CALCIUM/TRITIMETRIC

Contents

DETERMINATIVE METHOD

A. INTRODUCTION

1. Theory	Calcium is solubilized by acid hydrolysis forming calcium ion. The resultant hydrolyzate is diluted to a specific volume and an aliquot reacted with excess EDTA in alkaline media in the presence of cyanide and a hydroxy naphthol blue indicator. EDTA readily forms a chelated complex with the calcium ion. Excess EDTA is then titrated with calcium carbonate to a permanent purple end point. If phosphates are present they must be removed by passing an aliquot through an ion exchange column before the final titration steps.
2. Applicability	This procedure is applicable to the determination of calcium or bone in meat and poultry products.

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B. EQUIPMENT

Apparatus

a. Laboratory fume hood.

b. pH meter (Orion Model 701), or equivalent.

c. Volumetric labware: burets, flasks, pipets, etc.

d. Magnetic stirrer (Corning PC-353), or equivalent.

e. Hot plate.

f. Filtration funnel and filter paper (Whatman #4), or equivalent.

g. Glass beads.

h. Watch glass—about 80 mm diameter.

i. Chromatographic column—19 mm × 400 mm, fitted with a coarse-porosity scintered glass frit and teflon stopcock (Kontes #420540-0224), or equivalent.

j. 300 mL tall form beaker.

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C. REAGENTS AND SOLUTIONS

Reagent and Solution List

a. 0.0200M EDTA (Disodium dihydrogen ethylene-diamine tetraacetic acid dihydrate: Dissolve 7.44 g Na_2H_2 EDTA.2 H_2O (99+% purity) in H_2O in 1 L volumetric flask, dilute to volume with H_2O, and mix. Weigh accurately 3 separate amounts of ACS primary standard $CaCO_3$ (about 80 mg) to give about 40 mL titration with 0.02M EDTA and transfer to 3 separate 500 mL Erlemeyer flasks. To each, add 50 mL H_2O and enough HCl (1+3) to dissolve the $CaCO_3$. Dilute each to about 150 mL with H_2O and add 15 mL 1N NaOH disregarding any ppt or turbidity. Add about 200 mg hydroxy naphthol blue indicator and titrate with EDTA solution from pink to deep blue end point, using magnetic stirrer. Add last few mL EDTA solution dropwise.

$$\text{Molarity EDTA solution} = \frac{\text{mg } CaCO_3}{\text{mL EDTA} \times 100.09}.$$

Use the average molarity value of the three determinations.

b. Hydroxy naphthol blue indicator (Mallinckrodt 5630).

c. Potassium hydroxide-cyanide solution: Dissolve 280 g KOH in 500 mL water. Cool to room temperature, add 66 g KCN, dissolve, and dilute to 1 L. (Use care in handling KCN, as HCN is formed upon contact with water and acids.)

d. HCl (1+1) and (1+3).

e. NaOH (1+5) and 1N.

The following reagents are required only if phosphates are present:

f. Amberlite IRA-93 resin (Rohm and Haas).

g. 5% (w/v) sodium carbonate.

h. HCl (3+22).

i. 10% KOH (w/v in water).

j. Phenolphthalein indicator: 1% (w/v) in ethanol.

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D. STANDARDS

Preparation

0.02M $CaCO_3$ (ACS Primary Standard): Weigh 2.000 g $CaCO_3$ (dried at 100° C for 2 hr) into 1 L volumetric flask. Add 500 mL distilled water and 10-12 mL HCl (1 + 1). Heat just to boiling to dissolve. Dilute to volume with distilled water. Determine the relative strength ratio of the standardized EDTA solution to the $CaCO_3$ solution just before titrating samples (refer to section C, item a) as follows: Pipet 3 separate 25.0 mL portions of EDTA solution into separate 250 mL volumetric flasks. Dilute each to about 100 mL with H_2O and add 15 mL 1N NaOH, disregarding any ppt or turbidity. Add about 200 mg hydroxy naphthol blue indicator and titrate with the $CaCO_3$ solution from deep blue to pink end point, using magnetic stirrer. Add last few mL $CaCO_3$ solution dropwise.

$$\text{EDTA to } CaCO_3 \text{ ratio} = \frac{25.0 \text{ mL EDTA}}{\text{mL } CaCO_3 \text{ titrated}}$$

Use the average ratio from the three titrations.

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F. ANALYTICAL PROCEDURE

1. Determination

a. Weigh 10.0 g sample into a 300 mL tall form beaker.

b. Add 30 mL HCl (1 + 1), several glass beads, cover with watch glass, and place on hot plate in a fume hood.

c. Slowly bring to a boil and digest for about 20 min.

d. Cool to room temperature, filter into 200 mL volumetric flask. Wash filter paper with water until 200 mL filrate is obtained, stopper, and mix.

At this point, if phosphates are present, proceed with removal outlined in section F.2.

e. Pipette 20 mL aliquot into 400 mL beaker, add about 50 mL water. (Use 10 mL aliquot for samples containing greater than 0.85 percent calcium.)

f. On a magnetic stirrer in a fume hood, add 200-300 mg hydroxy naphthol blue indicator, and adjust the pH to 12.5 $\pm$ 0.2 with KOH-KCN solution. (If pH exceeds 12.7, go back to step e, as $Ca(OH)_2$ will be precipitated and it is insoluble.)

g. Add 10-25 mL 0.02M EDTA (Amount depends on amount of calcium present. Must be in excess by at least 3 mL. Color should be green.) Mix on magnetic stirrer.

h. Titrate with 0.02M $CaCO_3$ to a permanent purple end point.

2. Removal of Phosphates

(Required only if product has been dipped, soaked, or injected with or in phosphate solutions.)

a. Initial and regeneration of resin—Mix, in a beaker, approximately 35 g amberlite IRA-93 resin with three 250 mL portions of 5% sodium carbonate.

b. Wash with distilled water until washings indicate by phenolphthalein the absence of base.

c. Treat resin with three 250 mL portions of HCl (3 + 22), mixing thoroughly after each treatment.

d. Rinse with water until color is removed; transfer to chromatographic column with water. (The column is ready for use after water has drained to top of resin. The exchange capacity for phosphate is about 1,500 mg, so a number of aliquots can be passed through the column before regeneration is necessary. Before each use, rinse column with about 250 mL of water until elute is colorless.)

e. Transfer exactly 100 mL of sample solution from section F.1.d to a beaker. Adjust pH to 3.5 with 10% KOH, added drop by drop, using a pH meter and a magnetic stirrer.

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F. ANALYTICAL PROCEDURE (Continued)

f. Pass entire solution through the resin column, at a rate of 2-3 mL/min, into a 250 mL volumetric flask.

g. Wash the beaker and column into the volumetric flask by passing through two 50 mL portions of water, the first at 2-3 mL/min, the second at 6-7 mL/min. Finally, freely pass enough water through column to get 250 mL total eluate. Stopper and mix.

h. Pipet 50 mL aliquot into 400 mL beaker and proceed as in section F.1.f-h.

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G. CALCULATIONS

1. Procedure

$$\% \text{ Calcium content} = C = [A - (B \times R)](0.08)\left(\frac{M}{0.0200}\right)$$

Note: If 10 mL aliquot was used, multiply by 2 to obtain percent calcium content.

$$\% \text{ Bone for poultry} = (C - 0.015)F$$

where

A = mL 0.02M EDTA

B = mL 0.02M $CaCO_3$

0.015 = correction for natural calcium in poultry tissue

F = 6.25 for young chickens

= 4.55 for turkeys and mature chickens

R = EDTA to $CaCO_3$ ratio (from standardization)

M = molarity of EDTA

When analyzing mechanically deboned poultry that includes product from different age groups, calculate the bone multiplier as follows: (% of young chicken)(6.25) + (% of mature chicken)(4.55).

$$\text{Bone content of ''conventionally'' cooked poultry} = \frac{[(C) - 0.015](F)}{1.4}$$

NOTE: Raw deboned poultry contains approximately 23% solids. Conventionally cooked poultry will result in a 30% shrink of the fresh product, yielding approximately 33% solids. The factor 1.4 equates the bone content of conventionally cooked poultry to that of raw deboned poultry.

If inspector designates % solids processed other than by ''conventional'' cooking methods, bone content of such products $= \frac{[(C) - 0.015](F)(23)}{\% \text{ solids}}$

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G. CALCULATIONS (Continued)

For nutritional analyses:

United States Recommended Daily Allowance (USRDA) = 1.0 g

$$\text{mg/serving} = \frac{[A - (B \times R)](0.8\ \text{mg})\left(\frac{M}{0.0200}\right)(f)}{Wt}$$

where

A = mL 0.02M EDTA

B = mL 0.02M $CaCO_3$

R = EDTA to $CaCO_3$ (from standardization)

f = serving size converted to appropriate dimensions (i.e., ounces to grams, etc.)

Wt = weight of sample in aliquot taken

2. Reference

a. Wilson and Co. Method WC-29R1, 11/17/64.

b. JAOAC, 49, 287 (1966).

c. JAOAC, 50, 195, 219 (1967).

d. Hart and Fisher, "Modern Food Analysis," Springer-Verlag, NY (1971).

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H. HAZARD ANALYSIS

1. Method Title

Calcium, Titrametric Determination.

2. Required Protective Equipment

Safety glasses, plastic gloves, and lab coat.

3. Procedure Steps

	Hazards	*Recommended Safe Procedures*
Preparation of KOH-KCN solution	Chemical burn and HCN formation.	Perform mixing with care in a fume hood.
Digestion	Chemical burn and respiratory distress.	Perform digestion in fume hood.
pH adjustment	HCN formation.	Perfrom in fume hood.

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J. QUALITY ASSURANCE PLAN

1. Performance Standard

Compound	*Analytical Range (%)*	*Acceptable Recovery %*	*Repeatability % CV*	*Reproducibility % CV*
Calcium	0.03-0.85†	98-102	± 0.016‡	± 0.022‡

† 3-85 mg calcium in a 10 g sample.
‡ Standard deviation.

2. Critical Control Points and Specifications

Record	*Acceptable Control*
EDTA	EDTA 99% pure—0.200 ± 0.0005M
$CaCO_3$	Primary standard—0.200 ± 0.0005M
EDTA/$CaCO_3$	1:1 (NOTE: The actual ratio must be known within 0.001.)
End point	Color must be purple and persist for at least 1 min.
pH adjustment	pH 12.5 ± 0.2. If solution exceeds 12.7, analyst *must* go back to step F.1.e.
Phosphates removal (only required if carcasses have been soaked in phosphate solutions).	Pass through column—Amberlite IRA-93.
Calculations	Recheck.

3. Readiness To Perform

a. Familiarization.

i. Phase I: Prepare standards, perform standardization.

ii. Phase II: Ten samples replicated on two different days, each analyst.

iii. Phase III: Check samples for analyst accreditation.

b. Acceptability criteria.

See section J.1 above.

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J. QUALITY ASSURANCE PLAN (Continued)

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency:Initially, minimum of 1 check sample biweekly per analyst.

ii. Blind samples or random replicates chosen by supervisor or Laboratory QA Officer after initial analysis.

iii. Records are to be maintained by analyst and reviewed by the supervisor and Laboratory QA Officer.

b. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop all official analyses for that analyst.

ii. Investigate and identify probable cause.

iii. Take corrective action.

iv. Repeat Phase III of section J.3 if cause was analyst-related.

5. Sample Acceptability and Stability

a. Matrices—Mechanically separated (species)—MS(S); mechanically separated (kind)—MS(K).

b. Sample receipt size, minimum: Varied; enough to obtain matrix for all required quantitative results.

c. Condition upon receipt: Not spoiled or rancid, not leaking.

d. Sample storage:

i. Time: Indefinite.

ii. Condition: Frozen.

6. Sensitivity

a. Lowest detectable level (LDL): NA.

b. Lowest reliable quantitation (LRQ): 0.03%.

c. Minimum proficiency level (MPL): 0.03%.