Copper in Edible Oils: Trace Amounts Determined by Atomic Absorption Spectroscopy[1]

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ABSTRACT

The application of atomic absorption spectroscopy to trace copper analysis in fats was investigated. Copper spectral resonance lines 3248 A and 3274 A possess the most useful sensitivities applicable to fats and vegetable oils. These sensitivities were determined to be 0.15 and 0.25 ppm in oil, respectively, with detection limits about tenfold lower. Data show that copper absorption depends strictly on the amount of oil aspirated into the flame. In soybean oil-solvent mixtures, aspiration rates decrease logarithmically and linearly with increasing fat content. The accuracy and precision of atomic absorption techniques were established by comparing results from atomic absorption with those from two independent techniques. Error estimates were determined by analysis of copper-hydrogenated soybean oil diluted to low copper levels. The standard deviation, relative standard deviation and analytical error were 0.0108 ppm, 5% and 2.5%, respectively, over a range of 0.05-0.40 ppm copper in soybean oil.

INTRODUCTION

The detrimental effect of metals, particulary iron and copper, on keeping qualities and flavor stability of highly unsaturated fats is well known (1). Early work at the Northern Laboratory showed that copper, if in active form, can seriously affect the flavor stability of soybean oil at levels as low as 30 ppb. Over the past few years there has been considerable interest both here and abroad in copper-containing catalysts to hydrogenate soybean oils (2-4). Such is the background for continued interest in trace metal determination in edible fats.

Since atomic absorption was introduced (5), procedures have been developed for measuring nearly every metal. Despite the many advances in technique and refinements in instrumentation, little work has been published on the application to trace metal analysis in fats.

Piccolo and O'Connor (6) reported preliminary data for analysis of sodium, calcium, copper, iron and zinc in a variety of vegetable fats. Their work did not include an extensive study of copper. Although several procedures have been published for sodium, potassium, calcium and magnesium in edible oils (7,8), methods have not been finalized for heavy metals including iron, chromium, nickel and copper. Persmark (9) reviewed the possibilities and limitations of metal determinations in fats by atomic absorption.

EXPERIMENTAL PROCEDURES

Atomic Absorption Equipment

The atomic absorption spectrophotometer used in this study was a Perkin-Elmer Model 303 equipped with recorder readout facilities capable of expanding the scale 100X. The burner was a three-slot Boling-type of laminar flow design operated with an air-acetylene mixture for fuel. A water-jacketed constant-temperature bath was constructed to hold a sample during analysis and was maintained at 40 C.

Materials

Bis(1-phenyl-1,3-butanediono) copper(II) (standard reference material 1080) was purchased from the National Bureau of Standards. Its copper content was 16.5%.

Methyl isobutyl ketone (MIBK) was purchased from Aldrich Chemical Co. and was redistilled. The fraction boiling at 115-116 C was used.

Soybean Oils

Soybean oil was a commercially refined, bleached and deodorized salad oil. Copper-hydrogenated samples were prepared in the pilot plant of the Northern Laboratory.

Standards

A 500 ppm copper standard in soybean oil was prepared according to the data sheet supplied with the National Bureau of Standards' copper chelate except soybean oil replaced lubricating oil. Working standards were prepared by dilution of the stock solution with additional soybean oil. Although published reports indicate that standards prepared from some fat-soluble metal chelates are unstable (10), our copper standards have remained the same for more than a year. A typical standard curve for copper is shown in Figure 1.

Instrumental Parameters

Except where the effects of variable lamp current, slit width and flame type were studied, the instrument was operated with a lamp current of 15 mA, slit setting 4 and a slightly luminous flame. Wavelength was 3248 A.

Sensitivity Determinations

Copper sensitivities were determined by aspirating standards of copper in soybean oil as 25% solutions in MIBK and by plotting absorbance versus concentration. Sensitivity was taken as the copper concentration (oil basis) giving 1% absorption.

Measurement of Aspiration Rates and Absorption Maximum

Tared samples containing various concentrations of oil in MIBK were aspirated for 3 min into a slightly luminous flame. Aspiration rates were calculated from the resulting weight loss.

The optimum oil-to-solvent ratio was determined by aspirating a soybean oil-copper standard, diluted to various concentrations with MIBK, into a slightly luminous air-acetylene flame. When solutions of MIBK containing different amounts of fats are aspirated into the flame, the flame appearance and temperature change. To minimize the effect of temperature, absorption readings were taken after manually adjusting the fuel flow to give a uniform, slightly luminous flame for all aspirations.

RESULTS AND DISCUSSION

Since the copper contents of vegetable fats are usually quite low, high sensitivity is required. To achieve maximum sensitivity, the instrument should be operated at the optimum lamp current, spectral slit width, air-to-fuel ratio

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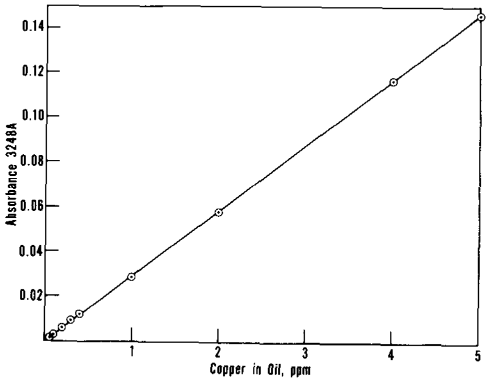

FIG. 1. Standard curve for copper in soybean oil; 25% fat in methyl isobutyl ketone (MIBK) by weight.

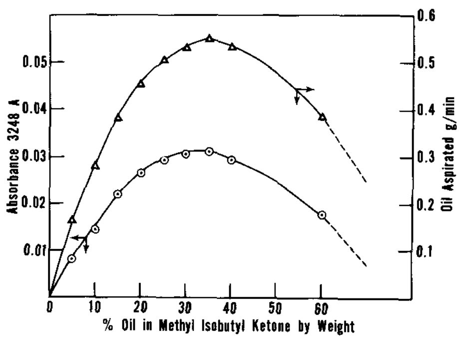

FIG. 2. Effect of soybean oil concentration on copper atomic absorption. Upper curve, oil aspirated-g/min vs. fat concentration in MIBK. Lower curve, absorbance versus fat concentration. Sample contained 1.0 ppm copper.

and resonance line. Slavin (11) points out that instrumental parameters have little effect on copper absorption in aqueous solution. Since fats are generally analyzed as solutions containing organic solvents, it seemed desirable to study the effect of instrumental settings on the copper absorption of fat dissolved in MIBK solutions.

In aqueous solution, copper absorption is almost independent of lamp current (11). Our results indicate that increasing lamp current slightly decreases copper absorption from fat-MIBK systems. When a 1.0 ppm copper in soybean oil (25% by weight in MIBK) was aspirated, settings of 5, 10, 15, 20 and 25 mA gave 8.9, 8.8, 8.2, 7.7 and 7.3% absorption, respectively. Because data indicate that increasing lamp currents lowered absorption, lamp currents were kept low.

Spectral slit width up to about 20 A has little effect on copper absorption at the 3248 A line. Also, fuel-to-air ratio has little effect on copper absorption. Lean, slightly luminous and rich flames gave essentially the same absorption when a 1.0 ppm copper-soybean oil standard was aspirated into the flame as a 25% solution in MIBK. Evidently for fat-MIBK mixtures the instrumental parameters, such as lamp current, spectral slit width and fuel-to-air ratio, have little effect on atomic absorption of copper.

The only instrumental problem encountered with oils in the copper atomic absorption method was a baseline drift caused by the close proximity of the sample to the flame. In effect, this proximity changed the sample aspiration rate slightly which, in turn, changed the baseline. Analyses carried out for low copper levels require that the scale be expanded. This expansion also increases baseline and noise problems.

The baseline problem can be quite serious at low metal concentrations since the initial amount of absorption is small. To overcome baseline drift, a small water-jacketed constant temperature bath was constructed to hold the sample during analysis. In addition, another constant temperature bath was used to equilibrate other samples before analysis. Improvements in baseline stability are realized because the sample is insulated from temperature effects of the flame. In addition, by aspirating samples at 40 C instead of room temperature, some increased sensitivity is realized since the sample viscosity is decreased and more sample is aspirated into the flame.

The copper spectrum first investigated by Allan (12) offers two resonance lines useful for trace copper analysis in fats. Listed in Table I are sensitivities determined for the 3248 and 3274 A copper resonance lines. Data for aqueous solutions are included for comparison. The data show sensitivities (parts per million for 1% absorption) of 0.15 and 0.25 ppm (oil basis) for the 3248 and 3274 A absorption lines, respectively. The detection limits (1% above noise) are about tenfold lower. Thus, with the 3248 A line, about 0.015 ppm copper can be detected in edible oil when the oil is dissolved as a 25% solution by weight in MIBK. Although the 3248 A line should be used for maximum sensitivity, the 3274 A line may be more useful when analyzing for copper in fats heavily contaminated with copper catalyst residues.

Vegetable oils present several unique problems in trace metal analysis by atomic absorption. Because fats are too viscous to aspirate directly into the flame, their viscosity must be reduced by dilution with some organic solvent. Solvents for atomic absorption work must have low volatility and low surface tension and must be combustible in the flame. Solvents used for fats by other investigators include MIBK (6), ethyl acetate (12) and *n*-heptane (13). We chose MIBK because of its low volatility and ready availability.

Slavin (11) points out that most workers find it necessary to match standards and samples with respect to materials present in excess of 1% in solution. This matrix matching technique is particularly necessary in fat analysis by atomic absorption since large amounts of fat are usually present in solution. The primary reason for matrix match-

TABLE I

Sensitivity of Copper Atomic Absorption Lines

	Sensitivity, ppm/1% absorption		
		Soybean oil	
Wavelength, A	Water[a]	On oil basis	In MIBK[b] solution, 25%
3247.5	0.1	0.15	0.038
3274.0	0.2	0.25	0.063

[a] Reference 12.

[b] Methyl isobutyl ketone (MIBK).

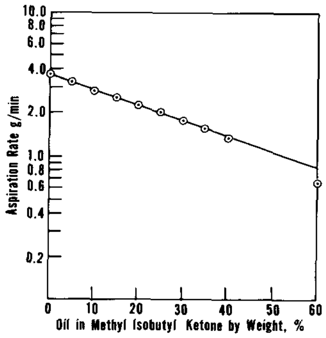

FIG. 3. Effect of oil concentration on aspiration rates of oil-MIBK solutions.

TABLE II

Comparison of Methods for Copper in Hydrogenated Soybean Oil

	Method, ppm copper in oil		
Oil	Atomic absorption[a]	Neutron activation[b]	Colorimetric[c]
A	4.77	4.55	4.70
B	3.40	3.25	3.21

[a] Determined as 25% oil solutions in MIBK.

[b] Performed by commercial laboratory.

[c] By low temperature dry ashing and zinc dibenzyl dithiocarbamate method (14).

ing in fat-solvent systems is to ensure that samples and standards have the same viscosity, density and surface tension. One of the prime difficulties encountered with analyzing trace copper in fats is to obtain a copper-free oil for preparing standards and for establishing a baseline as a reference point in the analytical procedure. Others (6) have used molecularly distilled oil to prepare standards for trace metal work, but in our experience one such sample contained detectable amounts of copper.

We prepared copper standards by dissolving a fat-soluble chelate (National Bureau of Standards No. 1080) in soybean oil. The particular soybean oil we used for preparing standards, establishing the recorder baseline and performing dilution studies had a copper content of 0.041 ppm as independently determined by colorimetric and neutron activation analysis. The recorder must be set at zero while burning a sample of the blank or reference oil so that any copper present in the blank will not affect the analysis and only the difference between the reference oil and the unknown will be detected. All data here are reported as copper content above the value of the reference oil.

An optimum oil-to-solvent ratio should exist for maximum absorption because increasing the metal concentration should logically increase the absorption signal until the viscosity is increased to such an extent that less fat-metal is aspirated into the flame.

The optimum oil-to-solvent ratio was determined by aspirating different dilutions of a 1.0 ppm copper solution in soybean oil into the flame. The effect of increasing oil concentration on copper absorption, plotted in Figure 2, shows that, indeed, an optimum oil-to-solvent ratio exists at 35% oil in MIBK for this particular burner and aspirating air pressure.

To account for the results presented in Figure 2, aspiration rates of the oil-to-MIBK mixtures were determined at 40 C (Fig. 3). By increasing the oil-to-solvent ratio, viscosity is increased and, consequently, less solution is aspirated into the flame. The relationship between the aspiration rate and oil concentration is a logarithmic function with the equation of the line being $\log y = 0.5682 - .0111p$ where y = aspiration rate (g/min) and p = percentage oil by weight in MIBK. The decrease in aspiration rate is uniform in the range 0-40% oil.

TABLE III

Copper Contents of Soybean Oil by Atomic Absorption

Calculated copper content, ppm	Found by atomic absorption, ppm[a] 1	2	3	4	Mean
0.4	0.412	0.386	0.410	0.408	0.404
0.3	0.315	0.315	0.304	0.300	0.308
0.2	0.213	0.218	0.207	0.210	0.212
0.1	0.093	0.093	0.091	0.091	0.092
0.05	0.059	0.060	0.045	0.050	0.054

[a] Determinations 1 and 2 as 25% oil solutions in MIBK; 3 and 4 as 10% oil solutions in MIBK. Standard deviation (1σ) over entire range = 0.011 ppm. Relative standard deviation, 5%, which includes both analytical and random error. Analytical error, 2.5%.

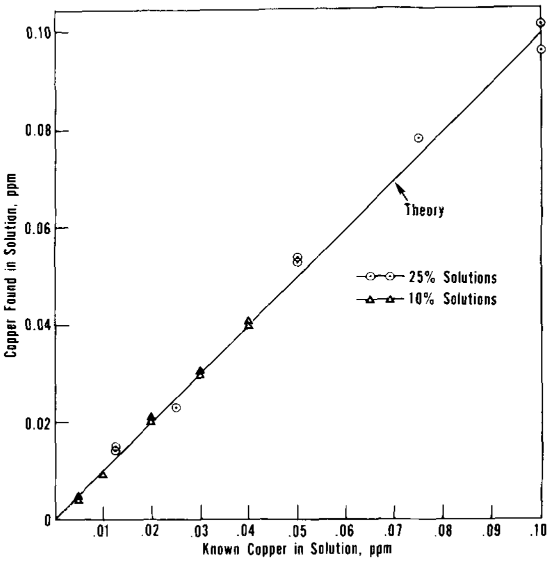

FIG. 4. Recovery of copper by atomic absorption from 10% and 25% oil solutions in MIBK.

Confirmation that 35% oil represents the optimum fat concentration for copper absorption is found in the upper curve in Figure 2 where the amounts of oil aspirated per minute (calculated from the aspiration rates) is plotted against absorbance. Under these conditions the maximum amount of oil (and therefore copper) is aspirated at 35% oil in solvent. Potassium in peanut oil reportedly gives maximum absorption at 30% oil in MIBK (8).

Our results indicate that a fairly wide range of oil-to-solvent ratio would be suitable for copper analyses. Several factors must be considered when choosing an optimum oil-to-solvent ratio. High oil-to-solvent ratios increase the troublesome burner clogging problem in which unburned oil accumulates in the burner head, which must be cleaned. The sensitivity of the method must also be considered. Higher oil-to-solvent ratios increase sensitivity and the amount of absorption obtained, but evidence presented later indicates that lower oil-to-MIBK ratios favor better accuracy and precision of analytical determinations. As a compromise, we adopted 25% oil by weight in MIBK as a standard procedure for copper analyses.

The accuracy and reliability of atomic absorption for copper in soybean oil were checked by two other methods. Two samples of copper-hydrogenated soybean oil analyzed by atomic absorption were also analyzed by neutron activation and colorimetry (Table II). The agreement between atomic absorption data and that obtained by the other methods is excellent, thus providing an independent check on the reliability of atomic absorption for copper in soybean oil. Few or no data have been reported on the accuracy and precision of copper determinations by atomic absorption. Our previous report indicated that relative errors were of the order 9% (1σ) for duplicate samples ranging in copper content from 0.05 and 5.0 ppm added copper (14). These analyses were performed by a commercial laboratory.

To obtain an error estimate at low copper levels, oil A (copper content, based on atomic absorption data, 4.77 ppm, Table II) was diluted with lower copper soybean oil to give calculated copper contents of 0.4, 0.3, 0.2, 0.1 and 0.05 ppm (above the baseline oil), respectively. The diluted samples were analyzed as 10% and 25% oil solutions in MIBK (Table III). Statistical treatment showed no significant difference between analytical results for the 10% and

25% oil levels. The standard deviations were 0.008 and 0.013 ppm, respectively, and based on the F-ratio test, the two do not differe significantly. Therefore, data where pooled to obtain a standard deciation which was 0.011 ppm for the entire range of copper levels with a relative standard deviation of 5% that includes both analytical and random error. The analytical error is 2.5%. These results further confirm the precision and accuracy of copper determinations in edible fats by atomic absorption techniques.

While the flame is the heart of the atomic absorption spectrophotometer, it is also unfortunately the weakest part. Most problems associated with trace metal determination in fats have been attributed to the flame where measurements are made. Flame measurements are influenced by slight changes in gas flow, sample viscosity, ground state atom populations and variations in flame temperature. Reportedly these so called sample matrix errors are quite pronounced, particularly when fat concentrations are high (9). Sample matrix errors are extremely difficult to measure experimentally and their effects are evident only in analytical data. If high fat concentrations are responsible for poor analytical data, then lower oil-to-solvent ratios should decrease the effects of these matrix errors.

The recovery of copper from 10% and 25% oil solutions in MIBK (data from Table III) is plotted in Figure 4. Although the differences are small, lower oil-to-solvent ratios favor better accuracy and precision for analytical determinations because data points for the 10% fat solutions lie closer to the theoretical recovery line than data points representing the 25% solutions.

Even though matrix errors influence metal determinations by atomic absorption, well-refined fats have copper content very close to or below the detection limits. The analyst is faced with measuring minute amounts of absorption some 100-fold below the optimum level suggested by instrument manufacturers.

Our presented data indicate atomic absorption is readily adaptable to determining traces of copper in fats and to studying the effectiveness of metal removal treatments. Preconcentraton steps like ashing and extracting will have to be performed to render refined oils amenable to atomic absorption. These techniques are under investigation and will be reported later.

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