Analytical Method for Determining Copper in Edible Shortenings and Oils

RUDOLPH E. DECK and KAY K. KAISER, The Procter & Gamble Company, Winton Hill Technical Center, Cincinnati, Ohio 45224

Abstract

A reliable, practical method was developed for the quantitative determination of sub-ppm levels of copper in edible shortenings and oils. Copper is quantitatively extracted with a boiling solution of 0.01% EDTA in 25% HCl. The extract was analyzed with 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (Bathocuproine), a highly specific and exceptionally sensitive reagent for the colorimetric determination of copper. An average recovery of 99.8% was obtained on 22 samples containing added oil soluble copper standards. A standard deviation of 0.002 ppm was established at the 0.05 ppm level during routine application of this method to a variety of shortenings and oils.

Introduction

Quantitative analysis of copper in edible shortenings and oils is necessary, both in quality control and experimental development. Several methods are in the literature, but are either impractical or not reliable for everyday laboratory use. The method developed and described in this report is not only precise and accurate, but applicable to high volume analyses of copper in shortenings and oils with a minimum of time and equipment. While a single sample can be analyzed in 3 hr, six samples can easily be run by an analyst in one day.

List et al. (7) adopted a low-temperature dry asher, an apparatus in which oxygen plasma is generated in a radio frequency field under high vacuum, for ashing glyceride oils. The residues were analyzed by a colorimetric procedure using zinc dibenzyldithiocarbamate as a reagent. The relative error they reported of a single determination was ±13% and that of the mean of duplicate determinations was ±9%. They used pure copper nitrate as the reagent for preparing oils containing known levels (0.04–5.0 ppm) of copper. A sample commonly employed required 60–72 hr to be ashed in their system. For lower levels of copper, 15 g of sample were needed, so three to five residues had to be combined before assay. They showed levels of copper in unhydrogenated, commercially processed soybean oils varying from 0.03–0.10 ppm. Labuza and Karel (6) described a direct solvent extraction procedure for copper and iron in fatty acids and fatty methyl esters. The application of this method to shortenings and oils was not demonstrated. A recent symposium (8) included discussions on several analytical techniques for copper in edible oils. Atomic absorption spectrophotometry was shown to have a lower limit of 0.05–0.10 ppm when measuring copper concentration directly in fats. With several instrument modifications, limits of 0.01–0.05 ppm copper in fat have been reached.

Høgdahl and Melsom (5) determined copper in two samples of hydrogenated fat by neutron activation analysis. A sample of fat was irradiated for 24 hr and the radioactive copper was extracted with 6 N HCl from a toluene solution of the irradiated fat. The gross gamma activity was measured with a NaI(Tl) well-type scintillation crystal. A colorimetric measurement (as copper dibenzyldithiocarbamate) was also made and the absolute amount of copper in the sample calculated. The sensitivity reported is about 1–3 ppm with a reproducibility of ±7% when 2 g of fat are used.

Two papers describe determination of copper in dairy products. Cranston and Thompson (2) reduced the pH of milk with perchloric acid until copper-protein complexes were broken. After filtering off fat and precipitated protein, the copper was then concentrated by an ion exchange procedure and determined polarographically. The amounts of copper recovered were erratic, since varying quantities of sugars, salts and casein markedly influenced the polarographic method. This condition was corrected by using an internal standard. Willis (10) found it necessary to extract butter and butteroil with nitric acid and to analyze the extract for copper by atomic absorption spectroscopy. Results at concentrations of <0.1 ppm were quite scattered and appear to be the lower limit of this method.

Van De Bogart and Beinert (9) published an excellent review and technique paper for copper analysis of biological materials by wet ashing, evaporation of excess acid, reduction, neutralization, color development and concentration of the colored complex by extraction with a small amount of organic solvent.

There is a wide choice of colorimetric reagents for copper. Some of the more common ones, their properties and drawbacks are shown in Table I. The reagent of choice is Bathocuproine (2,9-dimethyl-4,7-

TABLE I
Color Reagents for Copper

Reagent	Solvent	λ Max. nm	Molar absorbance	Disadvantages
Diethyldithiocarbamate	Water	440	8,000	Unstable in acid
	Isoamyl alcohol	440	12,700	Light sensitive
	CCl_4	435	14,700	
Dibenzyldithiocarbamate	CCl_4	435	16,200	Interference from Fe^{+++} and Ni^{+}
Dithizone	CCl_4	508	24,600	Not specific for copper alone
2,2′-Biquinoline (Cuproine)	Isoamyl alcohol	546	5,220	Low sensitivity
2,9-Dimethyl-1,2-phenanthroline (Neocuproine)	Isoamyl alcohol	454	7,950	Low sensitivity
2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline (Bathocuproine)	*n*-hexanol	479	14,160	

diphenyl-1,10-phenanthroline), a highly specific and exceptionally sensitive reagent for the colorimetric determination of copper. Details concerning this reagent can be found in a pamphlet by Diehl and Smith (3).

Experimental Procedure

Standards

Three types of materials were used as standards. The first was 99.99% pure ACS grade copper wire. The wire surface was cleaned before each use to remove oxides. The second type was copper soaps of two fatty acids, stearic and linoleic. In theory, the two samples should contain 10.08% and 10.21% copper respectively. A colorimetric determination of the copper content after dissolving in 1:1 HNO_3 showed 10.10% and 10.40% copper, respectively. The third material employed was an oil-soluble copper standard obtained from the National Bureau of Standards [bis(1-phenyl-1,3-butanediono)copper II, 16.5% Cu]. Their directions for preparing a known concentration in lubricating oils were followed, except that a vegetable oil was used in place of the lubricating oil.

Isolation of Copper

The major problem has been to quantitatively isolate only a few μg of copper from shortenings and oils. The methods investigated for this purpose were dry ashing and acid extraction. Wet ashing was not investigated due to safety hazards associated with this method when applied to edible oils.

The dry ashing procedure has a major disadvantage in that excessive heat is required to destroy all organic material. This can cause loss of volatile metals, for example, copper soaps. Adsorbed metals on the ashing vessel can cause both low results on a sample or contamination of subsequent samples or both.

Most success was obtained with an acid extraction procedure. All glassware must be cleaned with concentrated HNO_3, rinsed with deionized distilled water, and dried prior to use. A sample, weighed to contain approximately 1–10 μg copper, is refluxed with 50 ml of a 0.01% EDTA-25% HCl solution for 30 min. Acid washed carborundum chips are used to prevent bumping. This reflux step proceeds smoothly without emulsions forming to give a clean extract. After 30 min, the sample is cooled and quantitatively transferred into a 4 in. funnel containing an 18.5 cm Whatman No. 40 filter paper which had been wetted with deionized distilled water. The aqueous phase is collected in a 400 ml beaker and the oil remaining in the filter paper is washed with several small volumes of deionized distilled water, the washings collected in the 400 ml beaker, and the oil discarded. Ten milliliters ultra pure concentrated HNO_3 (Brinkman Instruments, Inc.) are added to the beaker and the solution evaporated to 5 ml on a hot plate and finally to dryness on a steam bath. After the sample is dry, add 5–10 ml deionized distilled water, heat on a steam bath, and quantitatively transfer the contents of the beaker to a 60 ml separatory funnel for colorimetric analysis of copper.

Colorimetric Determination of Copper

Less than 1 μg copper can be quantitatively determined by using the specific colorimetric reagent 2,4-dimethyl-4,7-diphenyl-1,10-phenanthroline (Bathocuproine, the G. Frederick Smith Chemical Company). Care must be taken to eliminate and avoid traces of copper contamination as shown below. Clean all glassware with concentrated HNO_3 and rinse thoroughly with deionized distilled water. Bathocuproine (0.3600 g) is dissolved in 100 ml freshly distilled *n*-hexanol and 100 ml of a 10% solution of hydroxylammonium chloride in deionized distilled water is shaken in a separatory funnel with 10 ml of the Bathocuproine solution and 6–8 drops ultra pure NH_4OH (Brinkman Instruments, Inc.). Discard the organic phase. This removes traces of copper from the aqueous hydroxylammonium chloride solution. One hundred milliliters of a 10% solution of ammonium acetate in deionized distilled water are made copper free by shaking in a separatory funnel with 5 ml of the copper free hydroxylammonium chloride, 5 ml of the Bathocuproine solution, and 5 ml distilled *n*-hexanol. Again, the organic phase is discarded.

To the sample in the 60 ml separatory funnel pipet 2 ml of copper free hydroxylammonium chloride and the pH of the solution is adjusted to 7 ± 0.5 with ultra pure NH_4OH. Ten milliliters copper free ammonium acetate, 1 ml of the Bathocuproine solution and 5 ml *n*-hexanol are pipetted into the same and the separatory funnel shaken vigorously for 2 min. Allow the phases to separate, draw off and discard the aqueous phase and save the organic phase. These samples can be filtered through 9.0 cm Whatman No. 40 filter paper if cloudy before determining the absorbance. A Beckman "B" spectrophotometer set at 479 nm is zeroed with *n*-hexanol in a 2 cm cell. The absorbances of the samples and a blank derived from all reagents as used in the above method are determined at 479 nm in 2 cm cells. A corrected absorbance is obtained by subtracting the absorbance of the blank from that of each sample.

The amount of copper present in the sample can be obtained from a calibration curve prepared from known amounts of pure copper wire dissolved in HNO_3. The concentrations of these solutions are plotted against the color developed (absorbance at 479 nm) with Bathocuproine reagent. The copper concentration could also be approximated by calculation using a molar absorbancy (E) of 14,100.

Discussion

In the dry ashing procedure, low recovery (0–84%) of added organic copper compounds was experienced due perhaps to volatilization. These data, shown in Table II, were obtained by adding the indicated type and amount of copper to 100 g of vegetable oil in a Vycor or platinum ashing vessel. Heat was applied to the bottom of the dish with a Fischer burner flame until the oil began to smoke. The vapors from the oil were ignited with a flame.

TABLE II
Copper Analysis by Dry Ashing

Added cupric salt	Ashing vessel	Muffle temp. C	Copper added μg	Copper found[a] μg	Recovery %
Stearate	Vycor	450	2.01	1.56	77.6
Linoleate	Vycor	450	2.04	1.73	84.3
Stearate	Vycor	450	8.06	4.67	57.9
Linoleate	Vycor	450	8.16	3.93	48.2
Stearate	Vycor	500	5.04	3.24	64.1
Stearate	Platinum	500	5.04	2.25	44.6
Stearate	Platinum	550	5.04	3.50	66.5
Stearate	Platinum	550	8.06	~18.00	?
NBS Standard[b]	Platinum	550	4.97	NF	0.0
NBS Standard[b]	Platinum	550	4.97	1.60	34.2

[a] Corrected for initial copper level in samples used.
[b] NBS standard, Bis(1-phenyl-1,3-butanediono) copper (II), oil soluble material containing 16.5% Cu, from National Bureau of Standards.

TABLE III
Copper Analysis by Extraction

	Copper added to vegetable oil ppm	Copper added to vegetable oil μg	Copper found[a] μg	Recovery, %
NBS Standard[b]				
	0.002	1.00	0.93	93.0
	0.010	1.00	1.00	100.0
	0.010	1.00	0.95	95.0
	0.010	1.00	1.05	105.0
	0.010	5.00	4.80	96.0
	0.010	5.00	4.35	87.0
	0.014	7.00	6.48	92.6
	0.040	4.00	4.20	105.0
	0.050	4.97	4.90	98.6
	0.050	4.97	4.90	98.6
	0.050	5.00	4.99	99.8
	0.050	5.00	5.10	102.0
	0.050	5.00	5.20	104.0
	0.100	9.95	9.60	96.5
	0.22	2.00	2.10	105.0
	0.22	2.00	1.85	92.5
	0.22	2.00	2.00	100.0
Cupric stearate				
	0.01	1.00	1.20	120.0
	0.01	1.00	1.05	105.0
	0.02	2.00	1.95	97.5
	0.03	3.00	3.00	100.0
	0.07	7.00	7.10	101.4
			Average	99.8

[a] Corrected for initial copper levels in samples used. Copper metal, 400 to 475 μg, well over limits normally encountered can be determined by this procedure.
[b] NBS Standard, Bis(1-phenyl-1,3-butanediono) copper (II) (oil soluble material containing 16.5% Cu), from National Bureau of Standards.

After the flame went out, the residue was placed in a muffle furnace at the indicated temperature until the carbon was gone. The ash was then analyzed for copper by the colorimetric procedure described.

Extracting copper with 0.01% EDTA in 25% HCl was found to give a quantitative extraction of copper from shortenings and oils. The extracts were analyzed for copper by the colorimetric procedure described. These data are presented in Table III. An average recovery of 99.8% added oil soluble copper standards was obtained with a standard deviation of 0.002 ppm established at the 0.05 ppm level during routine application of this method to a variety of shortenings and oils.

Using 25% HCl alone without added EDTA resulted in low recovery (88–90%) of added copper. Two somewhat similar methods are available for determining trace metals in petroleum distillates. Barney II (1) extracted an oil with sulfuric acid and then with a mixture of hydrochloric acid, acetone and water. The extract was analyzed by spectrographic analysis. Sulfuric acid chars edible oil so it cannot be used. Hackett (4) extracted mineral oils with alcoholic hydrochloric acid and colorimetrically determined the copper by extraction of the 2,2-diquinolyl complex with chloroform. The method operates satisfactorily when more than 10 μg copper are present. EDTA was not needed for a quantitative extraction of copper in mineral oils but was needed for edible shortenings and oils.

Less than 90% of added copper was extracted from shortenings and oils with 25% HCl alone. The addition of EDTA leads to a quantitative extraction of copper from shortenings and oils. Postulating the role of EDTA in assuring successful extractions of copper from shortenings and oils is beyond the scope of this report. Little, if any, information is available concerning the role of EDTA in highly acid media nor at the elevated temperature used in the heated system. One is that the EDTA becomes increasingly deprotonated, the extent of which is not known, making it a better chelating agent. The other is that a copper-EDTA complex would be less stable or more likely to dissociate at high temperatures.

The method as written is particularly applicable to the determination of copper in shortenings and oils at low levels (0.002–0.10 ppm), but could also be applied for analysis of copper in other media. It seems quite apparent that the isolation method could be extended to other metals (e.g., nickel in oil, hydrogenation catalyst).

ACKNOWLEDGMENTS

Initial work on the colorimetric analysis of copper, P. F. Vornheder: constructive advice, H. W. Wharton.

REFERENCES

1. Barney II, J. E., Anal. Chem. *27*, 1283–1284 (1955).
2. Cranston, H. A., and J. B. Thompson, Ind. Eng. Chem., Anal. Ed. *18*, 323–326 (1946).
3. Diehl, H., and G. F. Smith, "The Copper Reagents: Cuproine, Neocuproine, Bathocuproine," The G. Frederick Smith Chemical Company, Columbus, Ohio, 1958.
4. Hackett, C. E. S., Anal. Chim. Acta *12*, 358–362 (1955).
5. Høgdahl, O. T., and S. Melsom, Anal. Chem. *38*, 1414–1415 (1966).
6. Labuza, T. P., and M. Karel, J. Food Sci. *32*, 572–575 (1967).
7. List, G. R., R. L. Hoffmann, W. F. Kwolek and C. D. Evans, JAOCS *45*, 872–875 (1968).
8. Svenska Institutet för Konserveringsforskning, (SIK), Symposium, Metal Catalyzed Lipid Oxidation, Kallebäck, Göteborg, Sweden, October, 1967.
9. Van De Bogart, M., and H. Beinert, Anal. Biochem. *20*, 325–334 (1967).
10. Willis, J. B., Australian J. Dairy Technol. *19*, 70–74 (1964).

[Received September 10, 1969]