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Title: **Calcium, Inductively Coupled Plasma (ICP) Determination**		
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A. INTRODUCTION

1. Theory

   Calcium is solubilized by acid hydrolysis forming calcium ion. The resulting sample is diluted and the percent calcium is determined by Inductively Coupled Plasma (ICP) Atomic Emission Spectroscopy. The emission of calcium is read at a characteristic wavelength and compared to standard solutions for quantitation.

2. Applicability

   This procedure is applicable to the determination of calcium or bone in meat and poultry products.

B. EQUIPMENT

NOTE: Equivalent equipment may be substituted for the following items.

1. Apparatus
   a. Laboratory fume hood.
   b. Volumetric labware - burets, flasks, pipets, etc
   c. Magnetic stirrer (Corning PC-353).
   d. Hot plate
   e. Filtration funnel and filter paper (Whatman #4).
   f. Boiling beads or chips.
   g. Watch glass - about 80 mm diameter
   h. 200 and 300 ml tall form beakers.
   i. 15 ml polypropylene centrifuge tube
   j. Vortex mixer - Thermolyne Maxi Mix model M-16715 (Thermolyne Corp., Dubuque, Iowa).
   k. Robot-Coupe® - Robot Coupé® U.S.A., Inc., Jackson MS 39236-6627.

2. Instrumentation
   a. ICP, Perkin-Elmer model 3000DV

C. REAGENTS AND SOLUTIONS

NOTE: Equivalent reagents and solutions may be substituted for the following items.

1. Reagents And Solutions
   a. 6N HCl - Carefully mix 1 part HCl and 1 part de-ionized water.
   b. 1N HCl - Dilute 83 $\pm$ 3 mL HCl to 1 L with de-ionized water.
   c. 5% HNO_3 - Add 50 mL of re-distilled HNO_3 to 500 mL de-ionized water. Dilute to 1 L with de-ionized water.

D. STANDARDS

1. QC plus (100 µg/mL) - IV-19 from Inorganic Ventures.
   a. QC standard (25 µg/mL) - Mix 1 mL of QC plus with 3 mL of 1N HCl.
2. Calcium Stock Standard - 10,000 µg/mL, Fisher Scientific.
3. Calcium Calibration Standard Curve - Make a calcium calibration curve at 0, 25, and 50 µg/mL as follows:
   a. 0 µg/mL - Dilute 2 mL of re-distilled Nitric Acid with de-ionized water into a 100 mL volumetric flask. Transfer into 50 mL polypropylene centrifuge tubes.
   b. 25 µg/mL - Pipet 250 µl of 10,000 µg/mL Calcium stock standard into a 100 mL volumetric flask. Add 2 mL re-distilled HNO_3, and bring to volume with de-ionized water. Transfer into 50 mL polypropylene centrifuge tubes.
   c. 50 µg/mL - Pipet 500 µl of 10,000 µg/mL Calcium stock standard into a 100 mL volumetric flask. Add 2 mL re-distilled HNO_3, and bring to volume with de-ionized water. Transfer into 50 mL polypropylene centrifuge tubes.

   Note: Calibration curve levels may be adjusted as needed to bracket samples.
4. Standards Storage: Standards and Reagents may be stored at room temperature for 1 year.

E. SAMPLE PREPARATION

   a. For mechanically separated (MS) poultry sample- Transfer to a 6x12 sample bag. No further processing is necessary.

b. For muscle tissue, pre-desinewer MS beef; and MS pork- Process the sample in a Robot-Coupe® until uniform.

F. ANALYTICAL PROCEDURE

1. Determination

   a. Weigh 10.0 g sample into a 200 or 300 mL tall form beaker.

   b. Weigh 2 additional samples to be used as a blank and recovery. Fortify the recovery tissue with 1 mL of the Calcium stock standard (10,000 µg/mL)

   c. Add 30 mL 6N HCI, several boiling beads, cover with watch glass, and place on hot plate in a fume hood.

   d. Slowly bring to a boil and continue to digest for about 20 minutes.

   e. Cool to room temperature; filter into 200 mL volumetric flask. Rinse the beaker with approximately 40 mL of de-ionized water and add rinse to filter. Wash filter paper twice using approximately 15 mL of de-ionized water each time. Bring to final volume of 200 mL with de-ionized water, stopper, and mix.

   f. Pipet 1 mL of sample filtrate and 3 mL IN HCL into a 15 mL polypropylene tube, and mix well. Samples are now ready for the ICP.

      Note: Dilutions shall be made for sample results outside the linear range of the standard curve.

2. ICP Instrument Settings and Operational Conditions:

   The following are examples only. Parameters may be optimized to achieve maximum performance.

a.	Wavelength:	Ca 317.933
b.	Radial viewing height:	15 mm
c.	Argon torch flow:	15 L/minute
d.	Argon auxiliary flow:	0.5 L/minute
e.	Argon cross flow nebulizer:	0.80 L/minute
f.	Sample pump rate:	1.00 mL/minute
g.	Power:	1300 watts

3. ICP Quantitation Procedure

   Set up ICP according to manufacturer's instructions. Adjust torch position for maximum

response to manganese (Mn) calibration standard (1.0 mg/L). With the same standard perform a background equivalence check (BEC) and a coefficient of variation (CV) test for the manganese calibration standard. The BEC should be < 0.05 and the CV $\leq 2.0\%$ for ten replicates (or within manufacturer's specifications). This procedure should be followed each time the instrument is used.

G. CALCULATIONS

1. Procedure

The percent calcium results are determined from the instrument calibration curve, and reported on the ICP printout.

a. For raw poultry

% Bone = [(% Ca - Reagent Blank) - 0.015] x F

Where:

F (factor) = 6.25 for young chickens

= 4.55 for turkeys and mature chickens

b. For cooked poultry

Cooked poultry = % bone/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 FSIS Inspector designates % solids processed other than by "conventional" cooking methods, then the

$$\text{Bone content of such products} = \frac{[(C) - 0.015\,](F)(23)}{\%\ \text{Solids}}$$

where:

C = % Calcium - Reagent blank

c. $\% \text{ Recovery} = \dfrac{\% \text{ Ca found} - \% \text{ Ca in blank}}{\% \text{ Ca Added}} \times 100$

2. References

a. Calcium/Titrimetric Method, FSIS Food Chemistry Methods Guidebook, Pages CAL1-11, May 1993.

b. Trace Metals II Method, FSIS Nutritional Analysis Methods Guidebook, Pages 18-1 through 18-15, November 1996.

H. HAZARD ANALYSIS

1. Method Title — Calcium, Inductively Coupled Plasma (ICP) Determination.

2. Required Protective Equipment — Safety glasses, plastic gloves, and lab coat.

3. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Digestion	Chemical burn and respiratory distress.	Perform digestion in a fume hood
ICP instruments	Hot, corrosive, and irritating gases are produced.	Use an adequate exhaust vent when in operation.
Nitric Acid Hydrochloric Acid	Corrosive. Contact with fumes or liquid can result in burns and severe skin, eye, and respiratory irritation.	All steps using acids should be performed in a fume hood while wearing acid resistant gloves.

4. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Acids	See Above	All solutions can be neutralized and flushed down the drain according to local, state, and federal guidelines.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	*Analytical Range*	*Acceptable Recovery*	*Acceptable Repeatability (CV)*
Calcium	0-50 μg/ml	70-110 %	≤15 %

Regression coefficient for ICP standard curve: ≥ 0.995

2. Critical Control Points and Specifications

Record	*Acceptable Control*
a. Weight of sample	10.0 g
b. Final volume	4.0 mL

3. Readiness to Perform

a. Familiarization

i. Phase I: Standards- Triplicate standard curves on each of 3 consecutive days, which will include the following:

(a) 0 µg/mL

(b) 25 µg/mL

(c) 50 µg/mL

ii. Phase II: Fortified samples- Triplicate analyses on fortified samples at 0, 0.5, 1 and 2 times the concentration of interest on 3 different days. Alternately, triplicate analyses on previously analyzed samples on 3 different days.

NOTE: Phase I and Phase II may be performed concurrently.

iii. Phase III: Check samples for analyst accreditation.

(a) 12 fortified check samples.

(b) Report analytical findings to the Supervisor and/or Quality Assurance Manager (QAM).

(c) Letter from the QAM is required to commence official analysis.

b. Acceptability criteria.

Refer to section I.1 above.

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: 1 check sample per week per analyst as samples analyzed.

ii. Blind samples or random replicates chosen by supervisor or QAM after initial analysis.

iii. Records are to be maintained by the analyst and reviewed by the supervisor and QAM.

b. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability

a. Matrices -

i. mechanically separated meat and poultry,

ii. liver, kidney, muscle and

iii. meat products with more than 3% meat content.

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

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

d. Sample storage:

i. Time: Indefinite.

ii. Condition: Frozen.

6. Sample Set

a. Sample Set

i. reagent blank

ii. tissue blank

iii. blank tissue fortified at the level of interest.

NOTE: Reagent blank is required to help determine if there is any trace of contamination from glassware or reagent used.

7. Sensitivity

a. Lowest detectable level (LDL): NA.

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

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

J. WORKSHEET

An example of a worksheet, on the following page, can be removed from this book for photocopying.

% Calcium

Analyst: **Balance #**
Date started: **ICP #**
Date completed: **% rec =** **Standards**

rgt. Blank =

1NHCl
6M HCl

Sample I.D.	Sample weight (g)	Sample Volume (mL)	Aliquot (mL)	Dilution Volume (mL)	% Calcium	Corrected Calcium	Calcium mg/100 g

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