

1188

Determination of Calcium in Meat and Bone Meal*

By MILTON LAPIDUS† and EDWARD F. MELLON (Eastern Regional Research Laboratory, ‡ Philadelphia 18, Pa.)

Increased emphasis on the quality of materials used in feeds necessitates stricter quality control during their preparation to assure an acceptably uniform product. The present A.O.A.C. method for the determination of calcium in meat and bone meal is time consuming and therefore not ideally suited for routine control work. The Versene titration method was investigated to demonstrate its applicability for the determination of calcium in meat and bone meals. The disodium ethylenediamine tetraacetate (EDTA) direct titration method in conjunc-

tion with the murexide indicator has been used to determine calcium in the ash of plant materials and milk (1, 2).

The difficulty due to the interference from phosphate (3-5) has been circumvented by a number of methods (1, 2, 6, 7). The most practical method to remove phosphate rapidly without loss of calcium was by means of anion exchange resins (1, 2). Results of the oxalate-permanganate method agreed well with the recoveries of calcium from the anion exchange resin column as determined by the EDTA direct titration method for the determination of calcium (1, 2). However, the simplification of the method was overshadowed by the difficulty of determining the exact end point.

It is difficult to estimate visually the ex-

* Presented at the Seventy-first Annual Meeting of the Association of Official Agricultural Chemists, Oct. 14-16, 1957, at Washington, D.C.

† Research Fellow of the National Renderers Association.
‡ A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

act point of color change of the murexide indicator. The solution changes slowly from red to purple and the end point is not very pronounced. An effort has been made to improve the color change of the murexide indicator by incorporating naphthol green B. The color change is from olive green at the start of the titration to cold blue at the end point (8). A further improvement in determining the exact visual end point was achieved by the introduction of the indicator CalVer II.¹ The color change is distinct from pink through purple to pure blue at pH 12.5 (9, 10).

The method for removing phosphate from calcium by means of an anion exchange resin and the subsequent determination of calcium with the indicator CalVer II was applied to the determination of calcium in an acid digest of meat and bone meal.

METHOD

Reagents and Apparatus

a. *Standard Calcium Solution.*—Dissolve 2.4972 g reagent grade CaCO_3 , previously dried overnight at 110°C , in 200 ml 10% HCl. Boil 1 min. to expel CO_2 , cool, and dilute to 1 l with CO_2 -free distilled H_2O . 1 ml = 1 mg Ca.

b. *Standard Titrating Solution.*—Dissolve 10 g disodium dihydrogen ethylenediaminetetraacetate in 800 ml H_2O , adjust to pH 12.0 with KOH, and dilute to 1 l. Standardize this solution with 10 ml standard calcium solution. Determine following relationship: 1 mg calcium = x ml standard titrating solution.

c. *Calcium Indicator.*—CalVer II (Hach Chemical Co., Ames, Iowa).

d. *Potassium Cyanide Solution.*—10%. Dissolve 10 g reagent grade KCN in distilled H_2O and dilute to 100 ml.

e. *Potassium Hydroxide Solution.*—10%. Dissolve 10 g reagent grade KOH in distilled H_2O and dilute to 100 ml.

f. *Amberlite IRA-400 Column.*—To prepare resin, stir analytical grade Amberlite IRA-400 resin (100-mesh) batchwise with 3 volumes of 1N NaOH for 30 min., decant the alkali, repeat this treatment twice, wash once with distilled H_2O , stir with 3 volumes of 5% acetic acid for 1 hr, decant acid, and

repeat acid washing 2 more times with fresh 5% acetic acid. The resin is now in the acetate form; wash with distilled H_2O until pH of wash is that of the distilled H_2O . Store resin wet.

Add 30 g wet resin to 50 ml H_2O , stir gently, and let resin settle to remove air. Resuspend resin in H_2O by gentle stirring and run carefully into a 15×300 mm glass chromatographic column. Wash column thoroughly with distilled H_2O . Do not let column run dry. Regenerate resin by this same procedure after each run.

Preparation of Meat and Bone Meal Sample

To prepare homogeneous sample for analysis, extract 200 g meat and bone meal in Soxhlet apparatus with ether for 20 hr, air dry for 48 hr, grind in Wiley mill to pass 60-mesh sieve, and blend for 30 min. in Patterson-Kelley blender.

Acid Digest of Sample

Weigh, to ± 0.1 mg, approximately 1 g meat and bone meal and digest in a 100 ml Kjeldahl flask with 25 ml concentrated HNO_3 on a Kjeldahl digestion rack until brown fumes cease to be evolved. Cool flask, add 10 ml 72% HClO_4 , and digest until heavy white fumes appear and solution is clear. *Caution:* Neck of flask must not be put into opening of Kjeldahl fume duct during this HClO_4 fuming; otherwise explosion can be expected when HClO_4 fumes reach plastic or rubber tubing leading to exhaust pump. Transfer digest to a 50 ml volumetric flask and dilute to volume with distilled H_2O .

Removal of Phosphate

Add accurately measured 1–5 ml sample of the acid digest to the Amberlite IRA-400 column, let sample pass into resin, and follow with distilled H_2O . Do not let column run dry. Collect total effluent of 75–100 ml at rate of approximately 2 ml/min.

Calcium Determination

Transfer effluent to 3" casserole, adjust volume to approximately 100 ml, add 2 ml 10% KCN, and using pH meter, adjust solution to pH 12.5 with 10% KOH. Add 200 mg CalVer II and titrate, with stirring and under artificial light, with standard titrating solution until color change is permanently clear blue. (Magnetic stirrer makes very convenient stirring device. Anion exchange

¹ Mention of specific firms and products throughout this paper does not imply their endorsement by the Department of Agriculture to the possible detriment of similar firms or products not mentioned.

Table 1. Calcium recovery from standard solutions containing phosphate^a

| Ml Solution Added to Resin ^b | Calcium Concn. Mg/50 Ml | Mg Calcium Found | Mg Calcium Added | % Recovery ^c |
|---|-------------------------|------------------|------------------|-------------------------|
| 5 | 100 | 9.94 | 10.00 | 99.4 |
| 5 | 100 | 9.94 | 10.00 | 99.4 |
| 3 | 100 | 6.03 | 6.00 | 100.5 |
| 3 | 200 | 11.90 | 12.00 | 99.1 |
| 3 | 200 | 11.95 | 12.00 | 99.5 |
| 2 | 200 | 7.98 | 8.00 | 99.7 |
| 1 | 300 | 5.97 | 6.00 | 99.5 |
| 1 | 300 | 6.03 | 6.00 | 100.5 |
| 2 | 300 | 11.90 | 12.00 | 99.1 |
| 1 | 400 | 7.93 | 8.00 | 99.1 |
| 1 | 400 | 7.98 | 8.00 | 99.7 |
| 2 | 400 | 15.90 | 16.00 | 99.4 |
| 1 | 500 | 10.00 | 10.00 | 100.0 |
| 1 | 500 | 10.05 | 10.00 | 100.5 |
| 2 | 500 | 20.00 | 20.00 | 100.0 |

^a Molar ratio of Ca/P, 1.5.

^b Portion of 50 ml put on ion exchange column.

^c Mean recovery = 99.7%.

resin treatment can be omitted when EDTA titrating solution is standardized with standard calcium solution).

Results and Discussion

A series of 5 samples were prepared from CaCO₃ and NaH₂PO₄·H₂O to contain 100, 200, 300, 400, and 500 mg of calcium per 50 ml with a calcium-phosphorus molar ratio of 1.5 (this is approximately the ratio found in bone). Three portions of the acid digest of each of the 5 samples were put through Amberlite IRA-400 resin columns to remove phosphate, and the recovery of calcium was determined in the effluent by direct titration with EDTA (Table 1).

To demonstrate the reproducibility of the method, the calcium in 3 different weights of the same meat and bone meal product was determined. These samples were weighed, wet ashed, and analyzed for calcium, and this procedure was repeated on 3 different days. Comparisons shown in Table 2 indicate that the precision of this method is good. Because the necessity of the time-consuming step of precipitating calcium as the oxalate has been eliminated, the EDTA procedure is a rapid analytical method easily adapted to routine work.

The Amberlite IRA-400 in the acetate form removed the phosphate quantitatively

Table 2. Effect of weight of sample and time of analysis on determination of calcium in a meat and bone meal

| Wt Sample, ^a Grams | Mg Calcium Found | % Calcium ^b |
|-------------------------------|------------------|------------------------|
| 0.7555 | 115.8 | 15.32 |
| 1.0077 | 153.0 | 15.18 |
| 1.2582 | 190.9 | 15.17 |
| 0.7489 | 113.3 | 15.12 |
| 0.9970 | 152.1 | 15.25 |
| 1.2470 | 189.1 | 15.16 |
| 0.7657 | 116.7 | 15.24 |
| 1.0005 | 153.8 | 15.37 |
| 1.2666 | 192.8 | 15.22 |

^a Each set of three samples was determined on a different day.

^b Standard deviation is 0.08%.

and adjusted the acidity of the solution so that the effluents were approximately pH 4. Although the resin can remove phosphate from a larger volume of sample, the quantity of calcium should be kept below 20 mg in the sample titrated (1). The interference of iron can be eliminated by the addition of potassium cyanide to the effluent (pH 4). The potassium cyanide was not effective at an alkaline pH.

Summary

The EDTA direct titration method has been applied to the determination of calcium in meat and bone meal and in standard solutions containing calcium and phosphate. The results indicate that this method is of value in determining calcium routinely in meat and bone meal. One of the obvious advantages of this method is the short time necessary for analysis.

References

- (1) Gehrke, C. W., Affsprung, H. E., and Lee, Y. C., *Anal. Chem.*, **26**, 1944 (1954).
- (2) Jenness, R., *ibid.*, **25**, 966 (1953).
- (3) Cheng, K. L., and Brag, R. H., *Soil Sci.*, **72**, 449 (1951).
- (4) Gastler, G. F., *Proc. S. Dakota Acad. Sci.*, **28**, 77 (1949).
- (5) Willson, A. E., *Anal. Chem.*, **22**, 1571 (1950).
- (6) Collier, R. E., *Chem. and Ind.*, 587 (1955).
- (7) Verma, M. R., Bhuchar, V. M., and Lherattil, K. J., *Nature*, **179**, 1244 (1957).
- (8) Knight, A. G., *Chem. and Ind.*, 1141 (1951).
- (9) Hildebrand, G. P., and Reilley, C. N., *Anal. Chem.*, **29**, 258 (1957).
- (10) Barnard, A. J., Broad, W. C., and Flaschka, H., *Chemist & Analyst*, **45**, 86 (1956).