wall.

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