# A New Hydrolysis Procedure for Preparation of Orange Juice for Trace Element Analysis by Atomic Absorption Spectrometry

J. A. McHard, J. D. Winefordner,* and J. A. Attaway[1]

Hydrolysis of frozen concentrated orange juice with concentrated nitric acid has been evaluated as a sample preparation method prior to flame atomic absorption spectrometric measurement of certain elements in orange juice concentrate. The hydrolysis procedure has been applied specifically to the determination of calcium. Studies of matrix interferences show that the use of lanthanum as a releasing agent is not necessary or even desirable for the measurement of calcium with this procedure.

Primary among the problems of analysis for metals in foods is a suitable preparation procedure for the sample prior to the measurement step. In an on-going study designed to evaluate existing methodology for the determination of the elemental content of citrus juices, especially orange juice, it became evident that suitable methods, particularly methods for trace elements, either were not applicable or were not available.

A commonly used procedure for the flame atomic absorption analysis of calcium is to dry ash the sample, if it is organic in nature as foods are, and dissolve the ash in hydrochloric acid (AOAC, 1970). Dry ashing is not a generally suitable preparation procedure because a number of elements are incompletely recovered (Christian, 1969; Gorsuch, 1959; Pijck et al., 1961; Smith, 1965); this ashing step is likely to leave insoluble matter, particularly incompletely oxidized organic substances, and may be accompanied by the loss of volatile elements and/or substantive reaction of other elements with the surface of the ashing vessel; Pijck et al. (1961), for example, found that iron was only 80% recovered after dry ashing at 500°C and some elements, e.g., As and Hg, were not recovered to any detectable extent. In some instances, the vessel used for ashing may contain the element under investigation and provide a source of contamination resulting from the pitting and corrosion of the surface during ashing. Calcium contamination, for example, could conceivably occur in this manner since some porcelain materials are made from calcium containing starting materials. Also, the final ashing step is often carried out with open crucibles in dusty furnaces.

Acid hydrolysis procedures using 5 *M* or stronger hydrochloric acid have been proposed (Simpson and Blay, 1966; Szarski, 1971) with reportedly good results for many elements in foods. A serious disadvantage to using hydrochloric acid for hydrolysis is that the hydrolysis product must be filtered and the copious, pulpy, agglomerate nature of the hydrochloric acid hydrolysate causes it to be difficult to filter; also, the large amount of undissolved pulp may retain some of the elements being determined.

The use of 5–10 *M* nitric acid has been suggested (Rowe, 1973) as a substitute for hydrochloric acid because the hydrolysate consists then of a nitrate matrix providing a more suitable mixture for flameless furnace techniques of atomic absorption. Many elements form volatile chlorides but nonvolatile nitrates. The investigation reported on in this article revealed that, under the experimental conditions used, the nitric acid hydrolysate is easily filterable in sharp contrast to the situation resulting from hydrochloric acid hydrolysis. Also, the nitric acid hydrolysate leaves very little undissolved material, and the recovery of calcium is excellent.

Another major problem faced by the analyst in both flame and flameless atomic absorption spectrometry is the influence of the matrix on the measured signal (interferences). With regard to calcium, the flame emission signal from solutions with identical amounts of calcium present in each has been shown to be enhanced or depressed by a number of anions and cations (Herrmann and Alkemade, 1963). In particular, in both flame atomic emission and atomic absorption, the depressive effect of phosphate on the calcium signal has received considerable attention (Christian and Feldman, 1970; David, 1959, 1960; Slavin, 1968; Willis, 1960, 1961; Yofé et al., 1963; Yofé and Finklestein, 1958).

The study reported here outlines a modification of the nitric acid hydrolysis method mentioned above and describes its application to the determination of calcium in orange juice by flame atomic absorption. Data will also be given to show that at the comparative levels of calcium and phosphorus in orange juice and the relatively low concentrations of both these elements in the final mixture used for the absorption measurement, a "releasing" agent for phosphorus need not be added and that the addition of such an agent as lanthanum in a nitric acid matrix actually gave lower values.

## EXPERIMENTAL SECTION

**Apparatus.** A single beam spectrometer (Model IL 151, Instrumentations Laboratories, Inc., Lexington, Mass.) was used for all absorption measurements. All readings were taken from the digital readout system supplied with the instrument. A multielement hollow cathode source of Ca, Mg, and Al (Varian Techtron, Palo Alto, Calif.) was used in all studies and was operated at a lamp current of 8.5 ± 0.5 mA.

**Reagents.** The calcium solutions were prepared using Baker and Adamson reagent-grade calcium carbonate powder. A stock solution in dilute nitric acid containing 1000 μg/ml of calcium was prepared and diluted as needed. Nitric, phosphoric, and hydrochloric acids were analytical-reagent grade. Dextrose was Fisher-certified anhydrous *d*-glucose. A stock solution containing 200 μg/ml of potassium and 4 μg/ml of sodium was prepared from reagent-grade nitrates. Lanthanum oxide was 99.9% $La_2O_3$ from Alfa Products. All solutions were made in deionized water. All glassware and containers were acid washed before use.

**Procedure.** Two different sample preparation procedures were used, and the results were compared.

Department of Chemistry, University of Florida, Gainesville, Florida 32611.

[1]Present address: Florida Citrus Commission, Lake Alfred, Florida.

In the sample preparation, which will be called preparation A, 2.5-g samples of frozen orange juice concentrate were dried in a 20-ml platinum crucible under a heat lamp and were protected from contamination by a large glass petri dish cover. The temperature during the drying step was approximately 90°C, and the heating period was 7 hr. After cooling to room temperature, 2 ml of concentrated nitric acid was added and the samples allowed to stand (under cover in a hood) overnight. The acid was added as an ashing aid (Gorsuch, 1959) and seemed to facilitate the conversion to ash at temperatures below 500°C; the following morning, the acid was evaporated at 90°C in a fashion similar to the initial drying step, except that apparent dryness was achieved in approximately 3 hr. The samples were then heated gradually in a muffle furnace over a period of 6 hr to a temperature of 475°C. During this period, the samples were removed once or twice, allowed to cool, a few drops of water were added, and the samples were dried under a lamp and returned to the furnace, which had been allowed to cool to 300°C each time prior to return of the samples. This ashing procedure does not differ significantly, except for sample size and the addition of the nitric acid ashing aid, from the ashing procedures commonly used in food analysis (AOAC, 1970). The ashed products were then dissolved directly in their respective platinum crucibles with approximately 1 ml of "dilute" nitric acid (one part concentrated nitric acid to three parts water) and transferred with a dropper to 5-ml plastic tubes provided with snug-fitting covers. The crucible and dropper were rinsed with successive small portions of deionized water, adding the rinsings to the plastic container until the total weight of the sample matrix had reached 5 g. The reason for keeping the sample volume small was to provide a concentrated ash solution for the determination of the minor trace elements, such as, Cu, Mn, Zn, and Fe, as well as the major elements K, Ca, Mg, and Na. For the calcium analysis reported here, 0.5 g of this mixture was diluted to 50 g with water containing enough nitric acid to make the final 50 g approximately 0.1 *M* in acid. One of the orange juice brands used in the analytical study was selected as a "reference" and three 0.5-g samples were taken from this "reference" and diluted in the above manner. To one of the dilutions was added enough calcium to increase the calcium content by 0.5 μg/ml (final analyte matrix), to a second the added amount corresponded to 1.0 μg/ml, and to the third, none was added.

In preparation B, 10-g samples of the frozen concentrate in 100-ml volumetric flasks were allowed to hydrolyze with 10 ml of concentrated nitric acid overnight at room temperature. The flasks were loosely covered with clean 10-ml beakers to allow escape of nitric acid fumes and to avoid contamination; the following morning the flasks containing the partially hydrolyzed samples were heated (still covered with the beakers) in a water bath at 75°C for 5 hr. At the end of this heating period, the flasks were cooled to room temperature, diluted with deionized water to the 100-ml mark, and well mixed.

The hydrolyzed and diluted samples in the 100-ml flasks were filtered into clean, dry, plastic containers through rapid-filtration paper to remove the small amount of pulp which remained undissolved. At the beginning of the filtration step, a small portion of liquid which first passed through the filter paper was discarded to avoid dilution by the water used to pre-rinse the filter paper and funnel. In this procedure, three samples of the "reference" were also hydrolyzed. Amounts of calcium were added to two of the three "reference" samples prior to hydrolysis to correspond in concentration, at the final dilution used for measurement, to the concentrations added to the reference samples resulting from preparation A. It should be noted that the addition of calcium to the "reference" samples was made to begin with in preparation B but was made at final dilution in samples prepared by preparation A. For the analyte matrix, the samples from the filtrates from preparation B were diluted again by taking 2 ml and diluting to 40 g in 60-ml plastic bottles.

In all, seven orange juice brands which, during the rest of this discussion, will be referred to as brand R (reference brand), and brands I, II, III, IV, V, and VI, were analyzed by preparations A and B. Five of the brands, brand R and brands I, II, III, and IV, were prepared according to preparation A. Three brands (brand R and brands V and VI) were hydrolyzed by preparation B and diluted.

Measurements of the calcium absorbance were made at 422.7 nm by aspiration of the final solutions at a rate of 3.5 to 4 ml/min into a slightly fuel-rich acetylene air flame. The beam from the lamp was set, as recommended by the instrument manufacturer's instruction manual, at approximately 1 cm above the top of the burner. The burner used was a three-slot Boling type.

## RESULTS AND DISCUSSION

At the start of this project, it had been estimated from the prior literature (Birdsall et al., 1961; Kefford and Chandler, 1970; Rakieten et al., 1952; Roberts and Gaddum, 1937) that the calcium concentration in single-strength orange juice would be approximately 100 μg/ml. Preliminary experiments indicated that calcium gave a signal-to-noise ratio of about 200/1 at 2 μg/ml, so this level was chosen as the working concentration. Because frozen concentrated orange juice (FCOJ) is four times the strength of single-strength orange juice (SSOJ), the original sample of concentrate must be diluted by a factor of 200 to 1 in order to obtain a solution containing approximately 2 ppm of Ca. It was also estimated from the above-cited references that the phosphorus content of single-strength orange juice seldom, if ever, exceeded 350 μg/ml. This corresponds to a maximum molar ratio of P/Ca of ~4/1. The corresponding concentrations of phosphorus and calcium in the working matrix would be 8 μg/ml of P and 2 μg/ml of Ca. Because these concentration levels are low and because the final matrix was essentially aqueous 0.1 *M* $HNO_3$, the necessity of using a releasing agent for phosphorus was questioned.

Analytical curves for 0.1 *M* $HNO_3$ solutions with and without phosphate were prepared covering a range of calcium concentrations from 0.25 to 20 μg/ml. In the series with phosphate added, the amount of phosphate was kept constant at 7.5 μg/ml of P. This latter series, in addition to the added phosphate, was prepared containing dextrose, sodium, and potassium in concentrations to be expected from their reported values in orange juice (Birdsall et al., 1961; Rakieten et al., 1952). This, in effect, provided a simulated hydrolyzed orange juice matrix for evaluation and comparison. It should be noted that, although aluminum, which is another reported calcium suppressor, is present in orange juice, the levels of aluminum in the final measured solution would be of the order of only a few nanograms per milliliter. This is estimated from the reported ratio (Roberts and Gaddum, 1937) of Ca/Al as being between 10/1 and 100/1.

In Figure 1, the analytical curves obtained for the $HNO_3$ matrix and the simulated orange juice matrix are given. It can be observed that the two analytical curves follow each other very closely, and, therefore, the different matrices in these nitric acid solutions (both 0.1 *M* in acid)

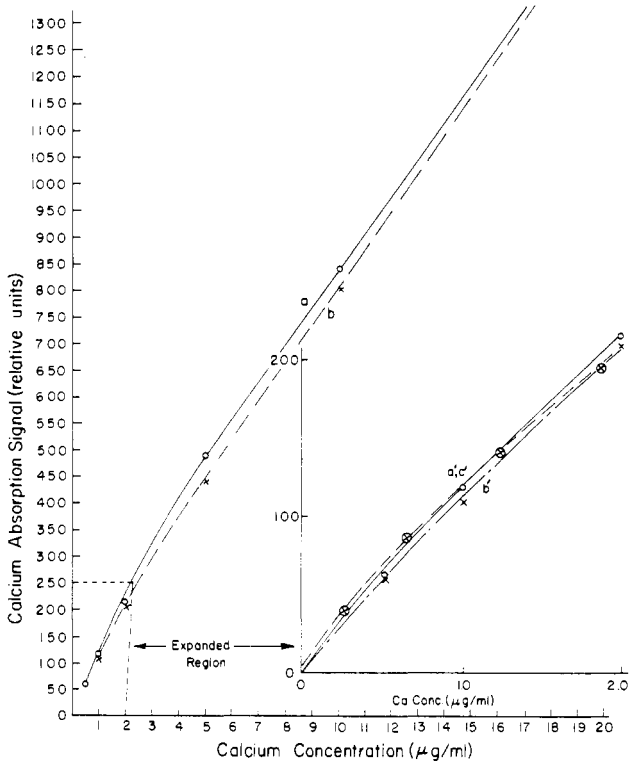


Figure 1. Analytical calibration curves for calcium: (a) calcium in 0.1 *M* $HNO_3$ matrix; (b) calcium in simulated orange juice matrix (not hydrolyzed) containing 0.1 *M* $HNO_3$ and 7.5 μg/ml of phosphorus. Insert shows expanded portion (0-2 ppm) of main figure: (a') calcium in 0.1 *M* $HNO_3$ matrix; (b') calcium in simulated orange juice matrix (not hydrolyzed) containing 0.1 *M* $HNO_3$ and 7.5 μg/ml of phosphorus; (c') same as b' except hydrolyzed prior to analysis.

show little, if any, interference effects.

**Effect of Added Phosphate.** As observed above, satisfactory analytical curves can be obtained even in the presence of phosphate. It was of interest, though, to evaluate the effect of phosphate added in incremental amounts to both nitrate and chloride matrices in the absence of any other additives. Two series of solutions were made, one in 0.1 *M* nitric acid and the other in 0.1 *M* hydrochloric acid. Both of these series of solutions contained 2 μg/ml of calcium but increasing amounts of phosphate. The results of this comparison are shown in Figure 2, and it can be seen that phosphate affects the calcium absorbance in both acid matrices but to a larger extent in the hydrochloric acid matrix. Three additional P/Ca ratios, i.e., ratios between 1 and 8, were studied for both the HCl and $HNO_3$ notices but were not included in the graphs in Figure 2 because a plateau (maximum) was reached for molar ratios exceeding 1/1. These results are similar to flame emission studies (Yofe et al., 1963; Yofe and Finklestein, 1958); however, the phosphate effect is much less marked in our case than that expected from these prior studies. The reasons for the lesser effect of phosphate in our studies are not readily apparent. One possible explanation is that the earlier studies of the phosphate effect were carried out at significantly higher calcium and phosphorus concentrations, resulting in significantly larger solute particles upon solvent evaporation of the aerosol droplets within the flame.

**Effect of Added Lanthanum.** Because essentially all methods prescribed for the flame atomic absorption determination of calcium call for the addition of large quantities of a releasing agent for phosphorus, the effect

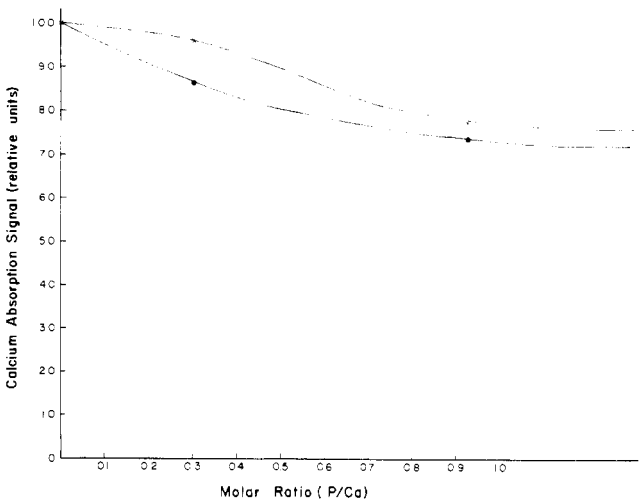


Figure 2. Influence of P/Ca ratio (mol $l.^{-1}$/mol $l.^{-1}$) upon calcium absorption signal (Ca present at 2 ppm and phosphate concentration varied): (a) calcium in 0.1 *M* $HNO_3$; (b) calcium in 0.1 *M* HCl.

Table I. Influence of Lanthanum upon Calcium Absorption Signal[a]

| Concn of P present, μg/ml | Concn of La added, μg/ml | Ca absorption signal, rel units |
|---|---|---|
| 0 | 0 | 100 |
| 7.5 | 0 | 77 |
| 7.5 | 1500 | 77 |
| 7.5 | 15000 | 52 |

[a] Concentration of calcium is 2.0 μg/ml in each case.

of adding lanthanum to the nitric acid matrix was investigated. In the series reported on above in the nitric acid matrix with added phosphate, two additional solutions were prepared. Both of these contained 2 μg/ml of calcium and 7.5 μg/ml of phosphorus as phosphoric acid. To one of these was added 1500 μg/ml of lanthanum and to the other 15000 μg/ml of lanthanum. The effect on the calcium absorbance is given in Table I.

A startling feature of these data is that lanthanum actually suppresses the calcium signal for the nitrate matrices at the largest lanthanum concentrations studied. Even at the lower lanthanum concentration (1500 μg/ml), there is no apparent enhancement. In the absence of phosphate in a chloride matrix, some of the data showed that lanthanum markedly enhanced the calcium signal. This is due in part, but not wholly, to the large blank observed at high lanthanum concentrations. There is restoration of the signal in chloride solutions containing phosphate, but the data obtained were erratic and difficult to interpret.

**Effect of Other Components of the Matrix.** It is impractical to eliminate the major inorganic constituent, potassium, from the sample matrix regardless of the method of preparation. In addition, sodium and magnesium are present at about 20 and 100 μg/ml, respectively, in SSOJ. No study of the influence of magnesium was made because its concentration approximates that of calcium in the usual situations, and magnesium acts essentially in the same way as calcium with respect to flame atomic absorption. It was further noted experimentally that addition of sodium and potassium at the expected concentrations for the prepared orange juice samples had little influence on the calcium signal when the sample was in a nitric acid matrix.

Orange juice at single strength has a total sugar content

Table II. Influence of Sugar in Measured Solution upon Calcium Absorption[a,b]

| Concn of P, μg/ml | Concn of dextrose, % | Calcium absorption (rel absorption) |
|---|---|---|
| 0 | 0 | 100 |
| 7.5 | 0 | 84 |
| 7.5 | 0.2 | 96 |

[a] Synthetic samples contain 2 μg/ml of Ca, 20 μg/ml of K, 0.4 μg/ml of Na. [b] The data in this table were verified on several different occasions.

of approximately 10% (Birdsall et al., 1961). At the dilutions used in the present study, assuming no degradation, the sugar concentration in the final analytical solution would be 0.2%. In the insert of Figure 1, the two analytical curves given in the body of the figure are expanded (0–2 ppm range which covers the range of interest for orange juice analysis), and an additional curve showing the influence of hydrolysis under the conditions of preparation B is also given. Although some degradation of the sugar was to be expected during the hydrolysis conditions used here, it was apparent during some work with a flameless furnace that significant amounts of sugar-related products remained as noted by the caramel-like odor evident during heating and the carbonaceous residue remaining after the burn cycle. Additions of dextrose to synthetic analyte mixtures at appropriate concentrations provided a significant restoration of the calcium absorbance in the presence of phosphate and gave better signal reproducibility. This effect was not evident in chloride solutions. The results of this study are shown in Table II. A more detailed study of the effect of glucose on the calcium atomic absorption signal has been given by Christian and Feldman (1970); these authors also report an enhancement for glucose concentrations exceeding $10^{-6}$ *M*.

**Results on Various Brands of Orange Juice.** The orange juice brands identified previously were compared as to their calcium content. Only brand R was evaluated by the two different sample preparation techniques. The concentration of calcium in brand R (reference brand) was determined by the method of standard additions using the additions procedure outlined under preparations A and B above. The calcium contents of the other brands were calculated by comparison of the absorbance values obtained at the same time and under the same conditions with the absorbance values from the reference. The extrapolation using the standard additions method is shown in Figure 3. Curve a shows the line for the reference brand obtained by the two different preparation procedures. Curve b represents the line for the reference prepared by ashing (preparation A) with 0.5% La added. Curve c shows the comparison for the reference prepared by hydrolysis (preparation B) with 0.5% La added. There are two noteworthy features represented by these extrapolations. First, the extrapolation data obtained from these two preparations without lanthanum added coincide exactly. This means that the hydrolysate matrix and the ashed matrix act very similarly toward the analyte and, also, since the calcium additions in the hydrolysis preparation were made at the beginning as opposed to final addition in the ashed preparation, the recovery is excellent for calcium by hydrolysis. Secondly, from curves b and c, it can be observed that lanthanum suppresses the signal (less slope to the line) in both preparations but more so in the hydrolyzed preparation. The calculated calcium concentrations from all the extrapolations differ by less than 10% even though the slopes are not the same. It

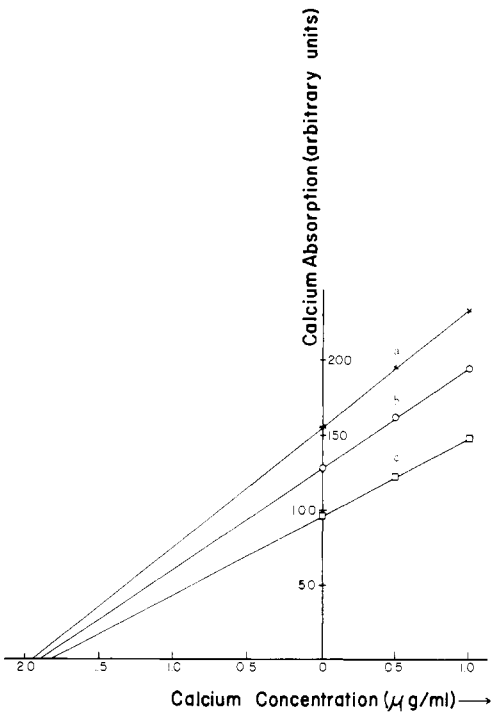


Figure 3. Standard addition plots for determination of calcium in reference brand orange juice: (a) reference brand prepared by preparations A and B, no lanthanum added; (b) reference brand prepared by preparation A, with 0.5% lanthanum added; (c) reference brand prepared by preparation B, with 0.5% lanthanum added.

Table III. Calcium Concentrations[a] of Several Orange Juice Samples by Preparation B

| Orange juice brand | Calcium concn, μg/ml |
|---|---|
| R | 97.5 |
| I | 91.5 |
| II | 87.5 |
| III | 88.0 |
| IV | 85.0 |
| V | 88.5 |
| VI | 97.5 |

[a] Calcium concentrations are for single strength orange juice.

seems evident, however, that the use of lanthanum is not desirable, especially when using preparation B.

Table III lists the values for calcium obtained on the seven brands by comparing the signals obtained with that of the reference prepared in the same procedure.

These samples of orange juice were all purchased from consumer sources in Florida during the period of October 1974 to March 1975. The values for calcium seem remarkably consistent, there being a variation from the average value of 90.8 μg/ml (based on SSOJ) by, at most, not more than 10%.

## SUMMARY

A hydrolysis procedure for the preparation of orange juice has been studied with special regard to its application to the determination of calcium by atomic absorption spectrometry. Future investigations of the application of this method will be performed to determine how widely useful it is for the determination of other major and minor elemental constituents of orange juice. The hydrolysis preparation (preparation B) is easy to carry out, requires little attention time, simple equipment, and lends itself readily to the preparation of a large number of samples at one time making routine collection of data more feasible

than by other previously used preparation techniques. Also, because exposure to containers and the environment is minimal in preparation B, contamination is minimal. Because of these factors, this preparation is preferred to preparation A as a method for orange juice.

ACKNOWLEDGMENT

The authors wish to thank Reuven Avni for valuable advice and consultation concerning the effect of phosphate on the determination of calcium, and S. V. Ting for consultation on the general nature of orange juice. Also, the gracious loan of an IL151 spectrometer by Instrumentation Laboratories is acknowledged.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 11th ed, Horwitz, W., Ed., Washington, D.C., 1970.
Birdsall, J. J., Derse, P. H., Tepley, L. S., *J. Am. Diet. Assoc.* **38**, 555 (1961).
Christian, G. D., *Anal. Chem.* **41**, 24A (1969).
Christian, G. D., Feldman, F. J., "Atomic Absorption Spectroscopy, Applications in Agriculture Biology and Medicine", Wiley-Interscience, New York, N.Y., 1970.
David, D. J., *Analyst* **84**, 536 (1959).
David, D. J., *Analyst* **85**, 495 (1960).
Gorsuch, T. T., *Analyst* **84**, 135 (1959).
Herrmann, R., Alkemade, C. T. J., "Chemical Analysis by Flame Photometry", Translated by P. T. Gilbert, Interscience, New York, N.Y., 1963.
Kefford, J. F., Chandler, B. V., *Adv. Food Res. Suppl.* **2**, Chapter 6 (1970).
Pijck, J., Gillis, J., Hoste, J., *Int. J. Appl. Radiat. Isot.* **10**, 149 (1961).
Rakieten, M. L., Newman, B., Falk, K. G., Miller, I., *J. Am. Diet. Assoc.* **28**, 1050 (1952).
Roberts, J. A., Gaddum, L. W., *Ind. Eng. Chem.* **29**, 574 (1937).
Rowe, C. J., "Food Analysis by Atomic Absorption Spectroscopy", Varian Techtron, Spring Vale, Australia, 1973.
Simpson, G. R., Blay, R. A., *Food Trade Rev.* **36**, 35 (1966).
Slavin, W., "Atomic Absorption Spectroscopy", Interscience, New York, N.Y., 1968.
Smith, G. F., "The Wet Chemical Oxidation of Organic Compositions", G. Frederick Smith Chemical Co., Inc., 1965.
Szarski, P., "Food Technology in Australia", AIFST Convention Paper, Melbourne, Australia, 1970, published May 1971, p 216.
Willis, J. B., *Spectrochim. Acta* **16**, 259 (1960).
Willis, J. B., *Anal. Chem.* **33**, 556 (1961).
Yofé, R., Avni, R., Stiller, M., *Anal. Chim. Acta* **28**, 331 (1963).
Yofé R., Finklestein, R., *Anal. Chim. Acta* **19**, 166 (1958).

Received for review June 13, 1975. Accepted September 15, 1975. This research was supported by the Florida Citrus Commission and by AFOSR-74-2574.

# Determination of Lead, Cadmium, and Zinc in Sugar

Nancy M. Morris,* Margaret A. Clarke, Verne W. Tripp, and Frank G. Carpenter

A new technique for eliminating matrix interference in the determination of lead, cadmium, and zinc in sugars by flameless atomic absorption is described. Yeast fermentation converts the sucrose to ethanol and carbon dioxide, both of which are easily volatilized. Reproducible results for these elements are obtained with this technique. Relative standard deviation for lead and cadmium was less than 10%, and for zinc less than 17%. Using this yeast fermentation technique, it was found that all three elements were present in raw and refined sugars at levels well below limits recommended by regulatory agencies.

Interest in the levels of heavy metals in various food products has increased greatly in the last few years. As analytical techniques become more sensitive, lower limits for toxic metals are often established and it becomes necessary to reevaluate many products. Atomic absorption spectrophotometry has been the method of choice for these studies. Manning (1973) reported the analysis of lead in milk using the graphite furnace and a single point calibration method. Huffman and Caruso (1974) used a tantalum ribbon and a carbon rod atomizer to determine lead in milk. Roschnik (1973) used flame atomic absorption to determine lead in ashed foods. Several studies of heavy metal constituents in molasses and other sugars have been conducted. Morriss and Nicol (1974) reported a direct atomic absorption method for trace constituents in molasses, using flame techniques and the method of additions. They successfully analyzed for copper, zinc, cadmium, lead, and iron at the high levels present in molasses. In 1972, Pommez and Clarke (published 1975) reported the results of a survey of trace elements in a raw and a refined sugar. The technique was extended by Clarke et al. (1973) to cover raw and refined sugars and the refinery liquors after various stages of the refining process. The graphite furnace was used to determine a number of heavy metals in sugar solutions and in refinery liquors. No matrix interferences were observed for iron, copper, manganese, chromium, and silver; however, a serious interference, attributed to the organic matrix in which the element was originally present, was encountered for lead. Yeast fermentation of the sugar to eliminate this matrix interference is reported in this paper.

Southern Regional Research Center, one of the facilities of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana 70179.

EXPERIMENTAL SECTION

**Apparatus.** The equipment consisted of a Perkin-Elmer Model 306 atomic absorption spectrophotometer, equipped with the HGA 2000 graphite furnace, a Model 56 recorder, and a deuterium background corrector. Details of the graphite furnace have been given by Kahn (1973).

**Reagents.** Standard solutions were prepared fresh daily from 1000-ppm commercial stock solutions. The sugars were obtained from the refinery, along with process liquors from various stages of the refining process. The liquors were all about 60% solids by weight.

**Procedure.** The yeast fermentation technique of Roberts and Rowland (1969) was used to break down all