Texas Tech University
Food & Nutrition Laboratory

Mineral Analysis

Combination of AOAC Official Method 985.35 & USDA wet ashing procedure

Note! Please make sure that all glasswares are acid washed with 1:3 HCl (1 part HCl to 3 parts water). ALWAYS WEAR GLOVES and LAB COAT. Change gloves as often as possible.

Make sure that QC Standards (NIST 1546, VPI Beef, VPI Chicken) fall within the range.

Reagents:

5% Lanthanum Chloride ($LaCl_3$)

Add 58.65 g of Lanthanum Oxide (La_2O_3) to 1L volumetric flask (acid washed). Add 400 ml of deionized water dissolve completely. Slowly and cautiously add 250 ml concentrated HCl under the hood) to dissolve the La_2O_3, bring to volume with deionized water. Stir thouroughly with a stir bar. Seal with parafilm.

20 % Nitric Acid (HNO_3)

Under the hood, add 500 ml deionized water to 1L volumetric flask. Slowly add 285.71 ml concentrated HNO_3, bring to volume with distilled water. Stir thoroughly with a stir bar, cover with parafilm.

Sample Stock preparation (applicable for high-fat matrices like chicken, pork and beef):

Place 10 g sample in previously acid washed crucibles.

Dry sample in 100°C oven for at least 16 hours or overnight.

With gloved hands (or use tongs), remove the crucibles from the drying oven and place in muffle furnace for 2 days at 550-600°C.

Turn off furnace and let cool. Ash should be white and free from C.

Add 3 ml deionized water and 3 ml concentrated nitric acid.

Slow dry on hot plate under the hood, set thermostat between 4 & 5. If C particles are still present, repeat adding 3 ml deionized water and 3 ml concentrated nitric acid then dry until all C particles are digested. Cool.
Re-ash in the muffle furnace, set at 550°C for 12-24 hrs. Cool. Remove the crucibles from the muffle furnace.

With eppendorf pipetter, add 10 ml 20% Nitric acid. Let stand for at least 5 hours (at most overnight).

Transfer to 50-ml volumetric flask, rinse crucible with dH20, add washing to vol flask and bring to volume with deionized water. Cover vol flask with parafilm and shake vigorously. Filter through a long stem funnel lined with filter paper (Whatman grade 40; 12.5 mm) into 50 ml centrifuge tube. **This will be the Na, K, Ca, Mg, Zn, P & Fe stock.**

Sample stock can be aspirated directly and diluted when necessary with deionized water.

Determination:

1. Calcium (Ca)

Standard preparation:

Place 0, 125.0, 250.0, 375.0 and 500.0 µl Ca standard (Sigma) into 100-ml volumetric flasks with eppendorf pipetter. Add 5 ml of $LaCl_3$ solution.. Bring to volume with deionized water. Cover with parafilm and shake well.

Unknown preparation:

Note! Vortex sample stock before use.

Place 1 ml of $LaCl_3$ and 20.5 ml deionized water with eppendorf pipetter into pre-labeled 50 ml culture tube. Add a predetermined aliquot of unknown (sample stock).

Cover with parafilm and vortex. Make sure that sample does not come in contact with parafilm while vortexing.

Read standards and unknown on Shimadzu Atomic Absorption Spectrophotometer (refer to instrument manual for operation, settings, etc.) Use 0 (zero) as the blank. Results are expressed in concentration (ug/ml).

Calculations:

$$\text{Ca (mg/100g)} = \frac{\text{concentration (ug/ml) x SV x DF x CF}}{\text{wet weight (g)}} \times 100$$

SV = stock volume (ml)

DF = dilution factor =(total volume of water + $LaCl_3$) / aliquot (ml)

CF = correction factor = 0.10

2. Magnesium (Mg)

Standard preparation:

Place 0, 12.5, 25.0, 37.5, and 50 µl Mg standard (Sigma) into 100-ml volumetric flask with eppendorf pipetter. Add 5 ml of $LaCl_3$ solution. Bring to volume with deionized water. Cover with parafilm and shake well.

Unknown preparation:

Follow Ca procedure.

Read standards and unknown on Shimadzu Atomic Absorption Spectrophotometer (refer to instrument manual for operation, settings, etc.) Use 0 as the blank. Results are expressed in concentration (ug/ml).

Calculations:

Follow Ca Calculations.

3. Potassium (K)

Standard preparation:

Place 50.0, 100.0, 150.00, and 200.0 µl K standard (Sigma) into 100-ml volumetric flasks with eppendorf pipetter. Bring to volume with deionized water. Cover with parafilm and shake well.

Unknown preparation:

Place 20 ml deionized water with eppendorf pipetter into pre-labeled 50 ml culture tube. Add a predetermined aliquot of unknown (sample stock).

Cover with parafilm and vortex. Make sure that sample does not come in contact with parafilm while vortexing.

Read standards and unknown on Shimadzu Atomic Absorption Spectrophotometer **using flame emission** (refer to instrument manual for operation, settings, etc.). Use deionized water as the blank. Results are expressed in concentration (ug/ml).

Calculations:

Follow previous calculations.

4. Sodium (Na)

Standard preparation:

Place 10.0, 20.0, 40.0, and 60.0 µl Na standard (Sigma) into 100-ml volumetric flasks with eppendorf pipetter. Bring to volume with deionized water.
Cover with parafilm and shake well.

Unknown preparation:

Follow K procedure.

Read standards and unknown on Shimadzu Atomic Absorption Spectrophotometer **using flame emission** (refer to instrument manual for operation, settings, etc.). Use deionized water as the blank. Results are expressed in concentration (ug/ml).

Calculations:

Follow previous calculations.

5. Iron (Fe)

Standard preparation:

Place 125.0, 250.0, 375.0 and 500.0 µl Fe standard (Sigma) into 100-ml volumetric flasks with eppendorf pipetter. Bring to volume with deionized water. Cover with parafilm and shake thoroughly.

Read standards and sample stock on Shimadzu Atomic Absorption Spectrophotometer (refer to instrument manual for operation, settings, etc.). Used deionized water as the blank. Results are expressed in concentration (ug/ml).

Calculations:

$$\text{Fe (mg/100g)} = \frac{\text{concentration (ug/ml) x SV x CF}}{\text{wet weight (g)}} \times 100$$

6. Zinc (Zn)

Standard preparation:

Place 25.0, 50.0, 75.0, 100 µl Zn standard (Sigma) into 100-ml volumetric flasks with eppendorf pipetter. Bring to volume with deionized water. Cover with parafilm and shake well.

Unknown preparation:

Follow K procedure.

Read standards and unknown on Shimadzu Atomic Absorption Spectrophotometer (refer to instrument manual for operation, settings, etc.) Use deionized water as the blank. Results are expressed in concentration (ug/ml).

Calculations:

Follow previous calculations

7. Copper (Cu)

Standard preparation:

Place 125.0, 250.0, 375.0, 500.0 µl Cu standard (Sigma) into 100-ml volumetric flasks with eppendorf pipetter. Bring to volume with deionized water. Cover with parafilm and shake well.

Unknown preparation:

Sample stock can be aspirated directly and diluted when necessary with deionized water.

Read standards and unknown on Shimadzu Atomic Absorption Spectrophotometer (refer to instrument manual for operation, settings, etc.) Use deionized water as the blank. Results are expressed in concentration (ug/ml).

Calculations:

Follow previous calculations

8. Manganese (Mn)

Standard preparation:

Place 75.0, 150.0, 225.0, 300 µl Mn standard (Sigma) into 100-ml volumetric flasks with eppendorf pipetter. Bring to volume with deionized water. Cover with parafilm and shake well.

Unknown preparation:

Sample stock can be aspirated directly and diluted when necessary with deionized water.

Read standards and unknown on Shimadzu Atomic Absorption Spectrophotometer (refer to instrument manual for operation, settings, etc.) Use deionized water as the blank. Results are expressed in concentration (ug/ml).

Calculations:

Follow previous calculations

8. Phosphorus (P) – Colorimetric method

Reagents:

Molybdovanadate (prepare under the hood)

Dissolve 40 g NH_4-molybdate·$4H_2O$ in 400 ml hot deionized water and cool. Dissolve 2 g NH_4-metavanadate in 250 ml hot deionized water, cool, and add 250 ml 70% perchloric acid ($HClO_4$). Slowly pour molybdate solution into 2L volumetric flask. Gradually add metavanadate solution with stirring and bring to volume.

Phosphorus Standard solutions:

(1) Stock solution - 2 mg P/ ml. Dissolve 8.7888g KH_2PO_4 in deionized water and dilute to 1L.

(2) Working solution – 0.1 mg P/ ml. Dilute 50 ml stock solution to 1L.

Sample Stock preparation:

Follow previous procedure.

Standard preparation:

Transfer 0, 2, 5, 8, 10 and 15 ml working solution to 100 ml volumetric flasks. With eppendorf pipetter add 20 ml of molybdovanadate, dilute to volume with deionized water. Cover with parafilm and shake well.

Let stand for 10 min, pipette 3 ml of each standard into separate 10 mm culture tube. Read at 400nm on colorimetric spectrophotometer (Genesy 20; please refer to operation manual for settings) using 0 as the blank.

Determine concentration (mg of P/ml) from standard curve.

Unknown preparation:

A - Place 15 ml deionized water into 50 ml culture tubes. Add 3.084 ml of molybdovanadate and predetermined aliquot. Vortex. Let stand for 10 minutes.

B - Pipette 3 ml of A into 10 mm culture tubes. Read at 400 nm on Genesy 20 using 0 as the blank.

Calculations:

$$\text{P mg/ 100 g} = \frac{\text{concentration x DF x 100}}{\text{wet weight (g)}}$$