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# Determination of Heavy Metals in Foods

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The proposed method utilized a $V_2O_5$-catalyzed $HNO_3/H_2SO_4/H_2O_2$ digestion followed by pH adjustment to 7.0 ± 0.5. If a precipitate formed, it was filtered and analyzed separately after being dissolved in acid. Heavy metals were removed from the digest with a column of Chelex 100 chelating ion-exchange resin in the sodium form. The metals were eluted from the column with 1 *N* $H_2SO_4$ and were determined by atomic absorption. Sensitivity varied from 20 ppb for Zn to 0.20 ppm for Pb, and recoveries of added standards varied from 91.4% for Pb to 100.5% for Zn, with an overall average recovery of 95.2% and an average standard deviation of 3.03%. The heavy metal content of eight different types of foodstuffs has been determined. The proposed method can be used to determine Pb, Cd, Cu, Co, Mn, Ni, and Zn in biological materials in the ppm and ppb range on a single sample.

The concern with possible heavy metal contamination of foods has created a need for analytical methodology to detect these metals in trace amounts since existing methods can not be used as a general screening method. The methods for the determination of individual metals in foods usually are not satisfactory for the analysis of several metals in a single sample. The present official method of the Association of Official Analytical Chemists (1970) for cadmium in foods, for example, utilizes either wet or dry ashing of organic material, followed by pH adjustment, organic solvent extraction, and colorimetric determination. If this method is applied to a scheme for multimetal determination, the extraction of the different metals involves differential pH adjustment and is tedious, lengthy, and subject to a vast number of interferences. A method is needed to determine several metals in a single sample charge which will be sensitive, precise, and accurate as the direct determination of each single element.

Previously published methods (AOAC, 1970; Flann and Bartlet, 1968; Hoover *et al.*, 1969; Thiers, 1957) for heavy metal determination have utilized either wet or dry ashing techniques for the destruction of organic material. Dry ashing has been reported (Thiers, 1957) to cause loss by volatilization, adsorption on unburned carbon, or formation of insoluble silicates. Hoover *et al.* (1969) have investigated several methods for lead and conclude that the method of Flann and Bartlet (1968), which involves wet digestion followed by coprecipitation of lead with strontium sulfate, offers the best solution to the problem of isolation and concentration of lead.

Volatilization is offset in wet ashing with acids, since the oxidation takes place at relatively low temperatures. One problem with wet ashing using $H_2SO_4$ arises from the presence of various metals, since lead coprecipitates with calcium and other sulfates. Wet oxidation using $H_2SO_4$ and 50% $H_2O_2$ has been used for the destruction of organic material in the determination of metals for many years. The procedure does not cause loss of cadmium by volatilization (Analytical Methods Committee, 1969) and has been proven to be a rapid and smooth oxidation with low blank values in the determination of heavy metals (Analytical Methods Committee, 1967).

The most common methods of separation and concentration include volatilization, electrodeposition, liquid–liquid extraction, precipitation, and ion exchange. Galle (1971) used the chelating ion-exchange resin, Chelex 100, to remove and concentrate manganese, iron, cobalt, nickel, copper, zinc, and lead in oil field brines. He effectively separated the metals from the brine after adjusting the pH of the solution and resin to 4.5. The metals were eluted from the column with 1 *N* HCl. Biechler (1965) used Chelex 100 to separate trace amounts of copper, lead, zinc, cadmium, nickel, and iron from industrial waste waters. He found it necessary to use 8 *N* $HNO_3$ to remove cadmium quantitatively from the resin and that the resin could not be regenerated after this treatment. Freudiger and Kenner (1972) separated trace amounts of cobalt, copper, iron, manganese, nickel, lead, and zinc from the high sodium ion concentration resulting from the basic fusion of ore samples by the use of Chelex 100 after adjustment of the pH to 7 ± 1. The metals were eluted with 3 *N* HCl and determined by atomic absorption. They observed that elimination of the high concentration of the alkali metal ions before measurement by atomic absorption resulted in greatly improved reproducibility and accuracy. Dingman *et al.* (1972) have used polyamine–polyurea resins to study the effects of pH, equilibrium time, and resin cross-linking in a batch equilibrium study on the chelation of Cu, Ni, Zn, and Co.

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This paper is concerned with the separation and determination of trace amounts of cadmium, cobalt, copper, manganese, nickel, lead, and zinc in a single sample of various foods as a screening procedure. The digestion involves bringing the sample into solution with $HNO_3$ and completing the digestion with $H_2SO_4$ and $H_2O_2$ catalyzed by $V_2O_5$. The pH of the cooled digest is raised to 7 ± 0.5 by careful addition of concentrated NaOH. If a precipitate forms before or during pH adjustment it is filtered and analyzed separately by dissolving it in $HNO_3$ and determining the metals by atomic absorption. This step accounts for metals that are precipitated, gathered, or occluded due to the dilution and neutralization of the $H_2SO_4$ used in the digestion procedure. Heavy precipitates are obtained with products such as milk which contain relatively large amounts of alkaline earth metals. Iron also can be determined in this solution if desired. The filtered digest is poured through a column of Chelex 100, which retains the heavy metals and allows the alkali metals and anions to pass through. The retained metals are eluted with 1 *N* $H_2SO_4$ and determined by atomic absorption. A reagent blank is used to correct for trace amounts of metals in the large volumes of reagent used.

The proposed method provides for a relatively rapid, precise, sensitive, and accurate determination of seven metals in foods in one sample charge.

## EXPERIMENTAL SECTION

**Reagents.** Working standard solutions of each metal at the concentration desired were prepared in 1 *N* $HNO_3$ or 1 *N* $H_2SO_4$ from stock standards supplied by Beckman Instrument Co. The Dowex A-1 resin (Chelex 100, 50–100 mesh) was supplied by Bio-Rad Laboratories as reagent grade quality in the sodium form. Buffer systems described by Clark and Lubs (1917) were used for solutions whose pH value was between 2.2 and 7.0. $H_2SO_4$ was substituted for HCl in solutions whose pH value was less than 4.0. All other chemicals used were ACS reagent grade quality. Deionized water was used throughout.

**Apparatus.** A 2.5-cm i.d. Allihn filter tube with a 145–175 μm glass frit (Ace Glass Co. No. 7195-02) was used to hold the resin. A reservoir for the eluting solution was prepared by attaching a 300-ml French-style globe separatory funnel (Kimax No. 29044F) to the column with a rubber stopper. The flow rate was controlled by the stopcock of the separatory funnel. The metal analyses were performed using a Perkin-Elmer Model 303 Atomic Absorption Spectrometer with a Sargent Model SRG Recorder. Operating parameters were similar to those stated in the standard conditions section of the "Analytical Methods for Atomic Absorption Spectrophotometry," (Perkin-Elmer Corp., 1971).

**Pretreatment of Resin.** The Chelex 100 resin as received is agitated in a beaker with two separate 50-ml portions of 1 *N* $H_2SO_4$. The $H_2SO_4$ is decanted and the resin is washed three times by decantation with 50-ml portions of deionized water. The resin is then mixed thoroughly with 50 ml of 3 *N* NaOH and allowed to stand 5 min. After decantation of the NaOH, the resin is washed with two 50-ml portions of deionized water. The regenerated resin may be used immediately or stored until needed. After use in a separation, the resin is removed from the column and regenerated according to the above procedure.

**Preparation of Column.** Place the regenerated resin in the filter tube to a depth of 3.0 ± 0.5 cm and attach the separator. Equilibrate the column to pH 7.0 ± 0.5 by addition of 25 ml of 0.2 *N* $H_2SO_4$ and 100 ml of pH 7 buffer. Decant the sample into the separator and control the flow rate at 3–5 ml/min. When the level of the liquid is just above the top of the resin bed, follow with 300–400 ml of deionized water. Discard the effluent.

## PROCEDURE

**Sample Digestion.** Weigh a 50.0-g sample into an 800-ml beaker together with 20–30 mg of vanadium pentoxide and 5 or 6 glass boiling beads. Prepare a reagent blank using only the vanadium pentoxide and beads. Carefully add 100 ml of concentrated $HNO_3$ to each and allow the mixtures to stand about 1 hr. Control any frothing which occurs by addition of small amounts of deionized water. Heat the mixtures on a hot plate until oxide of nitrogen fumes are no longer evolved. If the sample is not completely dissolved, add additional $HNO_3$ to both sample and blank and repeat the boiling process until the sample is completely in solution. Cool to room temperature, add 30 ml of $HNO_3/H_2SO_4$ (1 + 1) to both sample and blank, and evaporate until charring begins. Cautiously add 50% $H_2O_2$ dropwise to each mixture until the sample solution is clear and is the same pale green color as the reagent blank. Since 50% $H_2O_2$ is a very strong oxidant, it must be added slowly in small amounts to prevent frothing and splattering. The use of rubber gloves is recommended.

**Separation, Concentration, and Determination.** Cautiously bring the solution volume to about 100 ml with deionized water. Chill in an ice bath and adjust the pH of the solution to 7.0 ± 0.5 by slow addition of 50% NaOH. If a precipitate forms, carefully filter the neutralized solution through Whatman 2V or equivalent paper. Save the filtrate for column separation. Leave the paper in the funnel and dissolve the precipitate with about 90 ml of hot 1 *N* $HNO_3$, catching the filtrate in a 100-ml volumetric flask. Cool the solution, dilute to volume, and determine the metals present by atomic absorption. If necessary, an aliquot can be evaporated to a smaller volume to increase the sensitivity before determination.

Pour the filtrate through the column as described under column preparation and, after the water wash, elute with 95 ml of 1 *N* $H_2SO_4$ into a 100-ml volumetric flask and di-

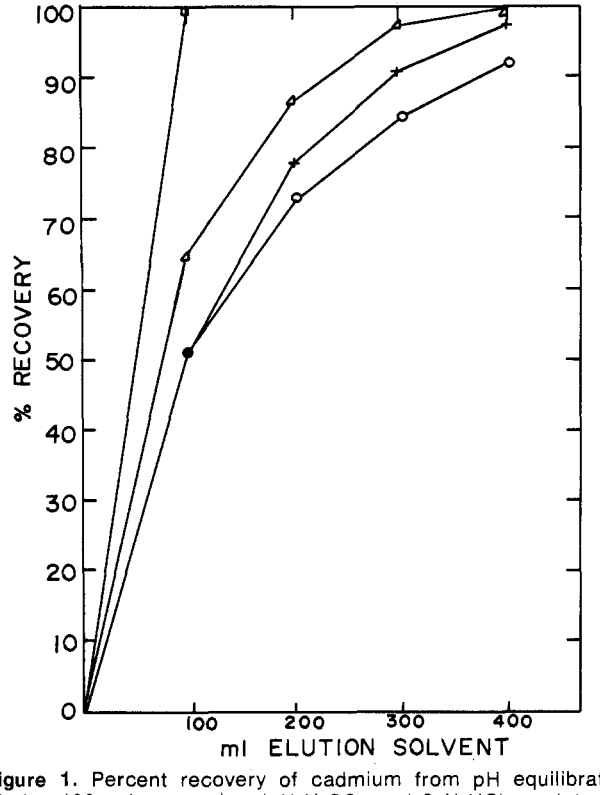


**Figure 1.** Percent recovery of cadmium from pH equilibrated Chelex 100 columns using 1 *N* $H_2SO_4$ and 3 *N* HCl as elutants. □, 1 *N* $H_2SO_4$ with column pH 3.5; △, 3 *N* HCl with column pH 3.4; +, 3 *N* HCl with column pH 6.1; O, 3 *N* HCl with column pH 7.0.

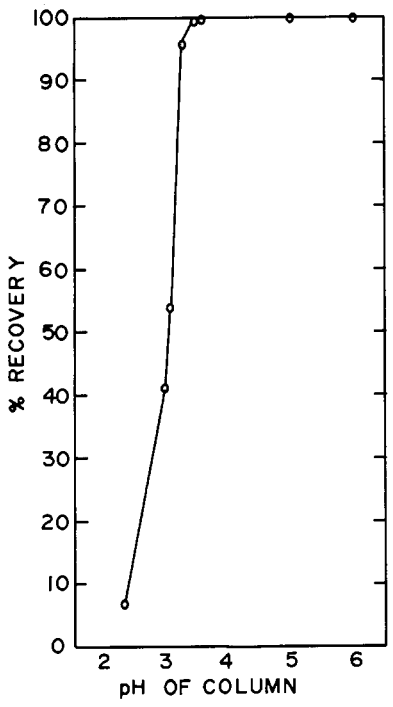


**Figure 2.** Percent recovery of cadmium at various column pH values using 95 ml of 1 $N$ $H_2SO_4$ as elutant.

lute to volume. If desirable, aliquots of both sample and reagent blank can be evaporated to a smaller volume to increase sensitivity before determination by atomic absorption. (Preliminary data obtained after this paper was submitted indicate that sensitivities can be increased for some foods by using 1 cm of resin in place of 3 cm, followed by elution with 25 ml of 2 $N$ $H_2SO_4$.) Correct all values for the amounts found in the reagent blank which has been carried through the column separation step.

**Elution Studies.** In all elution studies the resin was equilibrated to the proper pH by passage of the buffer before addition of the sample, and the sample solution was adjusted to the same pH.

The retention and elution of cobalt from the column was investigated at various pH values using cobalt standards and elution with 3 $N$ HCl. The retention of the metal by the column after digestion of food samples with nitric and sulfuric acid was studied by the addition of standards to the samples.

The retention of cadmium by the column at various pH values was investigated using 3 $N$ and 6 $N$ HCl, and 1 $N$, 2 $N$, and 3 $N$ $H_2SO_4$ as elutants.

The loss of lead by precipitation as lead sulfate during digestion or pH adjustment was investigated using various filtration and solution procedures.

The retention and elution of the metals of interest from the column using a mixed standard and elution with 95 ml of 1 $N$ $H_2SO_4$ were determined.

**Digestion Studies.** Recoveries of seven metals through the digestion procedure and column elution were determined for the proposed method and also for a rapid (20-min) digestion method using $HNO_3/H_2SO_4$ with a vanadium pentoxide catalyst.

**Table I. Analysis of Various Food Commodities for Lead, Cadmium, Cobalt, and Nickel**

| Commodity[a] | | Amounts found, ppm | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pb | | Cd | | Co | | Ni | |
| Dried fish | Ppt | 1.64 | 1.31 | 0.48 | 0.49 | | | | |
| | Col | BDL[b] | BDL | BDL | BDL | | | | |
| | Total | 1.64 | 1.31 | 0.48 | 0.49 | BDL | BDL | BDL | BDL |
| Spinach[c] | Ppt | 0.07 | 0.05 | 0.08 | 0.07 | | | 0.28 | 0.22 |
| | Col | BDL | BDL | BDL | BDL | | | 2.71 | 2.55 |
| | Total | 0.07 | 0.05 | 0.08 | 0.07 | BDL | BDL | 2.99 | 2.77 |
| Spinach[d] | Ppt | 0.55 | 0.60 | 0.13 | 0.08 | | | 0.07 | 0.08 |
| | Col | BDL | BDL | BDL | 0.01 | | | 0.18 | 0.12 |
| | Total | 0.55 | 0.60 | 0.13 | 0.09 | BDL | BDL | 0.25 | 0.20 |
| Spinach[e] | Ppt | 0.93 | 0.79 | 0.17 | 0.17 | | | | |
| | Col | BDL | BDL | BDL | BDL | | | | |
| | Total | 0.93 | 0.79 | 0.17 | 0.17 | BDL | BDL | BDL | BDL |
| Oysters | Ppt | 0.51 | 0.51 | 0.45 | 0.45 | | | 0.08 | 0.08 |
| | Col | BDL | BDL | BDL | BDL | | | BDL | BDL |
| | Total | 0.51 | 0.51 | 0.45 | 0.45 | BDL | BDL | 0.08 | 0.08 |
| Lettuce | Ppt | | | 0.04 | 0.04 | | | | |
| | Col | | | 0.02 | 0.02 | | | | |
| | Total | BDL | BDL | 0.06 | 0.06 | BDL | BDL | BDL | BDL |
| Potatoes | Ppt | | | 0.02 | 0.02 | | | | |
| | Col | | | BDL | BDL | | | | |
| | Total | BDL | BDL | 0.02 | 0.02 | BDL | BDL | BDL | BDL |
| Dried corn, 14% | Ppt | 0.15 | 0.12 | | | | | BDL | BDL |
| moisture | Col | BDL | BDL | | | | | 0.21 | 0.17 |
| | Total | 0.15 | 0.12 | BDL | BDL | BDL | BDL | 0.21 | 0.17 |
| Apples | Ppt | 0.28 | 0.27 | | | | | | |
| | Col | BDL | BDL | | | | | | |
| | Total | 0.28 | 0.27 | BDL | BDL | BDL | BDL | BDL | BDL |
| Milk | Ppt | | | 0.02 | 0.02 | | | | |
| | Col | | | BDL | BDL | | | | |
| | Total | BDL | BDL | 0.02 | 0.02 | BDL | BDL | BDL | BDL |
| St dev[f] | | 0.097 | | 0.01 | | | | 0.081 | |

[a] All commodities reported on wet weight basis except dried fish and corn. [b] BDL, below detectable level. [c] Untreated product grown in Canton, Texas. [d] Treated with Monzate G (manganese diethyldithiocarbamate). Grown in San Antonio, Texas. Harvested 3/30/72. [e] Treated with Monzate G, same field as d; harvested 4/4/72. [f] Calculated from the differences between duplicates by the method of Youden (1951).

**Recovery Studies.** Recoveries of seven metals added to the food samples were determined for the proposed method for six food products.

**Replication Studies.** Duplicate determinations were made for the seven metals using the proposed procedure on eight different food commodities.

## RESULTS AND DISCUSSION

The results of the investigation of the effect of pH upon the retention of cobalt from the column substantiated the work of Freudiger and Kenner (1972) in that cobalt is retained quantitatively by columns equilibrated to pH 4 and above before addition of the sample and can be eluted satisfactorily with 50 ml of 3 *N* HCl. However, it was noted that cobalt added to a dried fish sample before digestion was not recovered satisfactorily if a rapid but incomplete digestion, utilizing nitric and sulfuric acids with vanadium pentoxide as catalyst, was used. In this procedure any undissolved fat is removed by filtration and the sample is not completely oxidized. Using standard solutions, adjustment of the digest to pH 5.7, and elution with 1 *N* $H_2SO_4$, the average recovery for all seven metals was 99.0%, with a range of from 97.5% for cobalt to 104.2% for zinc. Addition of the mixed standard to a sample of dried fish before digestion by this procedure resulted in low recoveries of cobalt but satisfactory recoveries of the other metals. Investigation revealed that only a small amount of the cobalt was retained by the column and that the major portion was present in the effluent from the column. This effluent, a combination of the sample and wash solutions, would normally be discarded in the procedure. The brown color of the digest and of the first effluent from the column indicates the formation of a cobalt complex with incompletely oxidized carbonaceous material or with the nitric acid. A similar problem was encountered by Callahan *et al.* (1966) in the determination of cobalt in sea water due to cobalt complexes. As a consequence, the rapid or incomplete digestion procedure should not be used in samples containing cobalt.

The retention and elution of cadmium by the column was first investigated at pH values from 2.2 to 10.5 using 100 ml of 3 *N* HCl as the elutant. A maximum recovery of 65% was obtained for columns equilibrated to pH 3.4 and lower recoveries at other pH values. The minimum was 9.7% at pH 2.2. Use of 6 *N* HCl as elutant did not appreciably improve these values. Biechler (1965) also encountered difficulty in elution of cadmium from Chelex 100 columns with HCl and recommended use of 8 *N* $HNO_3$ to remove cadmium quantitatively from the resin. Elution with larger volumes of 3 *N* HCl at various pH values gave the results shown in Figure 1. It required 400 ml of 3 *N* HCl to remove the cadmium quantitatively from a column equilibrated to pH 3.4. At higher pH values, less than 100% of the cadmium was eluted with this volume of 3 *N* HCl.

These results indicate that both the chloride ion and the pH affect the retention of cadmium by the resin and the removal of cadmium from the resin. As a consequence, sulfuric acid was tried as the elutant. Cadmium is eluted completely from the column with 95 ml of 1 *N*

**Table II. Analysis of Various Food Commodities for Zinc, Copper, and Manganese**

| Commodity[a] | | Amounts found, ppm | | | | | |
|---|---|---|---|---|---|---|---|
| | | Zn | | Cu | | Mn | |
| Dried fish | Ppt | 170.45 | 150.75 | 1.09 | 0.53 | BDL[b] | 2.18 |
| | Col | 3.0 | 24.49 | 3.72 | 4.39 | 9.34 | 7.13 |
| | Total | 173.45 | 175.24 | 4.81 | 4.92 | 9.34 | 9.31 |
| Spinach[c] | Ppt | 10.60 | 10.07 | 0.93 | 0.64 | 4.83 | 6.34 |
| | Col | 0.41 | 0.63 | BDL | BDL | 12.93 | 12.10 |
| | Total | 11.01 | 10.70 | 0.93 | 0.64 | 17.76 | 18.44 |
| Spinach[d] | Ppt | 10.98 | 11.04 | 0.40 | 0.45 | 24.97 | 14.63 |
| | Col | 0.37 | 0.56 | 0.24 | 0.21 | 42.88 | 49.21 |
| | Total | 11.35 | 11.60 | 0.64 | 0.66 | 67.85 | 63.84 |
| Spinach[e] | Ppt | 7.20 | 7.50 | 1.16 | 1.24 | 9.08 | 10.17 |
| | Col | BDL | BDL | BDL | BDL | 11.47 | 10.51 |
| | Total | 7.20 | 7.50 | 1.16 | 1.24 | 20.55 | 20.68 |
| Oysters | Ppt | 652.00 | 643.50 | 30.26 | 33.35 | 1.90 | 1.71 |
| | Col | 39.50 | 57.20 | 11.89 | 9.02 | 2.26 | 2.87 |
| | Total | 691.50 | 700.70 | 42.15 | 42.37 | 4.16 | 4.58 |
| Lettuce | Ppt | 1.39 | 1.50 | 0.58 | 0.58 | 0.80 | 0.76 |
| | Col | 0.45 | 0.28 | 0.19 | 0.20 | 0.40 | 0.40 |
| | Total | 1.84 | 1.78 | 0.77 | 0.78 | 1.20 | 1.16 |
| Potatoes | Ppt | 3.77 | 3.95 | 0.67 | 0.86 | 0.75 | 0.73 |
| | Col | 1.25 | 1.10 | 0.47 | 0.34 | 4.40 | 3.95 |
| | Total | 5.02 | 5.05 | 1.14 | 1.20 | 5.15 | 4.68 |
| Dried corn, 14% moisture | Ppt | 1.58 | 4.41 | 0.48 | 0.34 | 0.38 | 0.20 |
| | Col | 14.44 | 11.40 | 5.62 | 5.36 | 4.73 | 4.91 |
| | Total | 16.02 | 15.81 | 6.10 | 5.70 | 5.11 | 5.11 |
| Apples | Ppt | BDL | BDL | 0.06 | 0.06 | 0.02 | 0.04 |
| | Col | 0.29 | 0.29 | 0.26 | 0.26 | 0.36 | 0.29 |
| | Total | 0.29 | 0.29 | 0.32 | 0.32 | 0.38 | 0.33 |
| Milk | Ppt | 1.45 | 1.63 | BDL | BDL | | |
| | Col | 2.11 | 2.00 | 0.15 | 0.15 | | |
| | Total | 3.56 | 3.63 | 0.15 | 0.15 | BDL | BDL |
| St dev[f] | | 2.099 | | 0.126 | | 0.971 | |

[a] All commodities reported on wet weight basis except dried fish and corn. [b] BDL, below detectable level. [c] Untreated product grown in Canton, Texas. [d] Treated with Monzate G (manganese diethyldithiocarbamate). Grown in San Antonio, Texas. Harvested 3/30/72. [e] Treated with Monzate G, same field as d; harvested 4/4/72. [f] Calculated from the differences between duplicates by the method of Youden (1951).

$H_2SO_4$, and 100% of the cadmium is recovered from columns equilibrated to pH 3.4 or above, as shown in Figures 1 and 2.

Elution studies for all seven metals of interest from a column equilibrated to pH 7 using 95 ml of 1 *N* $H_2SO_4$ showed that all the metals except lead were eluted satisfactorily. The elution of lead averaged only 38% of the amount placed on the column. The low recovery is probably due to the formation of lead sulfate inside the resin particle. Variation of the volume and concentration of the sulfuric acid by use of 50 ml of 2 *N* and 33 ml of 3 *N* did not appreciably affect the lead or other metal elution recoveries.

Due to the trouble with cobalt and lead, the procedure finally utilized comprises a complete digestion of the entire sample with nitric acid–sulfuric acid–hydrogen peroxide catalyzed by vanadium pentoxide. The digestion time varies according to the nature of material to be oxidized; however, this step usually can be completed in less than 4 hr. The vanadium pentoxide does not interfere, since it is not retained by the resin. The digest is adjusted to pH 7, filtered to remove any precipitate, and passed through a column of Chelex 100 equilibrated to pH 7. The column is eluted with 95 ml of 1 *N* $H_2SO_4$ and the metals are determined by atomic absorption. The regular blank corrections ranged between 0 and 0.03 absorbance units, with the majority being less than 0.01 absorbance units. Values are higher in solutions which have been concentrated by evaporation.

Quadruplicate recoveries of standards in the range of 1 to 15 ppm were determined and averaged 99.1% for all the metals, the range being 91.1 to 103.5%. The average standard deviation was 3.55%, with a range of 1.67 to 5.88. These data are tabulated and will appear in the microfilm edition.

Recovery of added standards in the range of 1 to 15 ppm to various food commodities averaged 95.2% for all the metals, with a range of 91.4 to 100.5%. The average standard deviation was 3.03%, with a range of 1.87 to 5.25. These data are tabulated and will also appear in the microfilm edition.

The results of duplicate determinations on eight different types of foodstuffs are shown in Tables I and II. The precision indicated by the low values of the standard deviations is satisfactory for a method of this type. The relatively high values for zinc (2.1 ppm) and Mn (0.97 ppm) are caused by the large amounts of these metals present in several of the samples.

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# Lactase and Other Enzymes Bound to a Phenol–Formaldehyde Resin with Glutaraldehyde

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A phenol–formaldehyde resin has been found to be an excellent adsorbent support for immobilizing enzymes. The enzymes can be held on the resin by treatment with the difunctional reagent glutaraldehyde. Enzymes that have been immobilized in this way include lactase ($\beta$-galactosidase), invertase, amyloglucosidase, $\alpha$-chymotrypsin, and pronase. The activity of the immobilized lactase (from *Aspergillus niger*) was 200 $\mu$mol of glucose produced/min/gram of drained enzyme resin at pH 4.0 and 45°. Over 99% hydrolysis of lactose occurred when a 3% solution of lactose at pH 4.0 was passed over a 1.2 × 10 cm column of the immobilized lactase at 30 ml/hr and 45°. A lactase column was operated continuously for more than 4 weeks with no detectable loss in activity. Similar columns of the immobilized lactase were operated continuously at 60/ml/hr for more than 4 weeks with no loss in activity.

Recent advances in fixed or immobilized enzymes have been reviewed by Silman and Katchalski (1966), Guilbault (1970), Goldstein (1970), Mosbach (1971), and Orth and Brümmer (1972). By procedures such as adsorption, encapsulation, and covalent bonding it is now possible to stabilize enzymes, overcome their high initial cost by repeated reuse, and remove them from the final product.

Of the many systems described for immobilization, many are quite complex and expensive because of the cost of the chemicals, the purity of the enzyme required, and the sequence of reactions involved in the systems. This paper describes a system which avoids some of these com-

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