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Determination of Ultratrace Metals in Hydrogenated Vegetable Oils and Fats

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ABSTRACT

Ultratrace levels of nickel, chromium, copper and iron occurring in hydrogenated vegetable oil products were estimated by dispersion of the samples in 4 methyl-2-pentanone and atomic absorption analysis by the graphite furnace technique. The principal goals in establishing the analytical methods were improved sensitivity to metals at low levels and applicability to limited amounts of products. Using reproducibility and linearity of response as criteria, optimum oil concentration in solvent and instrument parameters were established. For a series of commercial products, the method of standard additions was adopted to correct for matrix differences between the products and salad oil-based standards. The range for the metals was determined in five cooking oils: Ni, 29-207 ppb; Cr, 1-5 ppb; Cu, 13-37 ppb; and Fe, 138-301 ppb; in recovered oils from five margarines: Ni, 34-70 ppb; Cr, 2-12 ppb; Cu, 26-58 ppb; and Fe, 239-540 ppb; and in five solid shortenings: Ni, 592-2772 ppb; Cr, 8-35 ppb; Cu, 26-108 ppb.

INTRODUCTION

The transition metal content of edible oils has a significant impact on stability and shelf life of products (1). Salad-grade soybean oil has very low background levels of a variety of trace metals, but the potential exists for increased levels due to oil treatments, i.e., nickel from hydrogenation and copper, chromium and iron from processing equipment.

There have been few published reports on the transition metal content of edible vegetable oil products. Several studies (2-6) have reviewed the methods of measuring low levels of such metals and reported on developments in use of the graphite furnace atomic absorption spectrometer directly on the oil (2,6).

The present research has been directed further to assess graphite furnace atomic absorption spectrometry, to develop methods to obtain maximum instrument response, and to accurately determine several metals. A method requiring a minimum of sample quantity, handling and surface exposure was also desired. The developed method was used to determine the trace metal content of several commercial vegetable oil products.

PROCEDURE AND METHODS

Apparatus

A Perkin-Elmer Corp. Model 372 Atomic Absorption instrument was equipped with a model 2200 graphite furnace and a deuterium lamp background corrector.

Product Selection and Sample Preparation

Two containers with the same lot number for each of five cooking oils, margarines and solid shortenings were obtained at local retail outlets (designated subsamples). Margarine oils were recovered by melting samples in acid-washed beakers on a hot plate, allowing phase separation at 95-98 C in an oven overnight, drying the decanted oil layer on a rotary evaporator at 90 C for 1 hr and, finally, decanting the clear oil from the top of the granular material at 70 C. Cooking oils and margarine oils, recovered as above, were dispersed in redistilled 4-methyl-2-pentanone (methyl isobutyl ketone, MIBK) at 50% w/v in acid-washed glassware. Shortenings were sampled in a semisolid state using a core sampler fabricated from a plastic disposable tuberculin syringe. The final dispersion was likewise 50% w/v in MIBK. These dispersions required warming at the time of analysis to achieve a homogenous solution.

Standard Solutions

Organometallics in the form of *tris*(1-phenyl-1,3-butandieno) chromium III, *bis*(1-phenyl-1,3-butandieno) copper II and *tris*(1-phenyl-1,3-butandieno) iron III were dissolved in small quantities of 2-ethyl hexanoic acid and xylene, and diluted to make stock solutions in MIBK. Nickel cyclohexanebutyrate was dissolved as above, but was dispersed directly in a salad oil stock solution. Using two or more portions of each stock, singular working standards, which were 50% w/v salad oil in MIBK, were prepared at metal concentrations slightly above and slightly below the expected range for each respective sample.

Evaluations

Three design criteria were considered for measuring metals in each commercial fat: (a) reproducibility between containers, (b) repeatability among assays, and (c) uniformity of response at two levels of metal content. The standard

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error of the mean (SEM) and percent relative standard deviation (RSD) were used to evaluate analytical results.

RESULTS AND DISCUSSION

Furnace atomic absorption analysis was adopted because it offered the greatest sensitivity in currently available instrumentation for transition metal analyses. This single element analysis is noted for its selectivity, and can be optimized by adjusting time and temperature of charring and atomization. Establishing accuracy at the ultratrace level, however, is hampered by the absence of characterized reference materials.

In preliminary experiments, agreement in product metal content between laboratories, methods of preparation, operators, and between instruments by the same operator suggested that no serious systematic error existed in the adopted method for nickel. Absence of bias for chromium was less well established because of its extremely low level. Copper content, as that of chromium, was in near agreement for all combinations checked. Some positive bias in iron content was probably attributable to char-ashing resulting from atmospheric iron oxide.

To address the problem of precision, reproducibility between containers was evaluated by the method of standard additions. Also, direct analysis, which was subject to matrix effects, and method of additions, which was applied to correct such effects, were compared.

Direct Analysis

Products representing a laboratory-hydrogenated soybean oil, a commercial liquid fat, and a general-purpose cooking/salad oil of foreign manufacture were evaluated for Cr, Cu, Ni and Fe content by furnace atomic absorption spectrometry. Parameters studied were: (a) concentration of oil in MIBK; (b) instrument parameters involving charring conditions to destroy matrix; (c) the instrument readout for best reproducibility among atomizations; and (d) furnace conditions for atomization to allow a sizable response representative of the metal of interest.

Because sampling of vegetable oil into the furnace with no added solvent gave poor reproducibility, a compromise dilution of 50% w/v in MIBK was adopted to decrease viscosity. A nearly complete destruction of triglyceride matrix was required in the charring step to equalize and lower the background signal to a level compatible with background corrector capability, and to cause organometallics and inorganic species to attain the same ionic form. To achieve complete ashing at temperatures that assured quantitative retention of metals, a brief period of alternate gas charring was adopted. Thus, air was passed through the heated graphite tube at the same flow rate as that of the argon gas that shielded the outside of the tube. This procedure was recommended by Beaty and Cooksey for nickel determination in serum (7) and simulated the charring with blended gas, N_2/O_2, used by Kundu and Prevot (8) for lipid decomposition prior to copper analysis.

The instrument response was most reproducible in the integration mode that gave areas as product of absorbance and time in seconds. The internal gas flow was interrupted for 3 sec beginning with atomization, and the maximum power input feature was used in the time-controlled mode to attain greatest response for the quantity of metal present.

Based on these experiments, the following procedure was adopted. Aliquoting 20 μL of 50% w/v disperions of lipids in MIBK, evaporation of solvent and preliminary charring of lipid aliquots in the graphite furnace were the same for each metal determined. Time and temperature controls included a 30-sec dry cycle at 100 C, 6-sec ramp to 800 C with a 35-sec hold at that temperature, and a 10-sec charring with internal air flow at 300 cm^3/min. Not every element had the same maximum recommended temperature for final charring (9). The time of air-charring was doubled for copper because the recommended 900 C char did not cause as complete a destruction of organic matter as did the 1000–1100 C maxima for the other elements. Atomization was preceded by a 15-sec final char, which also served to clear air from the system. Time of atomization was 7 sec (10 sec for chromium) at 2700 C.

In a preliminary experiment designed to evaluate the adopted instrumental method, two series of blends of hydrogenated oils and a salad oil were prepared to test reproducibility and to determine if apparent metal content changed linearly with metal concentration. The specific metals evaluated were at similar levels in each of the two oils of the first series, so that only the problems of spurious contamination from glassware and of attaining reproducible instrument response were highlighted. The second blend series, which ranged about fourfold in concentration for iron and about sixfold for nickel, served also to select the most suitable wavelengths for analyses. For Ni, Cr and Cu, the primary absorption or resonance line was chosen for its greater sensitivity. Alternate lines of reduced sensitivity, 269.7 or 302.06 nm for iron, allowed analysis in a linear absorbance range such that one dispersion of each product sufficed for all metals of interest. Although a separate experiment suggested an alternate Ni line, 234.6 or 229.0 nm, to allow linear response from solid shortenings containing high levels of Ni, occurrence of Ni in these test liquids was low. For this blend series, the low and high average metal contents were 2 ppb for Cr and 200 ppb for Fe. From measurement of each metal in each flask on four different days, the limits of detection, 2s (s = standard deviation) were calculated. All blends with metal contents less than the overall means were judged as zero content. Because a plot of estimates of each metal against the proportion of high metal content oil in these blends was linear, an application of detection limits was judged to be conservative. Further, a series of cooking and recovered margarine oils were evaluated with a range of precision for the determinations of Cr, Cu, Ni and Fe in good agreement with the blending experiment. On this basis, the standard error was adopted as an indicator of presence or absence of metals in the products tested.

Modified Method of Additions

For each specific fat, several blends were made by aliquoting that analytical solution with one of two solutions of standards of each metal. The aliquoting was done directly in the furnace. As long as the total volume was constant, the amount of spreading in the graphite tube did not vary. Twenty-μL (total volume) samplings were made using 5 μL and 15 μL micropipettes, so that furnace blends represented 0.25 and 0.75 of each standard and the complement volume as analytical solution (sample). Atomization of these four blends (two levels of standards X two ratios)

TABLE I

Nickel Content of Cooking Oils by Two Laboratories[a] (ppb)

Sample	Laboratory 1	 2
1	168	135
2	41	93
3	245	340
4	46	135
5	112	85
SEM[b]	17	7
Overall mean	122	157

[a] Means of 4 determinations and 2 determinations, respectively.
[b] Standard error (analytical) applying to each of the means.

for each sample and analysis of 20-μL volumes of the standards yielded instrument readings which, when corrected for furnace blank and MIBK (10 μL) absorbance, gave Y values in absorbance-seconds for linear fitting.

To avoid extrapolation over a potentially nonlinear absorbance range, all readings were made in the same narrow metal concentration range. Two Y-intercepts for each sample were best estimates of the sample absorbance. The calculated values on the blend plot at X = 1.0 were best estimates of absorbance of standards. The metal concentration of sample was thus calculated as a proportion of that of the standard. As an option, atomizations of 20 μL of each sample and the base oil blank gave absorbances used in conjunction with those of standards for direct reading from an absorbance/concentration plot. The number of containers required for the traditional as opposed to the modified method of additions is markedly larger. When a doubling of precision was required, the modified method as applied to a group of samples was more efficient because the number of atomizations of standards that were shared in the calculation increased.

Two laboratories of major edible fat manufacturers cooperated with us in a test of validity of our methods of analysis. A limited number of fats, primarily crude palm kernel oils, was analyzed by one of the laboratories and by our laboratory for Cu, Ni and Fe contents. These samples were also analyzed by the direct analysis method repetitively throughout the period of the study. The results indicated that assays by furnace analyses were consistent. The mean for chromium, analyzed in three fats, was 14 ppb with a relative standard deviation among the assays (RSD) of 87%. Copper analyzed in the same three fats gave a mean of 68 ppb with RSD of 54%. Nickel in four fats averaged 170 ppb with RSD of 85%. Iron, analyzed in two fats, had a mean of 630 ppb and RSD of 95%. Both laboratories were in agreement.

To validate further the method adopted for Ni analysis, five subsamples of retail cooking oils were analyzed by a second industrial laboratory (Table I). The agreement between laboratories was satisfactory since a t-test of the overall means was not significant.

Application to Commercial Samples

The application of the method of additions to commercial cooking oils, margarine oils and solid fats served as a final test of the adopted analytical method and provided estimates of the content of trace metals. Simultaneous direct analysis was also run on the samples (Table II). Thus, an overview of metal content of five products from each of three commodity types on the grocer's shelf was also obtained. Data are averages of two containers of the same lot. Excluding iron, for which analysis was omitted on solid fats, metal contents of 30 subsamples are represented. For each of the metals analyzed and in turn for each of the commodity types, differences among manufacturer products were observed. Chromium analyses were in agreement between methods. Copper analyses were slightly higher by the direct method for all oils. Results of analyses of oils for Ni were consistent for the two methods, but somewhat larger variation for Cu between sources was ob-

TABLE II

Mean Trace Metal Content for 15 Products by Method of Additions A and by Direct Analysis B

		Metal (ppb)							
		Cr		Cu		Ni		Fe	
Product	Sample	A	B	A	B	A	B	A	B
Liquid cooking oils[a]	1	3.1	2.7	27.3	34.1	132.1	129	210	285
	2	2.0	3.6	13.2	25.2	29.2	106	138	339
	3	2.8	1.8	19.6	35.1	207.4	434	193	268
	4	5.2	2.6	30.6	48.3	90.3	231	301	276
	5	1.0	1.8	36.9	36.3	77.7	44.7	229	574
	SEM[c]	1.5	2.4	10.4	20.9	30.6	65.6	130	207
Margarine oils[a]	1	2.4	3.2	43.3	54.5	51.3	115	478	451
	2	11.9	7.0	47.9	61.4	60.0	39.5	540	512
	3	3.6	6.4	57.5	60.9	69.1	61.1	279	462
	4	3.2	2.0	25.9	30.1	69.6	136	319	423
	5	5.8	3.6	26.8	40.6	34.4	49.9	239	588
	SEM	1.5	2.4	10.4	20.9	30.6	65.6	130	207
Solid shortenings[b]	1	12.8	17.0	43.0	13.2	591.3	68.0		
	2	35.2	11.1	83.1	10.5	2445	1507		
	3	8.1	6.0	26.4	19.1	2772	2735		
	4	10.9	40.3	108.3	17.7	895.7	642.1		
	5	16.3	34.2	28.1	16.9	697.5	180.1		
	SEM	8.8	21.7	47.1	6.9	278.7	210.3		

[a] Means of 6 determinations A and of 3 determinations B.
[b] Means of 4 determinations A and of 2 determinations B.
[c] Standard error applicable to each mean (based upon container-to-container variability).

served by the method of additions. For both Cu and Ni, the direct analysis of solid shortenings gave lower values than the method of additions. Also for solid shortenings, larger variations among sources were noted for Cu by method of additions and for Ni by direct analysis. Iron analyses of oils were similar for both methods of analysis.

Because of decreased analysis time, direct analysis is probably the method of choice for all oils, but somewhat better precision was observed for the method of additions. In the light of apparent differences among products and between methods, the method of additions was preferred for analyses of Cu and Ni in solid shortenings. These methods were shown by preliminary experiments to give results consistent with those of other methods.

The range of metal contents and the respective container-to-container standard errors for Ni, Cr, Cu and Fe in cooking oils were 29-207 ± 31 ppb, 1-5 ± 2 ppb, 13-37 ± 10 ppb and 138-301 ± 130 ppb. Except for nickel which was uniformly low, these metals were found to also occur at these ranges in recovered margarine oils. The range of metal occurrence in solid shortenings was Ni, 591-2772 ± 279 ppb; Cr, 8-35 ± 9 ppb; and Cu, 26-108 ± 47 ppb.

The trace and ultratrace levels at which metals occurred in these samples necessitated that the analyses be replicated extensively. The relative standard deviation (RSD) for a determination of nickel or iron at the trace level was ∿30%, while for a determination at the ultratrace level of copper or chromium, the RSD was ∿150%.

A recently published IUPAC reference method for Ni in serum and urine (5) requires acid digestion, complexometric extraction of the metal, and measurement in a graphite-furnace absorption instrument. Their interlaboratory study indicated RSD of 23-24%. Our method, which requires no preanalytical manipulation, detects Ni in cooking oils at the same analytical range but with an increased RSD.

A number of extraction and complexometric methods have been reported for lipids (3). Most of these methods fail, however, with highly refined products with low levels of metals. The general problem is to transfer sufficient mass of metal into the instrument furnace to obtain a response. The average content of Ni, Cr, Cu and Fe in cooking or margarine oils exceeds the absolute sensitivities (9) by factors of 8, 2, 10, and 200, respectively. These limits in sensitivity are, however, established under the most ideal conditions with aqueous solutions containing only one cationic form and no associated organic matter requiring complete combustion. The solid fats averaged 148, 8 and 19 times the absolute sensitivities for Ni, Cr and Cu. The necessity of analyzing Ni in solid fats and Fe in cooking oils at alternate absorption lines of lower sensitivity is obvious in that this Ni content is 3 times the linear limit and Fe is 4 times the linear limit of the analyses at the primary lines.

Results of our analyses for nickel content of the commercial products (Table II) indicate that current post-hydrogenation processing effectively removes residual nickel catalyst from partially hydrogenated soybean oils used for salad/cooking oils and margarines, but removes it less effectively from more highly hydrogenated fats used in plastic shortenings.

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