Chapter-3

ANALYSIS OF FOOD COMPONENTS

❑Outlines:

- Introduction
- Solution preparation
- Moisture determination
- Ash determination
- Crude fat determination
- Crude protein determination
- Total carbohydrate
- Crude fiber determination
- Mineral (Iron)analysis
- Vitamin –A(beta carotene) analysis

Introduction

- Objectives:
  - By the end of the chapter students will be explain solution preparation and proximate analysis.

Cont'd…

- Solution:
  - ✓ A solution is a homogeneous mixture composed of two or more substances.
  - ✓ In such a mixture, a solute is dissolved in another substance, known as a solvent.
- Solute: the substance which dissolves in solution
- Solvent: the substance which dissolves another to form a solution.
- Saturation: it is the point at which a solution of a substance can dissolve no more of that substance and additional amounts it will appear as a precipitate.

Cont'd…

- Super saturation: It refers to a solution that contains more of the dissolved material that could be dissolved by the solvent under normal circumstances.

- Types of solutions are:

1. Percentage solution
2. Molar solution
3. Normal solution

Cont'd….

1. Percentage solution :

I. Weight per Weight % (w/w %)

II. Weight per Volume % (w/v %)

III. Volume per Volume % (v/v %)

I. Weight/weight solution%(w/w)

- This type of solution is rarely if ever prepared in the laboratory since it is easier to measure volumes of liquids rather than weigh the liquid on an analytical balance.

❖ Example :Find the percent by mass in which 41.0 g of NaCl is dissolved in 331 grams of water.

$$\frac{41\ g}{372\ g} \times 100 = 11.0\%$$

Cont'd…

II. Weight per Volume % (w/v %):

- Grams of solute
- 100 mL total solution
- Most common in biology but seldom used in chemistry manuals.
  - Example:

    20 g of NaCl in 100 mL of total solution

    =(20/100) x100

    = 20% (w/v) solution.

Cont'd…

III. Volume per Volume % (v/v %)

Means a fraction with:

- ✓volume of solute in numerator
- ✓ total volume in denominator

- Usually the "solute" here is a liquid as well
- Remember that volume in the denominator is the total volume of the solution.

2. Molar solution:

- Molar solution: it is a solution that contains 1 mole of solute in each liter of solution.
- Molecular weight:

- It is the sum of the atomic weights of all the atoms in a molecule, also called formula weight.

- Mole: it is gram/ molecular weight.
- Molarity:

- Molarity or molar concentration denotes the number of moles of a given substance per liter of solution.

Cont'd…

I.Molarity of solid solute:

- ✓ Example:Preparation of 1 M NaCl solution in 1000ml
- ❖ Calculations:
- ❖ Wt of solute(g)=M(mole/l)X M.wt(g/mole)x v(L)
- ❖ Where:
- ✓ M-the required molarity
- ✓ M.wt-molecular weight of solute
- ✓ V- tolal volume in liters

Example :

- How many grams of NaCl would you need to prepare 200.0 mL of a 5 M solution?

- Mass(g) = M x L x molar mass
- Mass (g) = (5mol/L) (0.2L) (58.44g/mol)
- Mass(g) = 58.44 g

II. Molarity of liquid solute:

- To prepare 1M sollution mole the following steps:

✓ Calculate the molecular weight of the solute

✓ Calculate the molarity of the solute using the following formula:

$$\text{Molarity(M)} = \frac{\% \text{ x Density x } 10}{\text{M wt.}}$$

Cont'd…

To determine how to dilute the stock solution, use the formula:

Formula to calculating dilution:

$$M_1V_1 = M_2V_2 \quad \text{or} \quad C_1V_1 = C_2V_2$$

where:

M_1 = Molarity before dilution
C_1 = Concentration before dilution
M_2 = Molarity after dilution
C_2 = Concentration after dilution
V_1 = Volume before dilution
V_2 = Volume after dilution

Activity-1

- How many milliliters of a 5 M stock solution of NaCl are needed to prepare 100 ml of a 0.4 M solution?

Cont'd…

Example 5:

How many milliliters of a 5 M stock solution of NaCl are needed to prepare 100 ml of a 0.4 M solution?

$$M_1 V_1 = M_2 V_2$$

$$(5) V_1 = (0.4)(100)$$

$$V_1 = 8 \text{ ml}$$

3.Normal solution:

- The definition of a normal solution is a solution that contains 1 gram equivalent weight(gEW.)per liter solution.
- Equivalent weight:

➢ An equivalent weight is equal to the molecular weight divided by the valence (replaceable H ions).

❖ Normal:

➢ A normal is one gram equivalent of a solute per liter of solution.

- Normality:

➢ Normality is the total number of gram equivalents of the solute present per liter of the solution.

How to calculate equivalent weight

- **Eq.wt for acids and bases**

$$\text{Eq.wt} = \frac{\text{M.wt of acid or base}}{\text{No of H+ in acids or OH- in bases}}$$

Examples:

- HCL the MW= 36.5 the EW = 36.5
- H_2SO_4 the MW = 98 the EW = 49
- H_3PO_4 the MW = 98 the EW = 32.7
- NaOH the MW = 40 the EW = 40
- Ca $(OH)_2$ the MW = 74 the EW = 37

- **Eq.wt for salts**

$$\text{Eq.wt} = \frac{\text{M.wt of salt}}{\text{No of cations X valency or No of anions X valency}}$$

- Cont'd…

Examples:

- KCL the MW= 74.55 the EW = (74.55 / 1 X 1) = 74.55
- $CaCl_2$ the MW= 110.98 the EW = (110.98 / 1 X 2) or (110.98 / 2 X 1) = 55.49
- Na_2CO_3 the MW= 105.98 the EW = (105.98 / 1 X 2) or (105.98 / 2 X 1) = 52.99

- **Normality of solid solute**

Examples:

- **Preparation of 1N NaOH solution**
- **Preparation of 1N $Ca(OH)_2$ solution**
- **Preparation of 1N KCL solution**

Calculations:

)=Wt of solute (g)= **N (Eq/l) X Eq.wt X V (L)**

- ***N: the required normality***
- ***Eq.wt: equivalent weight of the solute***
- ***V: total volume in liters***

Procedure:

- Dissolve ***40 g*** of NaOH in a ***1000 ml*** (1 liter) of water to prepare 1N NaOH solution.
- Dissolve ***37 g*** of Ca $(OH)_2$ in a ***1000 ml*** (1 liter) of water to prepare 1N Ca $(OH)_2$ solution.

Cont'd…

- Dissolve ***74.55 g*** of KCl in a ***1000 ml*** (1 liter) of water to prepare 1N KCl solution.

❑Normality of liquid solute:

- To prepare 1N solution make the following steps:

➢ Calculate the equivalent weight of the solute

➢ Calculate the normality of the solute using the following formula:

$$\text{Normality (N)} = \frac{\%\ \text{X density X 10}}{\text{Eq.wt}}$$

Cont'd…

- Calculate the volume of the solute needed to prepare 1N solution using the following formula:

- Subtract the volume of solute from the total solution volume
- Mix both volumes of solute and solvent to reach the total required volume.

Activity-2

- Prepare 1N HCl solution in 1liter

% percent	Density	M.wt
32	1.18	36.5

1.Normality of stock HCl?

2.Volume of HCl?

3.Required distilled water?

Answer:

- **Preparation of 1N HCL solution**

% percent	Density	M.wt
32	1.18	36.5

HCL normality = **10.345**

Volume required from HCL = **96.66** ml

Complete to 1 liter with **903.33** ml distilled water

Cont'd…

Apparatus are Commonly used in Preparation of Solution

Cont'….

Proximate analysis

- Quantitative estimation of Macronutrients is carried out by a method known as proximate analysis.
- Proximate analysis partition the compounds in feed into 6 categories based on chemical properties of the compound.

1.Moisture(water):

- Water is an important constituent of all plant and animal tissues.
- It is determined by drying a feed sample in air oven at 105^{o}C for a specified length of time.
- The loss of wt. is the moisture content of sample.
- The residue left after the removal of moisture is dry matter content of the sample.
- Moisture content is significant in the storage feeds.
- Ingeneral feeds with more than 11% moisture get mouldy &spoiled.

Cont'd…

Moisture Determination:

- **Materials:**

1. Hot air oven
2. Moisture dishes or Petri dishes
3. Desiccators
4. Analytical balance
5. Tongs
6. Grain flour

Cont'd…

- **Procedure:**

- Clean the moisture dish and dry at 130 ^{o}C for 1 hour and keep in a desiccator for about 15 to 20 minutes until it is cooled to ambient temperature
- About 2-5 g of a feed sample is weighed into a moisture dish previously dried and weighed.
- The sample is then dried in an oven for 105^{o}C for 4 hours to a constant weight or over night.
- Cool in a desiccator and weighed.

Calculation of % moisture

- %Moisture

$$= \frac{wt\ of\ sample - wt\ of\ sample\ after\ drying}{Wt\ of\ sample\ taken} \times 100$$

2. Ash determination:

- Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 550^{o}C in muffle furnace for 4hrs.

Materials

- ✓ Analytical balance,
- ✓ desiccator,
- ✓ Crucible
- ✓ muffle furnace,
- ✓ tongs,
- ✓ iron ring

Cont'd…

❖**Procedure:**

- Clean and dry the crusible
- Weight the dry crusible
- 2g of the sample is weight into a pre-weight crucible.
- The crucible is placed into muffle furnace at 550^{o}C for 4hrs or until whitish-grey ash is obtained.
- The crucible is then placed in the desiccator and weight.

- $\%Ash = \frac{wt\ of\ crucible+ash - wt\ of\ crucible}{wt\ of\ sample}$

3. Crude fat determination

- The ether extract of a feed represents the fat and oil in the feed.
- Soxhlet apparatus is the equipment used for the determination of ether extract.

❖ It consist of 3 major components :

1. An extractor: comprising the thimble which holds the sample.

2. Condenser: for cooling and condensing the ether vapor.

3. 250ml flask

Cont'd…

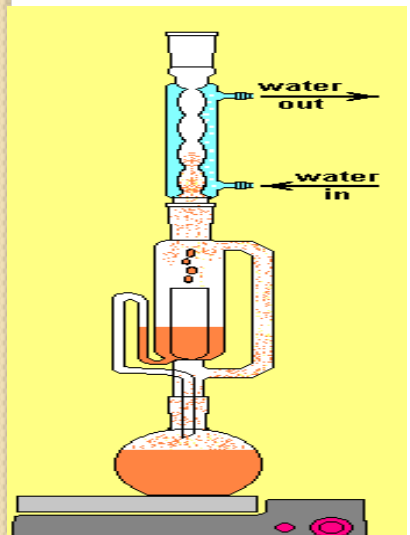
- **Materials are:**
  - ✓ Soxhlet extraction apparatus,
  - ✓ extraction thimbles (whatman cellulose),
  - ✓ desiccator,
  - ✓ evaporating dish,
  - ✓ Cotton
  - ✓ water bath,
  - ✓ analytical balance.
  - ✓ Food samples (oil seed or groundnut or maize flour)
- **Chemical:**
  - solvent (petroleum ether or n-hexane)

❑Procedure:

- ✓ About 150ml of solvent of boiling point of 60-80^{o}C is placed in the flask.
- ✓ About 5g of the sample is weighed into a thimble and the thimble is plugged with cotton wool.
- ✓ The thimble with content is placed into the extractor; the ether in the flask is then heated.
- ✓ As the ether vapor reaches the condenser through the side arm of the extractor, it condenses to liquid form and drop back into the sample in the thimble, the ether soluble substances are dissolved and are carried into solution through the siphon tube back into the flask.
- ✓ The extraction continues for at least 4 hrs.

- The thimble is removed and most of the solvent is distilled from the flask into the extractor.
- The flask is then disconnected and placed in an oven at 65°C for 4 hrs, cool in desiccator and weighed.
- %Crude fat

$$= \frac{wt\ of\ flask + extract - tare\ wt\ of\ flask}{wt\ of\ sample} \times 100$$



4. Crude Protein determination

- Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor.
- Crude protein is determined by kjeldahl method.
- The three basic steps involves:

I. Digestion,
II. Distillation and
III. Titration.

Cont'd…

- **Material/Equipment:**
  - ✓ Digester,
  - ✓ Distiller ,
  - ✓ Digital balance, titrate apparatus and sample
- **Chemicals :**
  - ✓ Concentrated H_2SO_4 (96-98%),
  - ✓ K_2SO_4, Na_2SO_4, $CuSO_4$ or NaOH pellets, HC1, H_3BO_3, bromocresol and methyl red indicator and distilled water

Cont'd….

- **Reagents and their preparations:**

1. *Sodium hydroxide* solutions (40%).
2. Boric acid (4%) with bromocresol green/methyl red indicator.
3. Add 5 ml of indicator solution (0.1% methyl red and 0.2% bromocresol green in alcohol) to 1 litre saturated boric acid solution.

Cont'd…

4. Hydrochloric acid (0.1N)

Chemical	% Percent	Density	M.wt
HCl	32	1.18	36.5

5. catalyst mixture (K_2SO_4 or Na_2SO_4 mixed with anhydrous$CuSO_4$ in the ratio of 10:1

I. Digestion:

- ✓ weigh about 0.1 -1g of the sample into kjeldahl flask and
- ✓ add 10-15mL of concentrated sulphuric acid,
- ✓ Often 1g of catalyst mixture made of Na_2SO_4 or K_2SO_4 with anhydrous $CuSO_4$ in the ratio of 10:1 is used.
- ➢ Na_2SO_4 or K_2SO_4 that speed up the reaction by raising the boiling point of H_2SO_4 and anhydrous$CuSO_4$ to speed the reaction.

- Place the digestion flask in the digester and bring the temperature to 350°C. Digest for about 2 hours.
- In the acid solution, ammonia is not liberated as gas because it exists as an ammonium sulfate salt.
- N (food) $\rightarrow$ $(NH4)_2SO4$
- Remove the flask from the digester and allow it to cool.
- If the digestion is complete, the digested sample is colorless or light blue.
- If it is light brown or yellow the digestion is incomplete

2. Distillation:

- After digestion is completed, it is cooled,
- add carefully 30mL of distilled water into the digestion flask and
- 25mL concentrated NaOH (40%) is added to neutralize the acid and to make the solution slightly alkaline.
- Immediately distil the contents inserting the digestion tube line into the receiver flask that contains 25mL of 4% boric acid solution.
- Collect about 150mL of the distillate.

3. Titration:

- ✓ Titrate the distillate against a standard acid (O.IN HCl).
- ✓ At the end point of the titration color is changed.
- ✓ Record the amount of HCl solution(ml) from burette.

- Cont'd…

$$\%N = \frac{V\,HCl(L)(Vs - Vb)\ \text{x}\,N\,HCl\,\text{x}\,14}{\text{sample wt.}}\ \text{x}\,100$$

Where :

- ✓ *V* is volume of HCI in *L* consumed to the end point of the titration,
- ✓ N is the normality of HC1 used often is about 0.1N and
- ✓ 14.00 is the molecular weight of nitrogen.
- ➢ To % of nitrogen is converted to % of protein by using appropriate conversion factors as follows:

% Protein = F x % N.

Where, F is the conversion factor

5. Total carbohydrate:

- %Total carbohydrate=100% - (H_2O + ash + fat + protein)

Or

%Available carbohydrate=100-(H_2O + ash + fat + protein+ fiber)

6. Crude fiber determination:

-Crude fiber is determined as that fraction remaining after digestion with standard solutions of sulphuric acid and sodium hydroxide under carefully controlled conditions.

- Materials are:
  - Defatted sample
  - Balance
  - Oven
  - Furnece
  - Fiber take plastic
  - Fiber take apparatuses
  - Hot plate

- Chemicals are:
  - ✓ H_2SO_4
  - ✓ NaOH
  - ✓ distilled water
  - ✓ Acetone

Procedure :

- Crude fiber: to measure the crude fiber content of the samples, AOAC (2000) method is followed.
- About 2.0 g of defatted sample is weighed in each fiber take plastic.
- A 1000 ml of 0.255N sulfuric acid solution is added to fiber take and allowed to boil on hot plate for 30 min by rotating and stirring periodically.
- After 30 min, 1000 ml of 0.313N sodium hydroxide solution is added in to fiber take and again allowed to boil for 30 min.
- The solution is then filtered.
- During filtration the sample is washed with hot distilled water.

Cont'd…

- The final residue is washed with:
  - ✓ 1% sulphuric acid solution,
  - ✓ hot distilled water,
  - ✓ 1% sodium hydroxide solution and
  - ✓ finally wash by acetone.

Cont'd…

Drying by oven

- Each of the crucibles with their contents is dried for 2 h at 130^{o}C and cooled in desiccators and weighed (W_1).

Ashing by furnace

- Then ash for 3 h at 550^{o}C in furnace and
- cool in desiccators.
- Finally the mass of each crucible weighed (W_2).

Cont'd…

calculation:

$$\text{Crude fiber (\%)} = \left(\frac{W1 - W2}{W3}\right) \times 100$$

Where:

W_1 = Crucible weight after drying

W_2 = Crucible weight after ashing

W_3 = Sample dry weigh

❖Vitamin analysis:

- Any group of organic compounds required in small amount to perform specific biological functions for normal maintenance of optimum growth and health of organism.
- Classification:

1. Fat soluble vitamins: A, D, E and K
2. Water soluble vitamins: C, B-complex

❑Materials/equipments are :

- ✓ Conical flask
- ✓ Food sample
- ✓ water bath
- ✓ Funnel
- ✓ Beaker
- ✓ Spectrophotometer
- ✓ cuvette

❑ Chemicals are:

- ✓ Ethanol
- ✓ distilled water
- ✓ petroleum ether (pet-ether)

❖Beta carotene determination: procedure:

- Pigment extraction for β-carotene analysis.
- This is carried out according to the method of the Association of Official Analytical Chemists (AOAC, 1980).
- The conical flask contain 50 mL of 95% ethanol,
- 10 gram of the sample place and maintain at a temperature of 70-80°C in a water bath for 20 minutes with periodicaly shaking.
- The ethanol concentration of the mixture bring to 85% by adding 15mL of distilled water and cool in a container of ice water for about 5minutes.

Cont'd…

- The mixture transferee into a separating funnel and 25 mL of petroleum ether (pet-ether) add and the cool ethanol pour over it.
- Swirl gently to obtain a homogenous mixture and it is later allowed to stand until two separate layers obtain.
- The bottom layer is run off into a beaker while the top layer collect in to a 250 mL conical flask.

Cont'd…

- The bottom layer transferee into the funnel and re-extracted with 10 mL pet-ether for 6 times until the extract became fairly red.
- The entire pet-ether collect in to 250 mL conical flask and transferee in to separating funnel for re extraction with 50 mL of 80% ethanol.
- The final extract measure and pour in to sample bottles for further analysis.

❖Measurement of spectrophotometer:

- The absorbance of the extracts measure using a spectrophotometer at a wavelength of 436nm.
- A cuvette containing pet-ether (blank) use to calibrate the spectrophotometer to zero point.
- Extract samples place in cuvette and reading take when the figure in the display window became steady.
- The operation repeat 6 times for each sample and average readings record.

Cont'd…

- The concentration of β-carotene calculate using Bear-Lamberts Law, which states that the absorbance (A) is proportional to the concentration(C) of the pigment.
- represent by the equation: $A \propto L$ (if concentration(C) is constant).

$$A = E * C * L;\ C = \frac{A}{EL}$$

- Where:
- C= concentration of carotene,
- A= absorbance
- E=extinction coefficient
- E of β-carotene =1.25x10^-2 L / µg
- L= thickness of cuvettes (path length) =1cm;

DETERMINATION OF TOTAL IRON IN SOLUTION USING AAS:

- The most common analysis method for the determining the concentration of metal ions in solution uses the atomic absorption spectroscopy.
- **settings:**
- Wavelength: 248.3 nm (Fe)
- **Reagents:**

1. Acetic acid/sodium acetate (AASA) buffer: 0.2M acetic acid, 0.2 M sodium acetate.
2. Standard solution of iron(II):

-Stock solution: 1000 ppm $Fe(NH_4)_2(SO_4)_2$ in AASA buffer

-Working solutions: 2, 4, 6, 8, 10 ppm in AASA buffer

Cont'd…

- 4. Standard solution of total iron:
  - Stock solutions: see 2, 3
  - Working solutions: 2, 4, 6, 8, 10 ppm in AASA buffer

❑ **Procedure:**

1. Prepare AASA buffer for solutions
2. Prepare stock solutions
3. Prepare working standards from stock solution.
4. Measure iron (II) concentration of all standards that contain iron (II)
5. Measure total iron concentrations of all standards, unknown sample and tap water using AAS by following the given instructions.
6. Clean up all glassware and wash AAS instrument by running it with distilled water for10 minutes.
7. Shut down AAS instruments.
8. Turn off gas and air supplies.

Acidity determination :

- **Materials:**

✓ conical flask

✓ pipette

✓ Titration apparatus

✓ A glass rod for stirring the milk in the dish

✓ A Phenolphthalein

✓ 0.1N Sodium hydroxide solution.

✓ Milk sample

Cont'd…

- **Procedure:**
- Nine ml of the milk measured into the conical flask,
- 1 ml Phenolphthalein is added and then slowly flow from the burette, 0.1 N Sodium hydroxide under continuous mixing, until a faint pink colour appears.
- The number of mls of Sodium hydroxide solution divided by 10 expresses the percentage of lactic acid.

- Thank you!!!