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Determination of Copper in Edible Oils by Direct Graphite Furnace Atomic Absorption Spectrometry

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ABSTRACT

A simple method for determination of copper in edible oils was developed, using a polarized Zeeman graphite furnace atomic absorption spectrophotometer and a pyro graphite tube. Edible oils were diluted with 2%lecithin-cyclohexane and analyzed directly by the standard addition method. The optimal ashing and atomizing temperatures were 900°C and 2700°C, respectively. A standard solution diluted with 2% lecithin-cyclohexane was superior to salad oil or cyclohexane. Results were best measured when the concentration of lecithin in cyclohexane was higher than 2%. The detection limit of this method was 2.0 ng/mL. The respective range of recovery of copper was 85.5~90.0% and 86.0 ~ 93.0% in salad oil and lard which were fortified with 10, 20 and 40 ng Cu/mL respectively using the standard addition method.

Key words: **graphite furnace atomic absorption spectrophotometer, copper, edible oils and fats.**

INTRODUCTION

Metal elements such as Na, K, Ca, Mg, Fe, Cu, Zn, Co, Mn, and Mo are essential nutrients in human body growth. However, metal elements sush as Hg, Pb, Cd, As, and Cu, could also have detrimental effects on health. The harmful effect induced by Cu only occurs when it is overdosed. In general, a “hazardous metal” is defined as a metal which could induce adverse symptoms on the human body when consumed even in trace amounts[1].

Metal-based contaminants are slightly decomposed once they are released into the environment. Human may be exposed to environmental metals through many different pathways. These contaminants could be consumed directly from drinking water or from marine products. Metals in soil can possibly be transferred into the human body when humans eat vegetables or other crops, which are cultivated in soil containing metals. Airborne metals can enter the human body through the respiratory system or by food contaminated by metals in the air. In general, the contamination levels through the above pathways are too low to induce the toxicity[1-2].

Copper is an essential element in maintaining body functions. It is regulated in the body via absorption, excretion, and a combination of processes. However, overdoses of copper could be

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toxic. The acute toxicity of copper can induce some symptoms such as nausea, vomiting, stomachache, diarrhea, dizziness, hepatosis, jaundice and hemolysis[1-2].

The tolerance level of copper in edible oil is set to be 0.4 ppm according to the Standard of Edible Oil announced by Department of Health in 1993. In Taiwan, the Chinese National Standard (CNS) No. 4529N6105 entitled "Method of Test for Edible Vegetable Oils: Determination of Lead and Copper"[3] is used to determine the copper content in edible oil. Sample oil is ignited and combusted using cotton as an ignition aide. The residue is ashed under high temperature and then dissolved in acid solution followed by extraction with solvent and concentrated prior to atomic absorption spectrophotometer detection. This operation, however, is complicated. The detection result is deeply affected by the extraction as well as concentration efficiency. The sensitivity using this standard method is usually unsatisfactory.

Graphite furnace atomic absorption spectrophotometer (GFAAS) is an instrument designed for trace element analysis. This instrument is widely used for the determination of heavy metals in food[4-13], body fluids[14], and environmental contaminants[19-20]. The GFAAS method allows minimal samples (only 20 μL) to be analyzed. The sample undergoes drying and ashing processes for removal of the substrate before GFAAS detection. The advantage of this method is that the sample can be directly introduced into the graphite furnace tube for analysis without digestion. In this study, GFAAS was conducted to determine the copper content in edible oil. The optimum ashing and atomizing conditions, limit of detection, and recoveries from various oils were researched in order to set up a precise method to detect trace amounts of copper in edible oils.

MATERIALS AND METHODS

I. *Reagents*

Copper standard in oil (1 g/kg) and cyclohexane were purchased from Merck Co. (Darmstadt, Germany). Lecithin (powder) from soybean was obtained from Nacalai Tesque (Japan). Pure water was prepared in our laboratory by Milli-Q SP (Millipore Co., USA.). Standard stock solution was prepared by diluting 1 g copper standard to a volume of 100 mL, with 2% lecithin-cyclohexane solution, which was made by dissolving 2 g lecithin in 100 mL of cyclohexane. All standard working solutions were prepared from a stock solution by diluting with 2% lecithin-cyclohexane.

II. *Devices and Instruments*

Polarized Zeeman Graphite Furnace Atomic Absorption Spectrophotometer Z-8000 equipped with a model SSC-100 auto-sampler, a pyro tube (type A 190-6003), and a copper hollow cathode lamp was the product of Hitachi Co. (Japan). A Thermostatic oven was obtained from Memmert Co. (Germany). All volumetric bottles and other glassware were Pyrex brand. Before use, they were washed with detergent and water, soaked with 50% (v/v) nitric acid overnight, rinsed with water, and dried.

III. *Methods*

GFAAS instrument parameters used in this study are listed in Table 1. The temperature setting for ashing and atomizing was according to the operation manual. These two temperatures were adjusted by introducing 20 μL of standard copper solution (20 ng/mL) to optimize instru-

Table 1. Instrument parameters for the determination of copper in edible oils using graphite furnace atomic absorption spectrophotometer

Parameter	Setting
Wavelength	324.8 *nm*
Slit width	1.3 *nm*
Mode	Peak height
Lamp	Hollow cathode
Lamp current	7.5 mA
Purge gas	Argon
Purge gas flow	200 mL/min. (30 mL/min. during atomization)

ment performance.

The temperature program of GFAAS is listed in Table 2. The drying temperature ramped from 80 to 110°C in 20 sec was used for removal of cyclohexane which boiling point is 80°C. To prevent oil samples from splattering, the drying temperature was gently increased from 110°C to 130°C in 20 sec and held at 130°C for another 20 sec. The initial ashing temperature was set at 400°C and held for 60 sec for complete removal of smoke as well as preventing oil spattering since the smoking point of oil is in the range of 350~450°C.

Testing the effect of the dilution reagent was conducted as follows: A copper standard was dissolved in soybean oil, cyclohexane, or 2% lecithin-cyclohexane to make a concentration of 20 ng/mL copper solution, which was then directly analyzed by GFAAS. To study the effect of lecithin concentration on absorbance, the copper standard was diluted to a concentration of 20 ng/mL with 0, 0.5, 1, 2, 3 or 4% lecithin-cyclohexane and then analyzed by GFAAS.

Determination of detection limit, based on 3 times of standard deviation, was conducted by consecutively analyzing soybean oil 10 times.

To determine the matrix effect on the slope of the calibration curve, five different oils (5 mL) including soybean oil, palm oil, olive oil, sunflower oil, and lard were individually spiked with a copper standard solution. The oil samples were then diluted to a series of copper concentrations (10, 20, and 40 ng/mL) with 2% lecithin-cyclohexane. Lard was liquefied at 80°C for 15 min before use. The calibration curve was plotted based on the absorbance versus concentration.

Comparison on the recovery obtained from linear calibration and standard addition methods was conducted as follows： Five mL of soybean oil or lard containing a copper standard was separately diluted to a volume of 10 mL with 2% lecithin-cyclohexane to make a series of concentrations including 0, 10, 20, and 40 ng/mL. The recoveries from both linear calibration and standard addition methods were calculated by deduction of blank samples without copper addition. A standard addition method was performed by spiking different concentrations of copper solution to the same volume of sample matrix. Figure 1 demonstrates a calibration curve after deduction of the absorbance of reagent blank using the standard addition method.

Table 2. Temperature program for the determination of copper content in edible oils by GFAAS

Step	Temperature (°C)		Ramp (sec)
	initial	end	
Drying	80	110	20
	110	130	20
	130	130	10
Ashing	400	400	60
	600	600	50
	900	900	30
Atomizing	2700	2700	7
Cleaning	3000	3000	5

RESULTS AND DISCUSSION

I. *Optimization of Instrument Conditions*

A 2% lecithin-cyclohexane solution containing 20 ng/mL copper was introduced to GFAAS in order to optimize both ashing and atomizing temperatures. The optimum ashing temperature was found to be 900°C over the temperatures from 750 to 950°C tested when the atomizing temperature at 2200°C was used (Figure 2). The maximum

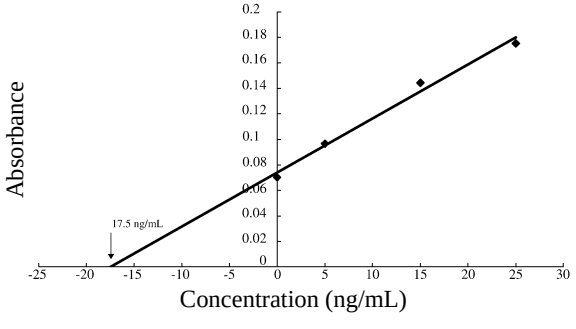

Figure 1. Copper content of palm oil by addition medthod.

absorbance was obtained at the atomizing temperature of 2700°C when the ashing temperature was set at 900°C as shown in Figure 3.

Using the above optimum temperatures to analyze a copper standard solution, the equation of calibration curve plotted by peak height versus concentration was regressed to be Y=0.0074X + 0.0315 with a relative coefficient of 0.9955. The limit of detection was calculated to be 2.0 ng/mL, which was determined by analyzing soybean oil, ten times consecutively.

II. *Effects of Dilution Reagent and Lecithin Concentration*

Three dilution reagents, soybean oil, cyclohexane, and 2% lecithin-cyclohexane were tested for comparison of the effect of a dilution reagent on copper detection. The copper standard was diluted to a concentration of 20 ng/mL and directly analyzed by GFAAS. Results showed that using 2% lecithin-cyclohexane was capable of getting the highest response for copper detection. The absorbances were 0.1734 ± 0.0112, 0.1038 ± 0.0013, and 0.0924 ± 0.0113 (mean ± S.D.) while using 2% lecithin-cyclohexane, cyclohexane, and soybean oil, respectively, as dilution reagents. Using soybean oil to dilute the copper standard gave the least response for copper detection. This could be due to the viscosity of soybean oil which is too high to completely mix with the copper standard. Cyclohexane is capable of reducing the viscosity when it is mixed with oil allowing the absorbance to be enhanced; however, the response on copper detection by using cyclohexane as a dilution reagent was still lower than that using 2% lecithin-cyclohexane.

To investigate how the lecithin concentration

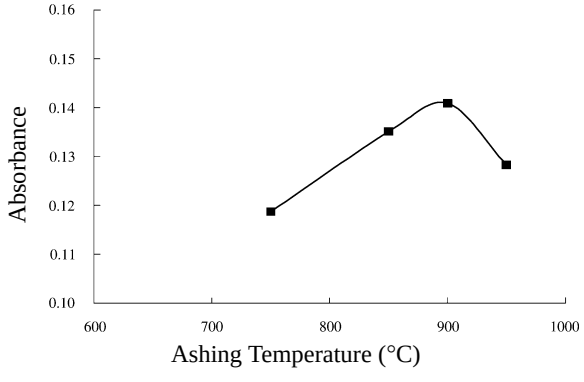

Figure 2. The optimal ashing temperature to measure copper in edible oils.

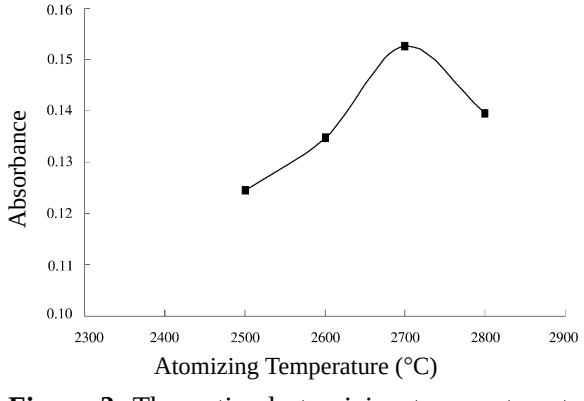

Figure 3. The optimal atomizing temperature to measure copper in edible oils.

Table 3. Ratios of slope of linear calibration curves obtained from various type of standard solution, edible oils and fat

Edible oil and fat	Slope	Ratio[a]
Standard solution	0.0085	1
Salad oil	0.0074	0.87
Palm oil	0.0081	0.95
Olive oil	0.0068	0.80
Sunflower seed oil	0.0074	0.87
Lard	0.0056	0.66

[a] Slope of curve from standard copper added in edible oil and fat was divided by slope of curve from standard copper in 2% lecithin-cyclohexane solution.

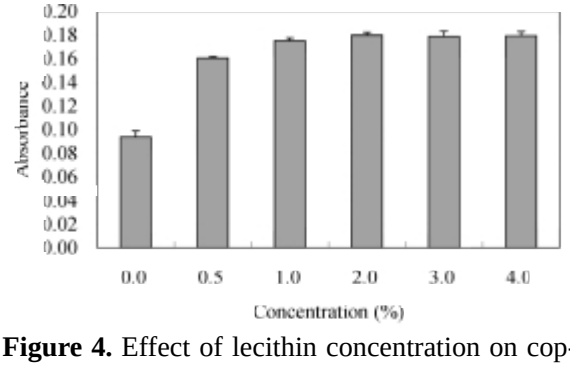

Figure 4. Effect of lecithin concentration on copper measurement in edible oils.

in the dilution reagent affected results, five concentration levels of lecithin in cyclohexane were prepared. The average absorbances of the copper standard in 0.5, 1, 2, 3, and 4% lecithin-cyclohexane solutions were detected to be 0.1600, 0.1750, 0.1795, 0.1785, and 0.1790, respectively (Figure 4); while the average absorbance of the copper standard in cyclohexane without lecithin added was 0.0935. Results showed that the lecithin concentration higher than 2% was capable of getting a better sensitivity of copper detection as shown in Figure 4. An oily copper standard can not be completely dissolved in cyclohexane resulting in a lower response of copper detection. Introducing lecithin, an emulsifier, to cyclohexane can greatly improve the absorbance because not only does lecithin-cyclohexane reduce the viscosity of the oil sample, but the phosphoric group in lecithin can also be a matrix modifier[21], which can improve the detection temperature as well as increase the background absorption so as to reduce interference. The action mechanism of lecithin is similar to that of $NH_4H_2PO_4$, which was used as a matrix modifier to investigate copper content in malt drink, in a repont according to Jaganathan and Kuger[22]. In the present study, 2% lecithin-cyclohexane was therefore selected as the dilution reagent for an oily standard.

III. *Slope Ratios of Calibration Curves of Copper Standard in Edible Oils*

Slope ratio of calibration curves between edible oil and a standard solution (2% lecithin-cyclohexane) was used to exam the interference level from matrices. The value of the slope ratio closing to 1 signifies that the analysis is less interfered by the matrix. A value less than 1 means the analysis could be interfered by the matrix, while a value of more than 1 symbolizes a synergistic effect occurs by introducing the matrix. Slope ratios of calibration curves of copper standard in different matrices were various (Table 3). As can be seen in Table 3, the slope ratios of calibration curves of copper standard in soybean oil, palm oil, olive oil, sunflower oil, and lard were 0.87, 0.95, 0.80, 0.87, and 0.66, respectively. These results indicate that the linear calibration method is less applicable to determine copper content in oil samples because

Table 4. Comparison of copper recoveries for salad oil and lard by linear calibration method and standard addition method

Copper added (ng/mL)	Salad oil					
	Linear calibration			Standard additions		
	Found[a] (ng/mL)	Recovery (%)	RSD[b] (%)	Found (ng/mL)	Recovery (%)	RSD (%)
10	7.1	71.0	4.7	9.0	90.0	2.1
20	13.0	65.0	4.1	17.8	89.0	3.3
40	22.7	57.8	2.9	34.2	85.5	2.7

Copper added (ng/mL)	Lard					
	Linear calibration			Standard additions		
	Found (ng/mL)	Recovery (%)	RSD (%)	Found (ng/mL)	Recovery (%)	RSD (%)
10	8.1	81.0	4.5	9.2	92.0	2.9
20	15.7	78.5	4.3	17.2	86.0	3.5
40	34.8	87.0	3.3	37.2	93.0	2.5

[a] Average of three determinations.

[b] Relative standard deviation.

the analysis using the linear calibration method is affected by matrices.

IV. *Comparison of Linear Calibration and Standard Addition Methods*

Both the linear calibration and standard addition methods were carried out to determine copper recoveries from soybean oil and lard. By using the linear calibration method, recoveries obtained from soybean oil spiked with 10, 20, and 40 ng/mL copper standard were 71.0, 65.0, and 57.8%, respectively, and from lard spiked with the same levels of copper were 81.0, 78.5, and 87.0%, respectively, with relative standard deviation less than 5% as shown in Table 4. By using the standard addition method, recoveries from soybean oil spiked with the three above levels of copper standard were determined to be 90.0, 89.0, and 85.5%, respectively, and from lard were 92.0, 86.0, and 93.0%, respectively (Table 4). These results demonstrate that the standard addition method is superior to the linear calibration method in terms of copper recovery from soybean oil and lard. According to published research, the standard addition method has been adopted to analyze copper content in infant formula[8] and heavy metals in fish oil[23]. Therefore, using the standard addition method to analyze copper metal in edible oils or fat is strongly recommended.

CONCLUSIONS

In this study, a standard addition method was developed to analyze copper in oil samples. Sample preparation was carried out by simply diluting oil samples with the same volume of 2% lecithin-cyclohexane solution prior to detection by a graphite furnace atomic absorption spectrophotometer. This developed method is capable of generating accurate results effectively without a complicated and hazardous digestion pretreatment of oil samples.

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以石墨爐式原子吸收光譜法測定食用油脂類中銅之含量

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摘　要

本研究探討以石墨爐式原子吸收光譜儀直接分析食用油脂類中之銅含量，將檢體以2% lecithin-cyclohexane溶液等量稀釋後，以偏極化茲曼石墨爐式原子吸收光譜儀直接檢測，得知最適灰化溫度為900°C，最適原子化溫度為2700°C。銅標準溶液之稀釋液為2% lecithin-cyclohexane優於沙拉油和cyclohexane。Lecithin在cyclohexane之濃度高於2%時，吸光度檢測狀況最佳。本方法之油脂樣品偵測極限為2.0 ng/mL，其次，添加銅10，20及40 ng/mL於大豆沙拉油中，使用標準添加法測定，其回收率為85.5%～90.0%。添加於豬油中，回收率為86.0%～93.0%。

關鍵詞：石墨爐式原子吸收光譜儀，銅，食用油脂。