Mineral Constituents in Corn and Wheat Germ by Atomic Absorption Spectroscopy[1]

W. J. GARCIA, C. W. BLESSIN, and G. E. INGLETT, Northern Regional Research Laboratory, Peoria, Illinois 61604

ABSTRACT

A rapid wet-ashing procedure, coupled with atomic-absorption techniques, provided a fast method of mineral analysis in corn and wheat-germ samples, with no chemical separations being involved. Samples (0.3- or 2.0-g.) were decomposed by wet-ashing to yield a clear solution. From this solution, potassium, magnesium, calcium, sodium, iron, copper, manganese, and zinc were determined by atomic-absorption techniques. Phosphorus was measured colorimetrically. Interference effects were evaluated in analyzing zinc in an ashed wheat-germ solution. The method has been applied to the analysis of various corn and wheat-germ samples, and results compare favorably with other methods.

In a study of air-classifying germ components (1), a reliable method of analysis was needed to measure its effect on individual mineral-element concentrations in different fractions. A method of germ-sample decomposition was needed that was rapid, with no incidental losses of elements. An additional requirement was that the ashed sample would, with a minimum of effort, give a clear solution that was directly compatible with atomic-absorption techniques. Chemical compositions of biological ashes vary widely, and behavior of various types of samples in ashing processes differ greatly. Corn and wheat germ, with major inorganic constituents of phosphorus (P), potassium (K), and magnesium (Mg), wet-ash easily with only concentrated nitric acid (HNO_3) at low temperatures.

Early investigations into the application of atomic absorption spectroscopy to the analysis of plant material for zinc (Zn), magnesium (Mg), copper (Cu), and iron (Fe), were made by David (2). He concluded that for Zn and Mg, atomic absorption was at least as accurate and sensitive as other methods then available and was considerably better in both rapidity and freedom from extraneous elements.

In a collaborative study of mineral elements in fertilizers involving approximately 20 laboratories, McBride (3) reported that chemical and atomic-absorption methods were comparable. In general, the precision for calcium (Ca), Cu, Fe, K, Mg, manganese (Mn), and Zn was acceptable for those samples of agronomic interest. In a subsequent report (4), McBride presented over-all statistical data on the precision of individual element determinations previously mentioned. Several high sodium (Na) values were attributed to: a) Contamination of glassware or reagents; b) attack on Pyrex by fluorine in the sample or the dissolution by acid, or c) both.

In an extensive study by Czerniejewski et al. (5), wheat, flour, and commercial mill fractions were analyzed for mineral constituents. They dry-ashed the samples

[1]Contribution from the Northern Regional Research Laboratory, Peoria, Ill. 61604. This is a laboratory of the Northern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

at 500°C. and determined the mineral elements by procedures involving various separation steps before measurement by colorimetric, flame-photometric, and titration techniques. Waggle et al. (6) gave results on mineral elements in flours and millfeeds made from nine different wheat mixes. Elemental results were obtained with a computer-programmed emission spectrometer with no mention of sample-decomposition procedures.

Zook et al. (7) used atomic absorption and colorimetry in determining Mg and eight trace mineral elements in wheats, wheat blends, flours prepared from wheats, prepared products from flours, and consumer products. Samples of approximately 55 g. were dry-ashed at 480°C. Ashed samples were extracted with dilute HCl and made to volumes of 50 or 100 ml. Extraction of the ash from these large samples required overnight treatment(s) on a steam bath.

When Pawluk (8) compared methods of analysis for Mg, reproducibility and accuracy were much better for atomic absorption than for the EDTA method. Other methods for Mg determination took too much time to be favorably compared. For Ca, results were good when compared to the oxalate and EDTA methods, if either lanthanum (La) or strontium was added for suppression of interferences. Reproducibility with the atomic-absorption method for iron determination (8) was much higher than with the *o*-phenanthroline method (9).

The primary advantages of using atomic absorption spectrophotometry are element versatility with no appreciable spectral interferences and only minor chemical interferences. Further assets derived from atomic absorption are elimination of many chemical separations and ease of sample preparation, with resultant time-conserving benefits. Precision and accuracy studies indicate that atomic-absorption techniques are generally equal to other standard methods and are probably superior in such specific determinations as Mg and Cu.

MATERIALS AND METHODS

Instrumentation and Reagents

Both a Perkin-Elmer Model 303 and a Varian Techtron AA120 atomic absorption spectrophotometers, with external recorders having noise-suppression and scale-expansion capabilities, were used for absorption measurements. Calibration and sample data were derived from absorption readings as indicated on the recorder. Single-element hollow-cathode lamps at the recommended current rating were used for all determinations. A three-slot burner was suitable for all elements with fuel (acetylene) and oxidizer (air) at optimum flow rates for each individual element.

Primary standard stock solutions were prepared from pure reagents, standardized compounds, and metals of high purity. Secondary working-standard solutions were prepared from primary stock solutions covering the desired concentration range for each element. Required reagents for the suppression of interference effects were added to the secondary standard solutions. All reagents and standards were transferred as soon as possible to polyethylene storage bottles to minimize dissolution of Na and K from glass containers. Double-distilled water, for dilution purposes, was collected in a polyethylene container to prevent contamination by Na and K from glass bottles.

Ashing Procedure

All nine elements can be analyzed with a germ sample that is only 0.3 g. in size, if the ashed sample is diluted to 25 ml.; however, for copper and iron, a 2-g. sample is more appropriate.

Germ sample (0.3 or 2.0 g) is transferred to a 50-ml. beaker and 5 ml. concentrated HNO_3 is added. With a cover glass on the beaker, the sample is placed on a hot plate at low heat for about 90 min. until the solution clears. Heat is increased and as the HNO_3 is evaporated, dropwise additions of concentrated HNO_3 are made as the sample starts to char. After a white ash appears, the walls of the cooled beaker are rinsed with double-distilled water and the beaker reheated on low heat or on a steam bath until contents are almost dry. Two milliliters concentrated HCl is added and warmed, with the cover glass in place, to get a refluxing action. The walls of the beaker are rinsed down with water and the mixture is evaporated to dryness. The refluxing procedure with concentrated HCl, followed by evaporation to dryness, is repeated twice.

Finally, 2.0 ml. concentrated HCl is added and warmed; then approximately 15 ml. water is added and heated for about 15 min. The cooled solution is transferred to a 50-ml. volumetric flask, made up to volume with water, and transferred immediately to a polyethylene storage bottle.

Phosphorus Analysis

The colorimetric molybdenum (Mo)-blue method (10), or an equivalent procedure, is more readily adaptable for the concentrations of P encountered in the ashed solution.

Briefly, an aliquot of the ashed solution and graduated increments of P standards (0.02 to 0.14 mg. P) are transferred to 25-ml. volumetric flasks. To the volumetric flasks are added, in order with mixing, the following reagents, prepared as previously described (10): Ammonium molybdate, hydroquinone, and sodium sulfite. On the basis of specified conditions (10) and a 1-cm. pathlength, an absorbance value of 0.3 is equivalent to about 0.06 mg. P. This colorimetric method is quite flexible if sample size, color-development volume, and cell pathlength are varied. No interference difficulties were encountered with germ samples when this procedure was followed.

At present there are no direct methods to determine P by atomic absorption, but it can be determined indirectly after it is combined with Mo (11). The Mo is determined by atomic absorption and the method does have amplification capabilities because of the relative combined weights of P and Mo. Because considerable work is involved in separations and because the reaction depends on an exact chemical combination, this phosphomolybdic acid method was not deemed worthwhile for germ samples.

Atomic-Absorption Techniques

Under the conditions given in Table I, absorption data were acquired by aspirating aqueous standard solutions and samples similar in acid concentration for at least 1 min. Double-distilled water was aspirated alternately between samples to compensate for noise signal and instrument zeroing. A composite standard-calibration curve was prepared from a series of single-element standard solutions aspirated before and after aspiration of samples. Figure 1 shows

TABLE I. GERM-SAMPLE WEIGHT REQUIREMENTS AND DILUTION FACTORS ASSOCIATED WITH ATOMIC ABSORPTION OPERATING CONDITIONS AND INSTRUMENTAL PARAMETERS

Element	Weight-Sample Required and Dilution Factor[a] g.	Resonance Line λA	Hollow-Cathode Lamp Current Mamp.	Optimum-Concentration Range of Aqueous Solution γ/ml.	Sensitivity Attained[b]
Zn	0.3, none	2139	15	0.3–3.0	0.033
Fe	2.0, none	2483	30	2–20	0.16
Mg	0.3, 75X	2852	6	0.2–2.0	0.014
Cu	2.0, none	3248	15	2–20	0.12
Ca	0.3, 2X	4227	10	2–10	0.082
Na	0.3, 3X	5890	10	0.3–3.0	0.030
K	0.3, 35X	7665	12	2–10	0.065
Mn	0.3, none	2974	5	0.5–5.0	0.047

[a]Further dilutions when original ashed solution volume was 50 ml.

[b]Microgram/milliliter/1% absorption.

calibration curves prepared from absorption data acquired from aqueous standards at the wavelength indicated. With the exception of Mn, where a scale expansion of about 2.4X was used, the standard curves represent nonscale expansion conditions. Absorption readings were converted to absorbance values for linear presentation.

Magnesium

Excellent determination of Mg is accomplished by atomic absorption and is probably the best technique available even when Mg is present at low concentrations. Interferences were not significant with ashed samples diluted 50 and 100 times for wheat- and corn germ, respectively. One of the highest sensitivities was obtained with aqueous Mg solutions (0.014 γ Mg per ml. per 1% absorption). Additionally, a very favorable signal-to-noise ratio was the result of optimum flame and optical conditions.

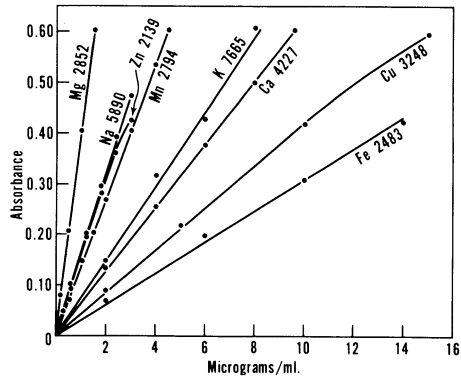

Fig. 1. Atomic-absorption calibration curves for individual elements at wavelength specified.

To prepare Mg stock solution, dissolve 0.2500 g. magnesium metal in concentrated HCl, and dilute to 250 ml. with water to yield a final concentration of 1 mg. Mg. per ml. in 1% HCl.

Potassium

The ashed germ sample was further diluted 50 times before aspiration. No interference from other elements was observed, but contact with glass containers should be kept to a minimum, especially after dilution.

For K stock solution, dissolve 0.4677 g. KCl in double-distilled water and dilute to 250 ml. to give a solution containing 1 mg. K per ml.

Calcium

Phosphorus, a major mineral constituent of germ samples, interferes with the determination of Ca. The formation of calcium phosphate prevents all the Ca from going to the ground state in the flame and results in low absorption values. To offset this detrimental P interference, La was added to both samples and standards so that both contained 1% La in a 5%-HCl medium. The La solution was prepared from lanthanum oxide (La_2O_3) reagent as directed (12). Approximately 15 ml. of ashed-germ sample solution plus 5.0 ml. La stock solution diluted to 25 ml. was used for the determination.

Initially, a La_2O_3 reagent was used that contained an excessive amount of Ca as an impurity. The contaminated La_2O_3 reagent (1% lanthanum solution) yielded an apparent blank value of more than 15 γ Ca per ml. instead of 0.18 γ Ca per ml. obtained with a different La_2O_3 reagent. It was advantageous to measure the absorption due to Ca in newly prepared La_2O_3 reagents before adding the solution to samples and Ca standards. Contamination of samples with Ca was thus avoided.

For Ca stock solution, dissolve 0.5000 g. $CaCO_3$ in a small amount of dilute HCl, boil off CO_2, neutralize with ammonia, and dilute up to 500 ml. with water. This solution contains 0.4 mg. Ca per ml.

Sodium

Although Na determination by atomic absorption is not the most sensitive technique, it is of the utmost importance that special precautions be exercised against the dissolution of Na from glass. With a range of working Na standards between 0.3 and 3.0 γ Na per ml., an increase in concentration of only 0.5 γ Na per ml. from extraneous sources can cause a significant error in the final results.

No detectable interferences were found.

To prepare Na stock solution, dissolve 0.6353 g. NaCl in double-distilled water and dilute to 250 ml. to give a solution which contains 1 mg. Na per ml.

Iron

The low sensitivity for Fe analysis in aqueous solution (0.16 γ Fe per ml. per 1% absorption) required a 2.0-g. germ sample. No interferences were found, and Fe standards and samples dissolved in either dilute HCl or HNO_3 gave essentially identical absorption values. No indication of P interference was evident with the Fe determination.

For Fe stock solution, dissolve 0.1422 g. standardized iron wire (99.80% Fe) in 20 ml. 1:3 HNO_3, boil to expel oxides of nitrogen, and dilute to 250 ml. This solution contains 0.5676 mg. Fe per ml.

Copper

Since Cu analysis also required 2.0 g. of germ, the same ashed solution used for Fe was suitable. But absorption values are low with the nondiluted aqueous ashed solution (50 ml.). If it is necessary to enhance these values, then either the ashed solution could be further concentrated or the Cu extracted selectively with ammonium pyrolidine dithiocarbamate (APDC) and methyl isobutyl ketone as the organic solvent (13).

To make a Cu stock solution, dissolve 0.7856 g. of clear uneffloresced crystals of $CuSO_4 \cdot 5H_2O$ in water, add 2.0 ml. HCl, and dilute to 200 ml. with water. This solution contains 1 mg. Cu per ml. Working Cu standards prepared from this solution were in good agreement in absorption values when compared to similar standards prepared by dissolving pure metallic copper.

Manganese

A 0.3-g. germ sample was satisfactory to measure Mn by scale-expansion techniques. No interference effects were evident.

For the Mn stock solution, dissolve 0.2503 g. of Mn metal (99.9% Mn) in 20 ml. 1:3 HNO_3, boil to expel oxides of nitrogen, and dilute to 250 ml. This solution contains 1 mg. Mn per ml.

Zinc

Although Zn is a minor inorganic constitutent in corn and wheat germ, excellent sensitivity to atomic absorption makes it possible to get accurate data from only 0.3 g. of ashed germ.

To determine possible differences, owing to matrix effects, between Zn standards and samples with higher solids content, the method of additions was followed. Increments of Zn standard were added to samples of ashed wheat-germ solution (Fig. 2). A straight-line relationship between the concentration of added Zn and absorbance was extrapolated to zero absorbance where the concentration of Zn of the sample solution is indicated. A value of 0.320 γ Zn per ml. arrived at by the method of additions agreed with a value of 0.312 γ Zn per ml. obtained by the direct method in similarly diluted samples. This agreement indicated that there were no appreciable interferences in the Zn determination with wheat-germ samples.

For the Zn stock solution, dissolve 0.2500 g. zinc metal in strong HCl and finally dilute to 250 ml. with water to give a 1% HCl solution that contains 1 mg. Zn per ml.

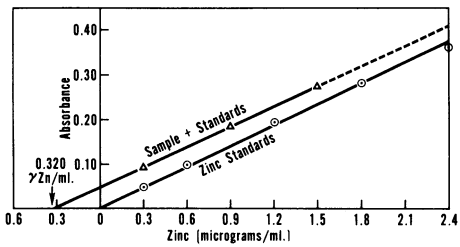

Fig. 2. Evaluation of interference effects in Zn determination for wheat-germ sample. The method of additions compensates for matrix differences between standards and sample solutions.

RESULTS AND DISCUSSION

Decomposing organic material without losing mineral elements is most important. Wet-ashing can be done on a considerably smaller sample than needed for dry-ashing. With a smaller sample, ashing time is reduced and the amount of silica (SiO_2) introduced from the sample is considerably less. Silica, if not removed or dehydrated, is known to give low results for Cu, Fe, and Zn because these minerals are adsorbed on the SiO_2. The presence of gelatinous SiO_2 in ashed sample solutions is often characterized by cloudy solutions. Evaporation of ashed samples with HCl serves to dehydrate the SiO_2 and also to ensure conversion of pyrophosphates to orthophosphates for a satisfactory colorimetric P determination. The absence of sulfuric acid serves to give a better matrix match between standards and samples. Low recoveries often result from coprecipitation or adsorption of mineral elements with calcium sulfate, which is formed when sulfuric acid is used for ashing. In turn, perchloric acid precipitates $KClO_4$.

After extensive study of ashing techniques, Gorsuch (14) concluded that wet oxidation is superior in terms of speed, low temperatures required, and freedom from loss by retention of trace elements on solid material in the system. Hoffman et al. (15) found that recoveries of Fe after dry ashing of bread were low unless NaOH served as an ashing aid.

The rapid ashing method we describe is directly compatible with atomic-absorption techniques, which involve elemental standards in the microgram/milliliter concentration range. When our procedure is followed, the ashed residue dissolved in dilute solutions of either HCl or HNO_3 gives a clear solution requiring no filtration. From this solution or dilutions thereof, mineral elements can be determined directly. With 10 to 20 samples, wet-ashing takes approximately 2.5 hr., with operator attention required at the end of the ashing process. It is possible to wet-ash a 0.3-g. germ sample and start determinations on it the same day.

To test the reproducibility of individual element determinations with only 0.3 g. of wheat-germ sample, seven replicate samples were wet-ashed and finally diluted to

TABLE II. MEANS[a] AND STANDARD DEVIATIONS OF MINERAL ELEMENTS BY PROPOSED METHOD IN DEFATTED WHEAT GERM E

Mineral Element	Mean Content in Germ	Mean Concentration in 25 ml. γ/ml.
P	1.39±0.06%	145.4
K	1.39±0.06%	145.4
Mg	0.408±0.022%	42.7
Ca	0.065±0.002%	6.77
Na	0.033±0.003%	3.43
Fe	149±13 p.p.m.[b]	1.56
Zn	215±13 p.p.m.[b]	2.25
Mn	248±10 p.p.m.[b]	2.59
Cu	16±1.0 p.p.m.[b]	0.167

[a]Mean of seven replicate 0.300-g. germ samples (moisture-free basis), wet-ashed and diluted to 25 ml.

[b]Scale expansion used on atomic-absorption instrument.

TABLE III. COMPARISON OF MINERAL-ELEMENT CONTENTS IN DIFFERENT WHEAT-GERM SAMPLES DETERMINED BY PROPOSED METHOD COMPARED TO OTHER METHODS[a]

Mineral Element	Proposed Method[b] Wheat germ C	Proposed Method[b] Wheat germ D	Published Wheat-Germ Data Waggle et al.[c] (6)	Published Wheat-Germ Data Czerniejewski et al. (5)
P, %	1.08	1.04	1.01±0.07	0.923
K, %	0.95	0.95	1.14±0.18	0.889
Mg, %	0.299	0.319	0.27±0.02	0.268
Ca, %	0.044	0.048	0.058±0.015	0.048
Na, %	0.010	0.027	0.024±0.009	0.0232
Fe, p.p.m.	98.0	102.5	53.6±7.0	66.6
Zn, p.p.m.	143.2	138.7	134.7±19.3	100.8
Mn, p.p.m.	148.4	163.7	135.5±20.1	137.4
Cu, p.p.m.	10.3	9.5	10.2±1.8	7.4

[a]Full-fat and moisture-free basis.

[b]Mean of determinations on triplicate samples.

[c]Mean values of determinations on nine different samples.

25 ml. Each solution was then analyzed for nine different elements (P, K, Mg, Ca, Na, Fe, Zn, Mn, Cu). The mean and standard-deviation values for individual elements are shown in Table II. Considering sample size, reproducibility was good for all elements. Values for Na indicate a greater deviation than for other elements. Two out of seven Na values were high, possibly caused by Na dissolved from glass beakers. If Teflon beakers were used during wet ashing, this extraneous source of Na would be eliminated. Although most of the sample solution is used up, the data demonstrate that it is possible to determine nine separate mineral elements by the proposed method with a good degree of reproducibility with only 0.3 g. of germ sample.

Losses of P during ashing were checked by adding Mg (15 mg.) as the nitrate to 0.3 g. of corn-germ samples before ashing. Essentially identical results were found for P with and without Mg.

Data from the proposed method on wheat-germ samples were compared with wheat-germ mineral data taken from Waggle et al. (6) and Czerniejewski et al. (5). The comparison, involving two different wheat-germ samples (C and D), is shown on Table III. Nine different wheat-germ samples in the Waggle report were converted to a moisture-free basis, and the mean value and standard deviation for each individual element were calculated. Results from the Czerniejewski report were taken as given except the value for Na was converted to a percentage basis.

Although different germ samples are compared by different methods, the levels of mineral elements present in wheat germ are generally in good agreement by all methods except for values for Fe (which by the proposed method are significantly higher). The differences in values for Fe, Zn, and possibly Cu might be the result of different ashing techniques.

Analytical results for mineral constituents in four commercial corn and wheat-germ samples of unknown geographical origins are listed in Table IV. The table reveals differences in mineral-element concentrations between two different corn and wheat germ samples. After being defatted in the laboratory, both corn and wheat germs contained approximately 20% starch, mostly of endosperm origin.

TABLE IV. ANALYTICAL RESULTS ON FOUR COMMERCIAL DRY-MILLED DEFATTED GERM SAMPLES[a]

	P[b] %	K %	Mg %	Ca %	Na %	Fe p.p.m.	Zn p.p.m.	Mn p.p.m.	Cu p.p.m.
Corn germ A[c]	2.33	2.34	1.23	0.018	0.063	192.5	200.1	52.7	16.1
Corn germ B[c]	2.34	2.36	1.10	0.019	0.092	209.0	202.9	59.5	15.3
Wheat germ C[d]	1.56	1.38	0.43	0.064	0.015	142.0	207.5	215.0	14.9
Wheat germ D[d]	1.49	1.35	0.46	0.069	0.038	146.4	198.2	233.9	13.6

[a]Moisture-free basis.

[b]By colorimetric method.

[c]Mean of determinations on duplicate samples.

[d]Mean of determinations on triplicate samples.

In our work the desired element concentration was reached primarily by dilution procedures. It was concluded that sample and standard matrix need not be closely matched with the germ-sample concentrations used. Atomic absorption could be extended in analyzing cereal grains for additional trace elements by including extraction and separation procedures.

The method described was most effective in studying (1) where individual mineral constituents were concentrated in air-classified fractions of corn and wheat germ. The method clearly established the effect air classification has on mineral elements.

Acknowledgments

We thank C. D. Evans and G. R. List for their helpful suggestions and advice.

Literature Cited

1. GARCIA, W. J., GARDNER, H. W., CAVINS, J. F., STRINGFELLOW, A. C., BLESSIN, C. W., and INGLETT, G. E. Composition of air-classified corn and wheat germ flours (abstr.). Cereal Sci. Today 16(9): No. 115 (1971).
2. DAVID, D. J. Determination of zinc and other elements in plants by atomic absorption spectroscopy. Analyst 83: 655 (1958).
3. McBRIDE, C. H. Determination of secondary and minor plant nutrients in fertilizers by atomic absorption spectrophotometry: Third collaborative study. J. Ass. Offic. Anal. Chem. 50: 401 (1967).
4. McBRIDE, C. H. Determination of secondary and minor plant nutrients in fertilizers by atomic absorption spectrophotometry. IV. J. Ass. Offic. Anal. Chem. 51: 847 (1968).
5. CZERNIEJEWSKI, C. P., SHANK, C. W., BECHTEL, W. G., and BRADLEY, W. B. The minerals of wheat, flour, and bread. Cereal Chem. 41: 65 (1964).
6. WAGGLE, D. H., LAMBERT, M. A., MILLER, G. D., FARRELL, E. P., and DEYOE, C. W. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. Cereal Chem. 44: 48 (1967).
7. ZOOK, ELIZABETH GATES, GREENE, F. ELLA, and MORRIS, E. R. Nutrient composition of selected wheats and wheat products. VI. Distribution of manganese, copper, nickel, zinc, magnesium, lead, tin, cadmium, chromium, and selenium as determined by atomic absorption spectroscopy and colorimetry. Cereal Chem. 47: 720 (1970).
8. PAWLUK, S. Soil analyses by atomic absorption spectrophotometry. At. Absorption Newslett. 6(3): 53 (1967).

9. SNELL, F. D., and SNELL, C. T. Colorimetric methods of analysis. Van Nostrand: Princeton, N.J. (1949).
10. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. AACC Approved methods (6th ed.). The Association: St. Paul, Minn. (1957).
11. KIRKBRIGHT, G. F., SMITH, A. M., and WEST, T. S. Determination of molybdenum in alloy steels by atomic-absorption spectroscopy in a N_2O-C_2H_2 flame.
12. ANONYMOUS. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer Corp.: Norwalk, Conn. (1966).
13. SPRAGUE, S., and SLAVIN, W. Determination of very small amounts of copper and lead in KCl by organic extraction and atomic absorption spectrophotometry. At. Absorption Newslett. 3(20): 37 (1964).
14. GORSUCH, T. T. Radiochemical investigations on the recovery for analysis of trace elements in organic and biological materials. Analyst 84: 135 (1959).
15. HOFFMAN, C., SCHWEITZER, T. R., and DALBY, G. Ind. Eng. Chem. Anal. Ed. 12: 454 (1940).

[Received January 25, 1971. Accepted November 1, 1971]