FE 271 FOOD CHEMISTRY

EXPERIMENT 6

ANALYSIS OF PROTEINS

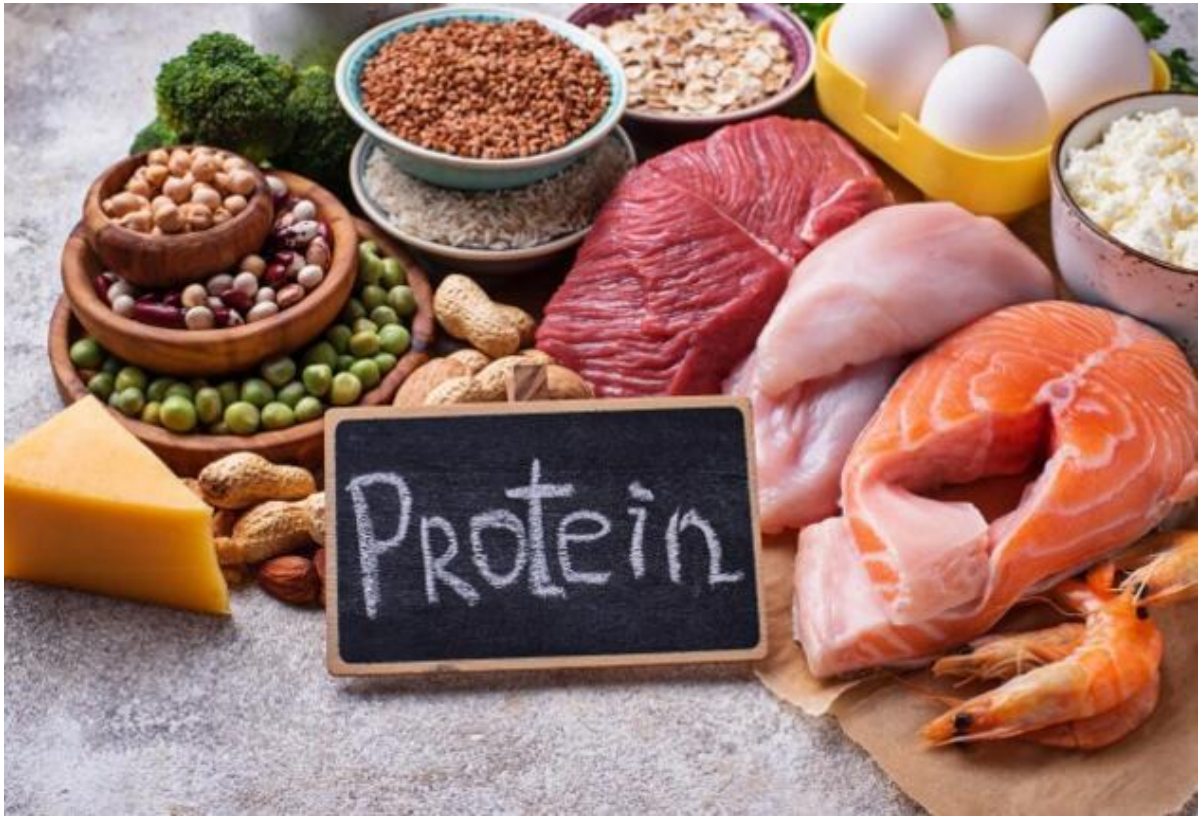
Proteins;

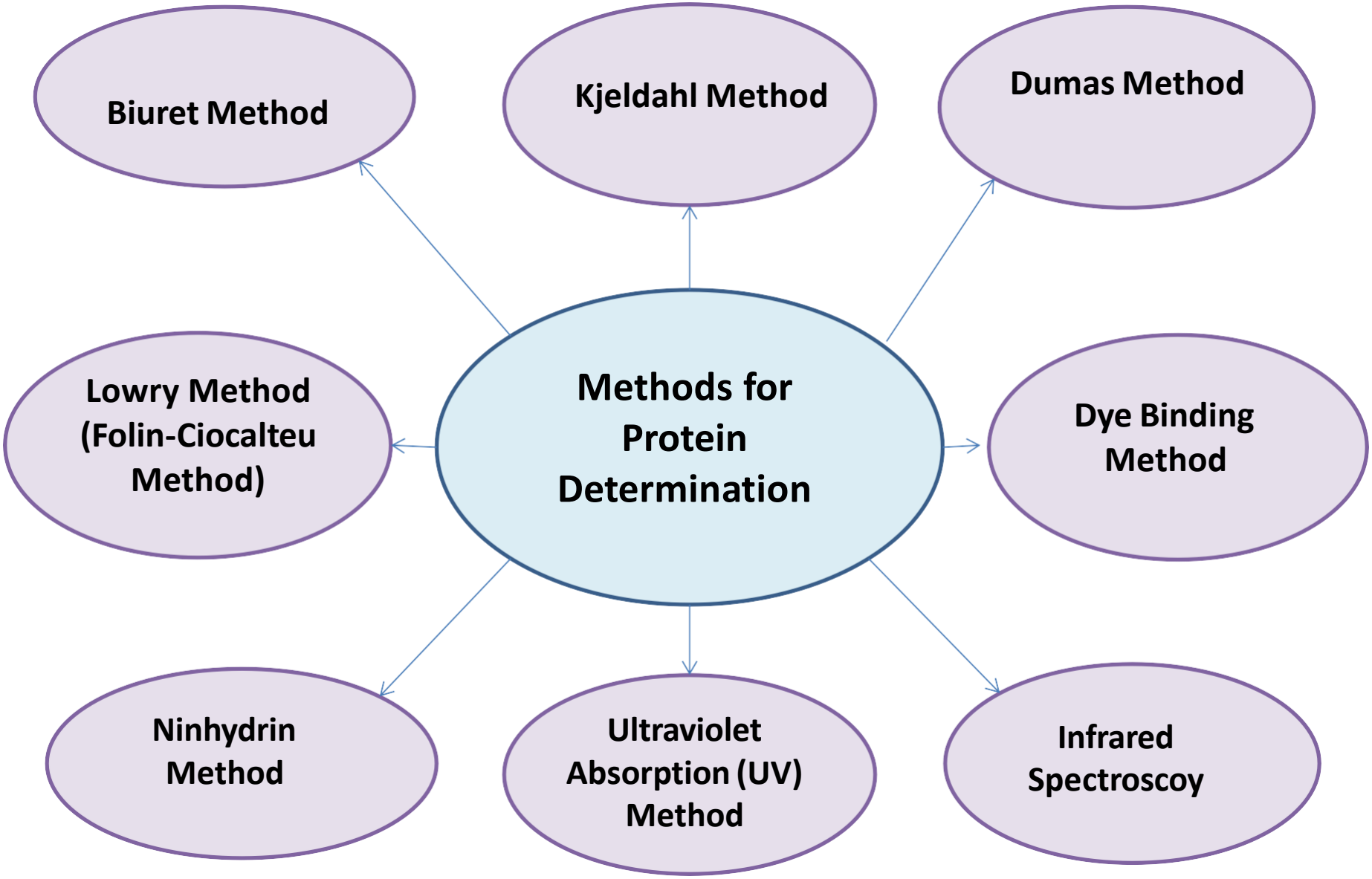
- ✓ essential components of every living cell, and are utilized in the formation and regeneration of tissue.
- ✓ contain nitrogen, hydrogen and oxygen: many contain sulphur, some contain phosphorus, and other elements such as zinc, iron and copper.
- ✓ Protein contents of some foods are seen in below table.

Milk (dry)	22-25 %	Rice	7.5-9 %
Milk (skim)	3.2 %	Wheat flour	9.8-13.5 %
Egg (dry)	35 %	Walnuts	15-21 %
Beef chuck roast	18.5 %	Potato	10-13 %

The aim of the experiment?

To estimate protein content of the sample.




Biuret Method
Kjeldahl Method
Dumas Method
Lowry Method (Folin-Ciocalteu Method)
Methods for Protein Determination
Dye Binding Method
Ninhydrin Method
Ultraviolet Absorption (UV) Method
Infrared Spectroscoy

- The choice of the method depends on several factor:

    a) amount of protein available

    b) concentration of the protein

    c) presence of interfering chemicals

    d) specifity of the assay

    e) ease and reliability

Kjeldahl Method

- This method is based on the conversion of nitrogen to ammonia and is the most common and well-accepted method for determining nitrogen in food. This method is very accurate and precise and composed of three steps:

1) Digestion:

The amino nitrogen in the sample is converted to Ammonium Bisulfate as the organic material in the sample is destroyed by digestion with boiling concentrated sulfuric acid. Potassium sulfate is added to raise the boiling point of the mixture (370-400°C), thus speeding the decomposition. A metal catalyst is also added to accelerate the digestion; i.e. mercury, copper, selenium.

Protein + H_2SO_4 +Heat+Catalyst------> $(NH_4)_2SO_4$ + CO_2 + H_2O + SO_4

2) Distillation: After digestion of sample, excess Sodium Hydroxide is added to the digestion mixture to neutralize the remaining Sulfuric acid and release the ammonia. When the sample containing ammonia, sodium hydroxide and sodium sulfate is heated, the ammonia is driven off as a gas. The Ammonia is distilled over into a Boric acid solution. It reacts with boric acid to form ammonium and borate ion.

$(NH_4)_2SO_4 + 2NaOH$ --------> $2NH_3(g) + 2\ H_2O + Na_2SO_4$

NH3(l) + H3BO3 ----------> NH4 + H2BO3-

3) Titration:

- The Ammonium is then determined by direct titration with a standart acid. We take the ammonium borate solution and titrate it to an endpoint with hydrochloric acid (we must know the normality of the hydrochloric acid used - usually 0.1N). This titration step has an endpoint around pH 5 and is detected by a change in color. This color change is the function of the pH indicator. The indicators used are methylene blue and methyl red.

- $H_2BO_3^- + H^+ \longrightarrow H_3BO_3$

The amount of HCl required to titrate the ammonium borate is equivalent to the amount of ammonium present (1 moles of ammonium contains 1 mole of nitrogen), which equals the moles of nitrogen.

Determination of Protein by Kjeldahl Method

Equipment:

Kjeldahl flasks (500 to 800 mL), Kjeldahl digestion unit with fume removal manifold, Kjeldahl distillation apparatus, erlenmeyer flask , analytical balance.

Reagents:

- Sulfuric acid (concentrated 95-98%),
- Sodium hydroxide,
- Potassium sulfate (K_2SO4),
- Anhydrous Copper sulfate (CuSO4),
- Boric acid H_3BO_3 (4%w/v)
- Methyl red indicator,
- Boiling chips.

Procedure

1) Digestion

- Weigh approximately 1 g ground sample into digestion flask, recording weight (W) to nearest 0.1 mg.

- Add 7 g potassium sulfate, 0.01 g anhydrous copper sulfate. Then add 12 mL sulfuric acid.

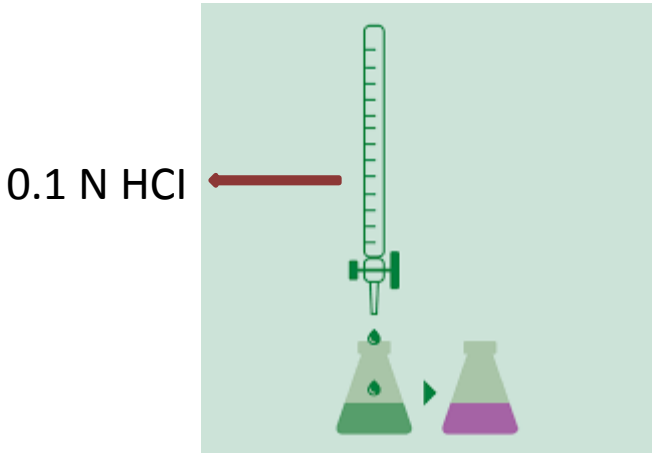
- Place flask on Kjeldahl digestion unit (adjusted to 400°C for 40 min).
- Heat until white fumes clear bulb of flask or clear green color of flask.

Distillation

- Prepare titration flask by adding 25 mL of Boric acid into erlenmeyer flask.
- Immediately connect flask to distillation apparatus and distill at about NaOH=5, H2O=5 boiling rate until at least 150 mL distillate is collected in titrating flask.
- Remove digestion flask and titrating flask from unit, rinsing the condenser tube with distilled water as the flask is being removed.

Titration

The Ammonium is then determined by direct titration with a standart acid. The ammonium borate solution is titrated with 0.1 N hydrochloric acid solution.



Calculation

- 1 mL 0.1 N HCl= 1.4015 mg NH_3
- Amount of g of NH_3 (g) = V (volume of HCl)* 1.4015 mg/ 1000
- **% Protein= g NH_3/g sample*100*factor**

Discussion

! your discussion must be answer these questions;

- ✓ What is your numerical result
- ✓ What is the predicted numerical result for your sample
- ✓ What is the gap between these two results and why it can be occur
- ✓ Why we apply very high temperature in the digestion part?
- ✓ Why we are use boric acid solution in this experiment?
- ✓ What is the importance of the anhydrous copper sulfate in this experiment?
- ✓ What are the critical steps in this experiment?
- ✓ What is the importance of this experiment in food industry

POST LAB QUESTIONS

- Which criteria are important for selection of suitable method for different types of food product?
- Give 5 example of different protein analyses method usage for specific food products.

DATA

Sample name:

Weight of sample:

Volume of HCl:

Factor:

Submitted by:

Group:

Group members:

Submitted to: