ANALYSIS OF ASH AND MINERALS
Ash content is a measure of the total amount of minerals present within a food.

Mineral content is a measure of the amount of specific inorganic components present within a food such as Ca, Na, K and Cl.
Importance of Ash Analysis

- Nutritional Labeling
- Quality
- Microbiological stability
- Nutrition
- Processing
Determination of Ash Content

- Ash is inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food.

- Principle:
  - Minerals are not destroyed by heating, and that they have a low volatility compared to other food components.
Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or the various traditional methods.

Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, *e.g.*, dried beef.
Sample Preparation

- Solid food are finely ground and mixed
- Sample with high moisture content are often dried to prevent spattering during ashing
- Sample with high fat content are usually defatted
- Mineral from grinders, glassware or crucible should be prevented to come into contact with sample.
- Deionized water is recommended to avoid incorrect results.
Types of ashing analyses

- dry ashing
- wet ashing
- low temperature plasma dry ashing
Procedure of Dry Ashing

Sample is put in muffle furnace (550-600°C)

Water and volatile compounds are vaporized and organic compounds are burned

Minerals are converted to oxides, sulfates, phosphates, chlorides or silicates
Dry Ashing

- Use a high temperature muffle furnace (500-600°C)
- Water and other volatile materials are vaporized and organic compounds are burned in the presence of oxygen to \( \text{CO}_2 \), \( \text{H}_2\text{O} \) and \( \text{N}_2 \).
- Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates
- The sample is weighed before and after ashing
The ash content can be expressed on either a dry or wet basis.

\[
\% \text{ ash (dry basis)} = \frac{\text{weight after ashing}}{\text{weight before ashing (dry)}} \times 100
\]

\[
\% \text{ ash (wet basis)} = \frac{\text{weight after ashing}}{\text{weight before ashing (wet)}} \times 100
\]
Advantages:
- Safe, few reagent are required, many samples can be analyzed simultaneously, not labor intensive, and ash can be analyzed for specific mineral content

Disadvantages:
- Long time required, muffle furnace are quite costly to run due to electrical costs, lost of volatile minerals at high temperature eg, Cu, Fe, Pb, Hg, Ni and Zn
Wet Ashing

- Primarily used in the preparation of samples for subsequent analysis of specific minerals.
- It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution.
- A dried ground food sample is usually weighed into a flask containing strong acids and oxidizing agents (e.g. nitric, sulfuric acid) and then heated.
Heating is continued until the organic matter is completely digested, leaving only mineral oxides in solution.

Advantages: little loss of volatile minerals due to low temperature used, more rapid than dry ashing

Disadvantages: labor intensive, requires a special fume-cupboard, low sample throughput
Low Temperature Plasma Ashing

- A sample is placed into a glass chamber which is evacuated using a vacuum pump.
- A small amount of oxygen is pumped into the chamber and broken down to nascent oxygen by application of an electromagnetic radio frequency field.
- The organic matter in the sample is rapidly oxidized by the nascent oxygen and the moisture is evaporated because of the elevated temperatures.
Advantages: Less chance of losing trace elements by volatilization
Disadvantages: relatively expensive equipment and small sample throughput
Other Ash Measurements

1. Soluble and Insoluble Ash in Water
   - An index of fruit content of preserves and jellies
   - A lower ash indicates extra fruit is added to fruit and sugar products

2. Ash Insoluble in Acid
   - Use to measure surface contamination of fruits and vegetables, wheat and rice coating

3. Alkalinity of Ash
   - Used as quality index of fruit and fruit juices
The most effective means of determining the type and concentration of specific minerals in foods is to use atomic absorption or emission spectroscopy.

Can quantify to concentrations as low as a few ppm.
Sample Preparation

- The sample need to be ashed using one of the method previously described
  - Reason: to isolate the minerals from the organic matrix surrounding them
- Presence of contaminants in the water, reagents or glassware
  - Recommended to use ultrapure water and/or reagents
  - Use the same glassware and reagents for blank
Gravimetric Analysis

- The element to be analyzed is precipitated from solution by adding a reagent that reacts with it to form an insoluble complex with a known chemical formula.
- The precipitate is separated from the solution by filtration, rinsed, dried and weighed.
- The amount of the element present in the sample is determined from the chemical formula of the precipitate.
Example: the amount of chloride in a solution can be determined by adding excess silver ions to form an insoluble silver chloride precipitate.

- It is known that Cl is 24.74% of AgCl.

Not suitable to determine trace elements in food because the balances are not sensitive enough to accurately weigh small amount of precipitate formed.
Colorimetric Methods

- Rely on a change in color of a reagent when it reacts with a specific mineral in solution which can be quantified by measuring the absorbance of the solution at a specific wavelength using a spectrophotometer.

- Example: yellow-orange color forms when vanadate-molybdate is added to phosphorus containing sample. The phosphorus then can be quantified by measuring the absorbance of the solution at 420nm and comparing with a calibration curve.
Titrations

- EDTA compleximetric titration
  - EDTA is a chemical reagent that forms strong complexes with multivalent metallic ions.
  - The complexes formed by metal ions and EDTA can be represented by the following equations:

\[
\begin{align*}
  m^{2+} + H_2Y^{2-} & \rightarrow mY^{2-} + 2H^+ \\
  m^{3+} + H_2Y^{2-} & \rightarrow mY^{-} + 2H^+ \\
  m^{4+} + H_2Y^{2-} & \rightarrow mY^{-} + 2H^+
\end{align*}
\]
Procedure

Sample is diluted with water

Made alkaline (pH 12.5 to 13)

Indicator is added

Titrate with EDTA
- EDTA forms complexes with minerals first then with the indicator – determine the end point of the reaction.
- The calcium content is determined by comparing the volume of EDTA required to titrate it to the end point with a calibration curve prepared for a series of solutions of known calcium concentration.
Redox Reactions

- Based on coupled reduction-oxidation reactions
- One of the half-reactions leads to a measurable change in the system that can be conveniently used as an end-point e.g. a color change
- E.g. permanganate ion is a deep purple color (oxidize form) while the mangenous ion is a pale pink color (reduced form)
Potassium permanganate is titrated into the aqueous solution of ashed food

\[\text{MnO}_4^- \text{ is converted to Mn}^{2+} \text{ as Fe}^{2+} \text{ still remaining in the food}\]

\[\text{All Fe}^{2+} \text{ converted to Fe}^{3+} \text{ and remaining MnO}_4^- \text{ leads formation of a purple color (end-point)}\]
Precipitation Titrations

- When at least one of a titration reaction is insoluble in precipitate
- Common method: Mohr for chloride analysis
- Silver nitrate is titrated into an aqueous solution containing sample and a chromate indicator
  \[ \text{AgNO}_3 + \text{NaCl} \rightarrow \text{AgCl (s)} + \text{NaNO}_3 \]
- End point: the first hint of orange color
Ion-Selective Electrodes

- The principle is similar with pH meters, but the composition of the glass electrode is sensitive to specific types of ion.
- Principle: ISE only sensitive to one type of ion (free ions)
  - Free ions can be obtained by ashing the food.
- Problem: interference from other types of ions
  - Can be reduced by adjusting pH, complexing or precipitating the interfering ions.
Two electrodes are dipped into an aqueous solution containing dissolved minerals. Concentration of the specific minerals are determined from a calibration curve of voltage versus log of conc.
Atomic Spectroscopy

Principle:
- Each element has a unique electronic structure
- Consequently, it absorbs or emits radiation at specific wavelength
- Absorption: electrons in the ground state are promoted to various excited states
- Emission: electrons in an excited state fall back to a lower energy level
Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is an analytical method that is based on the absorption of UV-visible radiation by free atoms in the gaseous state.
Procedure

Ashed sample is dissolved in an aqueous solution

Placed in the instrument where it is heated to vaporize and atomize the minerals

A beam of radiation is passed through the atomized sample

Absorption of radiation at specific w/length
Schematic Diagram of AAS

- hollow cathode lamp
- atomized sample
- lens
- monochromator
- detector
- readout
- amplifier

Instrumentation

- Radiation Source
  - The lamp emits radiation characteristic of the metal in the cathode
  - i.e. if the cathode is made of sodium, a sodium emission spectrum is produced.
  - When this radiation passes through a sample containing sodium atoms it will be absorbed because it contains radiation of exactly the right wavelength to promote transition from one energy level to another
  - Thus, a different lamp is needed for each type of element analyzed
Chopper

- Radiation arriving at the detector comes from two different sources
  1. Radiation emitted by the filament of the lamp
  2. Radiation that is emitted by the atoms in the sample

- Purpose: To isolate the signal generated by the light from that emitted by the atoms in the sample
Atomizer

- To convert sample to be analyzed into individual atoms
- Atomization process: exposing the sample to high temperatures
- involves 3 stages: water removal, conversion molecules into a gas and atomization
- Ionization of atom is undesirable
Two types of atomizers

- Flame-atomizers consist of a nebulizer and a burner
  - Nebulizer converts the solution into a fine mist or aerosol

- Electrothermal AAS
  - The sample is placed in a small graphite cup which is electrically heated to a temperature (2000 – 3000°C) to produce volatilization and atomization
- **Wavelength selector**
  - To select the radiation of the desired wavelength
  - Typical wavelength selectors: monochromatic or filters
- **Detector**
  - A photomultiplier tube that converts electromagnetic energy reaching it into an electrical signal.
Atomic Emission Spectroscopy

- Atomic Emission Spectroscopy (AES) utilizes the emission of radiation by a sample
- Several ways that energy can be supplied: heat, light, electricity and radio waves
Schematic Diagram of AES
Instrumentation

- The sample itself acts as the source of the detected radiation
- Radiation source or chopper are not needed
- Atomic emissions are produced when the electrons in an excited state fall back to lower energy levels
- Each atom have characteristic emission spectrum as they have different allowed energy levels
- The emitted radiation passed through a w/length selector and the intensity of the peak isolated is measured using detector.
Atomization-Excitation Source

- Two most common sources
  1. Flame - AES: similar to flame AAS
  2. Inductively Coupled Plasma (ICP) – AES: heat the sample in the presence of argon ions
    - Argon ions lead to the release of electrons that push the equilibrium towards the non-ionized form of the mineral
W/length selectors
- To isolate particular spectral lines
- Types of w/length selectors:
  - Filters – can only be used to measure the intensity at a particular fixed w/length (one type of mineral)
  - Gratings – can be used to measure the intensity at many different w/lengths (many different types of minerals)
Practical Consideration

- Sample must be ashed, dissolved in a suitable solvent (water or diluted HCl) before injecting it into the instrument.
- Veg oils can be analyzed by dissolving them in acetone or ethanol and injecting them directly into the instrument.
- Use very pure reagents when preparing samples for analysis.
- Use glassware that is in very clean and dry so that it contains no contaminating elements.
- No interfering substances present (absorb at the same w/length) in the samples.