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Title: Determination of Sodium and Calcium by ICP Atomic Emission Spectroscopy		
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Contents

A. INTRODUCTION

1. Summary of Procedure

   This method is for the analysis of the nutritional elements sodium and calcium. Inductively Coupled Plasma (ICP) emission spectroscopy is used. The sample is dry ashed, and the resulting decomposed sample is solubilized, diluted, and aspirated into a plasma. The emission elements are read at a characteristic wavelength and compared to standard solutions for quantitation.

2. Applicability

   This method is suitable for the quantification of sodium and calcium in fresh and ready to eat meat products at levels ≥ 3 ppm.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus
   a. Balance - accurate to ± 0.02 g, Sartorius, B1419-52A.
   b. Crucibles - Vycor® transparent, 50 mL, Corning, #1 294050b0.
   c. Muffle furnace and controller - capable of maintaining a temperature of 550 ± 10 °C, Thermolyne, #FA 1740 and #CP53640.
   d. Hot plate - capable of maintaining a surface temperature of 120 ± 10 °C, Thermolyne, #HPA2245M.
   e. Bottles - polyethylene, 125 mL or 250 mL suitable for storing standards, Nalge, #20030004 and #2003008.
   f. Centrifuge tubes - graduated, polypropylene with screw cap, 50 mL, Becton Dickinson Labware FALCON® Brand Blue Max™, #2098.
   g. Centrifuge tubes - graduated, polypropylene with screw cap, 15 mL, Becton Dickinson Labware FALCON® Brand Blue Max™
   h. Ultrasonic cleaner - Branson, 8821 OMT.
   i. Magnetic stirrer - Thermolyne, S7225.
   j. Magnetic stirring bar - Scientific Products, S8314-25.
   k. Stirring rod - polypropylene, Nalge, #61 690010.

l. Dispensers - Repipet®, 5, 10, and 20 mL, Barnstead Thermolyne 3005A, 3010A, and 3020A.

2. Instrumentation

a. ICP spectrometer- Perkin-Elmer Optima 3000 DV, with ICP Winlab Software.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The stability time frame of the solution is dependant on the expiration dates of the compounds used.

1. Reagents

a. Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$) - reagent grade, Mallinckrodt AR ® ACS.

b. Hydrochloric acid (HCl) - concentrated, Mallinckrodt AR®.

c. Nitric acid (HNO_3) - concentrated, Mallinckrodt AR®.

d. Distilled, deionized water 10 megaohm resistivity or better.

2. Solutions

a. $Mg(NO_3)_2$ 6.67% w/v

Using a graduated cylinder, dissolve 66.7 g $Mg(NO_3)_2 \cdot 6H_2O$ in 1000 mL of deionized H_2O and mix thoroughly. Store in a bottle at room temperature.

b. HCI solution, 1.00 N

Using a graduated cylinder, dilute 83.0 mL concentrated HCI to 1000 mL with deionized H_2O. Store in a bottle at room temperature.

c. 50% HNO_3 solution

Using a graduated cylinder, dilute 500 mL HNO_3 with 500 mL with deionized H_2O. Store in a bottle at room temperature.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. The stability time frame of the solution is dependant on the expiration date of the components used. In-house prepared standards shall be assigned an expiration date that is no later than the expiration date of the earlilest expiring component or no later than the stability stated in the method, whichever ends soonest.

1. Standard Information

   a. Sodium ICP Stock Standard (10000 µg/mL) - SCP Science, Champlain, NY.

   b. Calcium ICP Stock Standard (10000 µg/mL) - SCP Science, Champlain, NY.

   c. Yttrium ICP Stock Standard (1000 µg/mL) - SCP Science, Champlain, NY.

2. Preparation of Standard Solution(s)

   a. Yttrium Internal Standard (ISTD) solution:

      i. Yttrium Internal Standard Instrument Solution, 2 µg/mL (to infuse on Instrument using the T-Junction)

         Pipette 2.0 mL of 1000 µg/mL Yttrium stock standard into a 1 L volumetric flask. Dilute to volume with 1N HCl.

      ii. Yttrium Working Internal Standard Solution (to Spiking Samples Directly)

         Pipette 25 mL of 1000 µg/mL Yttirum Stock standard into a 50 mL volumetric flask. Dilute to volume with 1N HCl.

3. Preparation of External Calibration Curve

   Note: Other concentration levels may be used. This is an example of a five point calibration curve. If using the T-Junction to infuse the Yttrium Internal Standard, omit the Yttrium in the calibration curve preparation. Similar calibration solutions can be made for calcium analyses. Standard solutions can be combined if both Na & Ca need to be analyzed at the same time.

   a. Sodium ICP Calibration Standard 1 (0 µg/mL)

      Pipette 0 µL of 10000 µg/mL Sodium stock standard and 100 µL of Yttrium Stock standard into a 50 mL volumetric flask. Dilute to volume with 1N HCL. Mix well.

   b. Sodium ICP Calibration Standard 2 (6 µg/mL)

      Pipette 30 µL of 10000 µg/mL Sodium stock standard and 100 µL of Yttrium Stock standard into a 50 mL volumetric flask. Dilute to volume with 1N HCL. Mix well.

   c. Sodium ICP Calibration Standard 3 (12 µg/mL)

      Pipette 60 µL of 10000 µg/mL Sodium stock standard and 100 µL of Yttrium Stock standard into a 50 mL volumetric flask. Dilute to volume with 1N HCL. Mix well.

   d. Sodium ICP Calibration Standard 4 (24µg/mL)

      Pipette 120 µL of 10000 µg/mL Sodium stock standard and 100 µL of Yttrium Stock standard into a 50 mL volumetric flask. Dilute to volume with 1N HCL. Mix well.

e. Sodium ICP Calibration Standard 5 (48 µg/mL)

Pipette 240 µL of 10000 µg/mL Sodium stock standard and 100 µL of Yttrium Stock standard into a 50 mL volumetric flask. Dilute to volume with 1N HCL. Mix well.

E. SAMPLE PREPARATION

Process the sample until homogeneous.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

a. Weigh 1.00 ± 0.20 g of sample into a 50 mL Vycor® crucible.

Also, weigh an additional 1.00 ± 0.20 g portion of blank tissue for each of the following Quality Control samples as needed:

i. Negative Control

ii. Positive Control - Fortify with 60 µL of the 10000 µg/mL stock standard.

iii. Internal check sample - If required.

b. Prepare a reagent blank to be included with each analytical batch.

2. Extraction Procedure

a. Add 2 mL of 6.67% $MgNO_3$ solution to the samples and swirl gently.

b. Place the sample into a cool (<80 ºC) muffle furnace and raise the temperature of the oven according the following furnace control program:

Furnace Controller Program*

Step 1 Ramp = 3 ºC/min Level = 100 ºC Dwell = 360 min

Step 2 Ramp = 3 ºC/min Level = 150 ºC Dwell = 60 min

Step 3 Ramp = 3 ºC/min Level = 500 ºC Dwell = 480 min

Step 4 Ramp = end

*The sample must not be heated so rapidly that it ignites.

c. Remove the sample from muffle furnace and cool to room temperature.

d. A second ashing step may be required to remove any remaining carbon residue.

e. Add 2 mL 50% aqueous HNO_3 while washing down the sides of the crucible. Swirl gently to dissolve all of the ash.

f. Place samples on a hot plate to remove excess acid;

Caution: Avoid excessive spattering.

g. Replace sample in the cool muffle furnace and raise to 500 - 550°C. Maintain sample at this temperature for 1 hr.

h. Repeat this procedure until a carbon-free ash remains.

i. Remove sample from muffle furnace and cool to room temperature.

j. Add 5mL of 1N HCl and swirl gently to dissolve the ash.

k. Transfer the solution from the crucible to a clean 50 mL test tube using two portions of 1N HCl to a final volume of 50 mL.

l. Fortify sample with 200 µL of Yttrium Working Internal Standard and mix well.

Note: If using the T junction on the instrument omit this step.

m. Analyze sample by using ICP/AES according to section F.3.

3. Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

a. (Optional: In cases where the internal standard is not fortified directly into sample and calibration standards.) Set up ICP sampling pump with a T-fitting to mix ISTD with all solutions analyzed. Flow of ISTD solution should comprise approximately 50% of total input flow.

b. Program system software to quantitate on the basis of response relative to the ISTD concentration.

c. Calibrate the ICP using a ICP using the reagent blank and the calibration standard monitor the following wavelengths:

Wavelength: Na 589.592

Ca 317.993

d. Set up ICP according to manufacturer's instructions. Adjust torch position for maximum response of manganese. Perform a background equivalence check (BEC) and a coefficient of variation (CV) test for the manganese calibration standard (i.e., 1.0 µg/mL Mn). The BEC should be < 0.05 and the CV ≤ 2.0% for ten replicates.

4. Injection sequence/Sample Set

Note: Each sample set must contain one QA sample/20 samples.

a. Calibration Curve

b. Reagent blank

c. Tissue blank

d. Recovery

e. Check sample if needed

f. Samples

G. CALCULATIONS / IDENTIFICATION

Note: Instrument software can be programmed to perform all necessary calculations.

1. Using values for m, b determined for the calibration curve determine selected analyte concentration (CE, in µg/mL) in any extract having a relative response R using:

$$CE\ (\mu g/mL) = CE,\ \mu g/mL = (R-b)/m$$

2. Calculate selected analyte concentrations in digested controls and samples (CS) using:

$$CS\ (ppm) = \frac{CE \times VE \times D}{W}$$

Where

CE = Analyte concentration in final extract, in µg/mL

VE = Final sample extract volume in milliliters

D = Dilution factor (Diluted volume/aliquot volume), if secondary dilution was made.

W = Sample Weight in grams.

3. Manual Calculation

Use linear regression analysis to determine a standard curve (emission vs. concentration)

$$ppm\ (analyzed) = \frac{\mu g\ /mL (from\ curve) \times mL\ (final\ volume)}{g\ (sample\ weight)}$$

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — Safety glasses, plastic gloves, laboratory coat, heat-resistant gloves, and crucible tongs.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
$Mg(NO_3)_2$	Skin, eye, and respiratory irritant.	Use only in chemical fume hood. Wear suitable protective clothing, gloves, and eye/face protection.
HCl, HNO3	Skin, eye, and respiratory irritant. Corrosive. Contact with liquids can result in burns and severe skin, eye, and respiratory irritation.	Prepare solutions in a well-ventilated area such as a fume hood and dispense using repipettors wherever possible. Wear plastic gloves.
Equipment		
Muffle Furnace	HOT surfaces	Wear heat-resistant gloves. Use crucible tongs to remove and insert crucible.

3. Disposal Procedures

   Follow local, state and federal guidelines for disposal.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	*Analytical Range (ppm)*	*Acceptable Recovery(%)*	*Acceptable Repeatability (CV)*
Na	0 - 200	70 -110	≤ 15 %
Ca	0 -100	70 - 110	≤ 15 %

2. Critical Control Points and Specifications

	Record	*Acceptable Control*
a.	Sample weight	1.00 ± 0.20g
b.	Final muffle furnace temperature	500 ± 50 °C
c.	Completeness of ashing	No visible carbon residue
d.	Reagent blank	Absorbance should produce a response ≤ 1.0 ppm.

3. Intralaboratory Check Samples
   a. System, minimum contents.
      i. Frequency: One per week per analyst when samples analyzed.
      ii. Records are to be maintained.
   b. Acceptability criteria.
      Refer to I. 1.
      If unacceptable values are obtained, then:
      i. Investigate following established procedures.
      ii. Take corrective action as warranted.

4. Sample Condition upon Receipt
   Not spoiled or rancid

J. APPENDIX

1. Minimum Level of Applicability (MLA):

   Sodium ≥ 3 ppm

   Calcium ≥ 3 ppm

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Staff