Kingdom of Saud Arabia

King Abdulaziz University

Girls Collage of Science

Biochemistry Department

NUTRITION

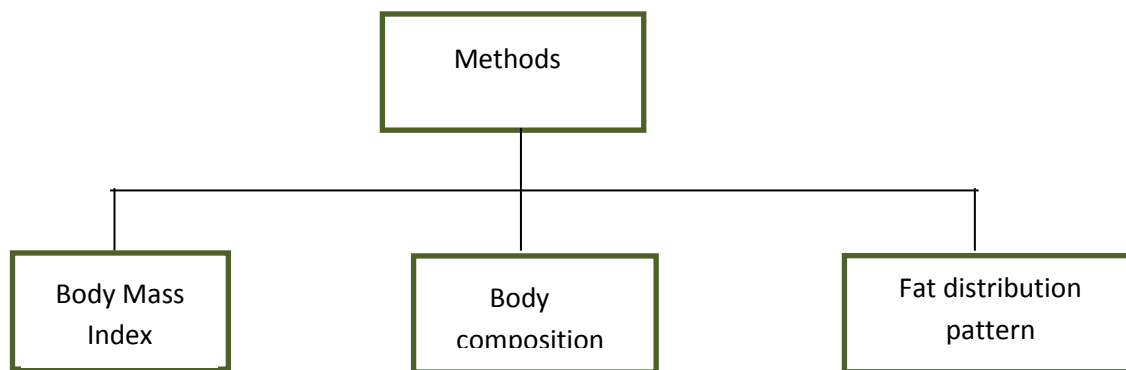# *BIOC 314*

Name:

Computer No:

Section:

Contents

1- Energy Balance and Body Weight Control

Nutritional Assessment

Nutrition assessment evaluates a person's health from a nutrition perspective by:

- History taking.
- Physical examination
- Biochemical analysis.
- Anthropometric measurements.

There are different methods that can help you evaluate the health of your current body weight.

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                    ┌──────────────┐
                    │   Methods    │
                    └──────┬───────┘
          ┌────────────────┼────────────────┐
┌──────────────┐   ┌──────────────┐   ┌──────────────────┐
│  Body Mass   │   │    Body      │   │ Fat distribution │
│    Index     │   │ composition  │   │     pattern      │
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1) Body Mass Index

Body Mass Index (BMI) is a number calculated from a person's weight and height. BMI is used as a screening tool to identify possible weight problems for adults. However, BMI is not a diagnostic tool. For example, a person may have a high BMI. However, to determine if excess weight is a health risk, a healthcare provider would need to perform further assessments. BMI is calculated as:

$$BMI = body\ weight\ (in\ Kg)\ /\ height\ 2\ (in\ m)$$

BMI for adults are interpreted as follows:

BMI	Weight Status	Comment
< 18.5	Under weight	Associated with increased risk of health problems and death
18.5 to 24.9	Normal	Healthy weight-for-height
25 to 29.9	Over weight	
30 to 39.9	Obese	Increased health risk
≥40	Morbid obesity	Major health risk

Note: Adult BMIs should not be applied to children, still growing adolescents,   older people, pregnant and lactating women and high muscular individuals.

Exercise (1): Calculate your BMI? What your value shows?

2) Body Composition:

Body composition is the proportions of muscle, bone, fat and other tissue that make up a person's total body weight. Direct measures of body composition are impossible in living human beings. Instead, researchers assess   body composition indirectly based on the following assumption: body weight = fat + lean tissue (including water).

3) Fat distribution patterns:

To evaluate the health of your body weight, it is also helpful to consider the way fat is distributed throughout your body. This is because your fat distribution is known to affect your risk for various diseases. Some people store fat in upper- body areas whereas others stores fat lower on the body. The picture below shows two types of fat patterning.

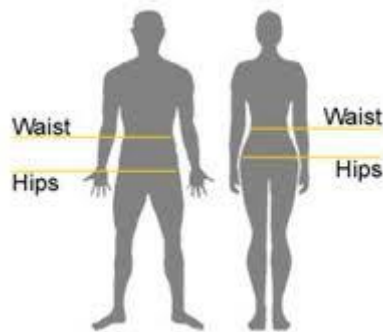Apple shape   Pear shape
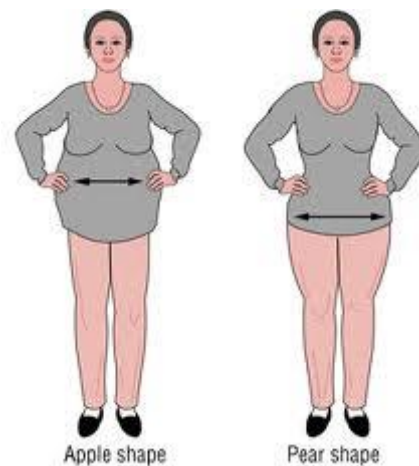
Apple shaped: increases a person's risk for many chronic diseases. • also it causes problems with the metabolism of fat and carbohydrate, leading to unhealthful changes in blood cholesterol, insulin and glucose.

Pear shaped: doesn't seem to significantly increase   a person's risk for chronic disease

To determine the type of fat patterning, flow these steps:

1-Measure your circumference of natural waist in inches (i.e. the narrowest part of your torso from the front).

2- Measure your hip circumference (in inches).

3- Divide the waist value by the hip value. This measurement is   called "waist-to- hip ratio".

A ratio higher than .90 (in men) and higher than .80 (in women) is an apple shaped fat distribution pattern.

Exercise (2): A 45 years old man, his waist circumference is 50 inches and his   hips are 40 inches: calculate his hip-to-waist ratio?

☐ What type of fat patterning does he have?

☐ Is he in risk for chronic disease? Why?

EXERCISE (3):
- Calculate your waist-to-hip ratio?

- What type of fat patterning do you have?

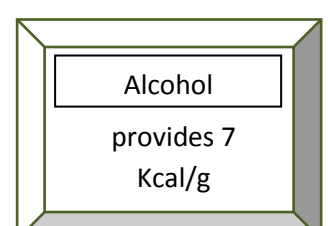- Are you at risk for chronic disease?

Energy Balance

To achieve or maintain a healthy body weight you must pay more attention to the important concept of ENERGY BALANCE. When the energy intake (in the form of food and beverages) equals the energy expended (through metabolism and physical activity) this means energy is balanced.

$$energy\ intake = energy\ expenditure$$
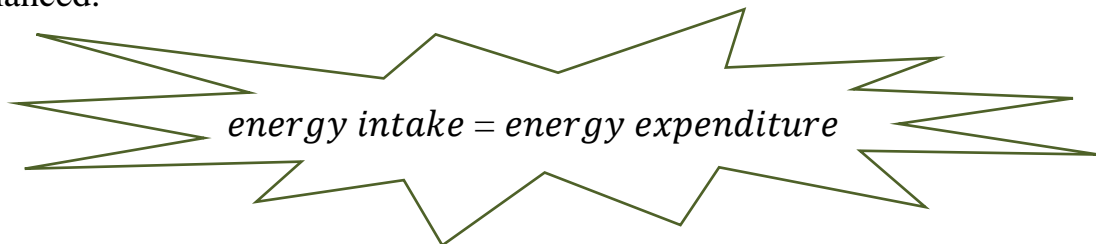
Energy intake

Energy intake is equal to the amount of energy in the food we eat each day. This value includes all foods and beverages and expressed as kilocalories per day (Kcal/day). The energy content of each food is comes from:

Carbohydrates	Protein	Fat	Alcohol
provides 4 Kcal/g	provides 4 Kcal/g	provides 9 Kcal/g	provides 7 Kcal/g
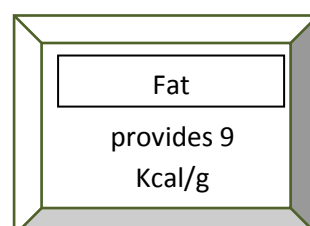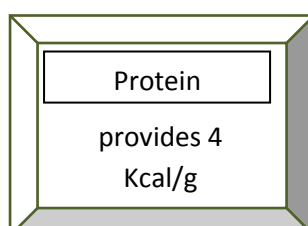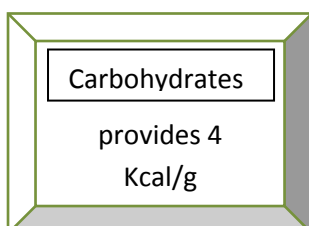
EXERCISE (4):

One cup of oatmeal contains 6 gm of protein, 25 gm of carbohydrates and 2 gm of fat. Calculate the total energy content of this oatmeal?

2 - COLORIMETRIC DETERMINATION OF SUGARS IN DATES: PHENOL-SULFURIC ACID COLORIMETRIC METHOD

Dates are part of a healthy diet. Actually it considered as a whole food likehoney. They contain sugar, fat and proteins, as well as important vitamins.Hence the great importance attached to them by the Prophet.

Dates are also rich in natural fibers. Modern medicine has shown that they are effective in preventing abdominal cancer. They also surpass other fruits in the sheer variety of their constituents. They contain oil, calcium, sulphur, iron, potassium, phosphorous, manganese, copper and magnesium. In other words, one date satisfies the minimum requirements of a balanced and healthy diet.

Dates are rich in several vitamins and minerals. When the level of traceelements falls in the body, the health of the blood vessels is affectedleading to an increased heart rate and a consequent inability to perform itsfunction with normal efficiency.

As dates are also rich in calcium, they help tostrengthen the bones. So, it prevents rickets and Osteomalasia.
Dates are also important in keeping up the health of eyes. It is quite effective in guarding against night-blindness, why?

Principle:

To determine the sugar content of a sample, the sugar solution is placed in a test tube; aqueous phenol and sulfuric acid were added. The heat of dilution completes the reaction in seconds, and after cooling the orange-yellow color is determined in a spectrophotometer.

Materials:

1- Spectrophotometer and colorimetric tubes.
2- Syringe dispenser for sulfuric acid or 5ml cylinder.
3- Pipette 1ml.
4- Blender or mortar.
5- Dates.

6- Phenol 5%: dissolve 50gm of redistilled phenol in water and dilute to 1-L.

7- Sulfuric acid, 96% Reagent grade.

8- Sugar solution, fructose, 0.07g/l.

9- Arabinose.

Method:

Preparation of date's homogenate:

1- Weigh accurately 1 gm of dates.

2- Homogenize in 50ml of distilled water in a warring blender.

3- Boil for 10 min.

4- Cool, and filter in a 100 ml volumetric flask, and complete to volume with water. Shake well to mix. Decolorize with charcoal if necessary.

5- Dilute 1ml of the above solution to 100ml and use it as unknown.

Measuring the sugar content:

1- Prepare sugar standard curve from 10-70 μg/ml (0.01-0.07 mg/ml).

2- Pipette 1ml of aqueous sample solution of sugar into colorimetric tube.

3- Add 1ml of 5% phenol and mix.

4- Add 5ml of sulfuric acid from a fast-flowing dispenser to each tube in order to produce good mixing and even heating.

5- Mix the contents of each tube by the same technique and let stand in air for 10 min.

6- Cool to room temperature.

7- Read the absorbance of the yellow-orange color at 490 nm for hexsose and 480 nm for pentoses.

8- Plot a standard curve of sugar ranging from 10-70 μg of sugar.

Calculation:

Determine the concentration of the sugar sample in gram sugar/gram fruit.

i- From the standard curve you have constructed.

ii- By using the following equation

Since $A_s/C_s = A_{un}/C_{un}$;

$$C_{un} = A_{un} * C_s / A_s$$

Result sheet:

3 - CHEESE: DETERMINATION OF IT'S CHLORIDE CONTENT

Sodium chloride is added to cheese to enhance its taste, and also as a preservative so that some types of cheese will keep well at room temperature. In the industrial preparation of certain cheeses, such as mozzarella, it is important to check that the quantity of salt added is of an optimum concentration.

Principle of Assay:

Sodium chloride is precipitated in acidic solution with excess silver nitrate; the excess unreacted silver nitrate is determined by back titration with a thiocyanate solution.

An iron salt is used as indicator to detect the end-point.The first excess of thiocyanate will react with iron (III) to produce a deep red color. The difference between the excess silver nitrate and that found by titration is equivalent to the amount of sodium chloride in the weight of cheese taken.

Procedure:

1- Weigh accurately a sample of cheese (about 1.5g) into a conical flask.
2- Add 10 ml water and heat the flask to about 75oC in water bath for 10 minutes. Add 25 ml of standard 0.05 M silver nitrate, using apipette. Add 5 ml conc. nitric acid (measuring cylinder and in hood).
3- Digest the cheese curd by boiling gently for 10 minutes.
4- Cool, and add about 50 ml water. Filter quantitatively.
5- Titrate with standard potassium thoicyanate using iron (III) alum (Ferric alum) indicator (2 ml).
6- The end-point is an orange tint, which should persist for fifteen seconds.Record the volume of KSCN as V.
7- The experiment can be repeated with different cheese for comparison. For each calculate the percentage of chloride (expressed as NaCl).

Calculation:

% NaCl = (25 – V ml) x 0.0029 x 100/1.5
Standard AgNO3 : 8.5 g/l ; standard KSCN 5 g/l.

Safety:

Concentrated nitric acid is very corrosive. Wear safety glasses and take greatcare handling the concentrated acid. Safety glasses should be worn throughout the method, especially during the sample preparation.

Result sheet:

4 - ETERMINATION OF FAT IN POTATO CHIPS

Recently, there has been a lot discussion of calories and fats in our foods in the news media. Most food labels list recommended serving sizes, sometimes with unrealistic quantities, of the food product and then lists the nutritional information based on that serving, assuming a 2,000 calorie per day diet.

information based on that serving, assuming a 2,000 calorie per day diet.Nutritional information usually includes calories, total fat, saturated fat, cholesterol, sodium, total carbohydrate (with separate listing of dietary fiber and sugars), and protein. The label information may also include vitamins and minerals.

Nutritionists recommend that no more than 30% of our daily 2000 calories come from fat. However, in our snack food and fast food world, we often eat a diet that contains a larger percentage of fat. So, the question arises, how much fat is in a snack bag of a food such as potato chips, or a standard serving of French fries?

In this experiment, you will determine the fat content of several different brands of potato chips.

(Note: This procedure can also be used for determination of the fat in French fries.)

Safety Precautions:
Wear safety goggles at all times in the laboratory.Hexane is flammable. Keep it away from flames or devices that may spark.

Disposal:
Dispose of all waste material in the proper waste containers.

Materials:

1- Potato chips. An assortment of brands, including regular, low fat,
baked, potato crisps
2- (Pringles, Stax), corn chips, and tortilla chips.
3- Hexane, C_6H_{14}
4- Graduated cylinder, 10 mL
5- Erlenmeyer flask, 250 mL
6- Beaker, 600 mL
7- Small plastic bag, quart size
8- Hot plate
9- Ring stand
10- Utility clamp

Procedure:

1- Obtain between 5 and 10 g of potato chips. Place them in a small plastic bag and crush them.

2- Determine the mass of a clean, dry 250 mL Erlenmeyer flask.

3- Add 5 g of crushed potato chips to the flask and determine the mass of the flask and crushed chips.

4- Measure 10 mL of hexane and add it to the flask containing the crushed chips.

5- Mix the hexane with the crushed chips by gently swirling the flask for about 1 minute.

6- Carefully, pour off the hexane into a waste container, without pouring any pieces of the crushed chips. A small amount of hexane will remain in the flask.

7- Measure 5 mL of hexane and add it to the flask containing the crushed chips.

8- Mix the hexane with the crushed chips by gently swirling the flask for about 1 minute.

9- Carefully, pour off the hexane into a waste container, without pouringany pieces of the crushed chips. A small amount of hexane will remainin the flask.

10- Measure another 5 mL of hexane and add it to the flask containing the crushed chips.

11- Mix the hexane with the crushed chips by gently swirling the flask for about 1 minute.

12- Carefully, pour off the hexane into a waste container, withoutpouring any pieces of the crushed chips. A small amount of hexanewill remain in the flask.

13- Set up a water bath under a hood using a 600 mL beaker on a hotplate. Heat the water to boiling.

14- Using a ring stand and utility clamp to hold the flask, heat the flask,with the crushed chips, in the hot water bath for about 5 minutes toevaporate any residual hexane.

15- Remove the flask from the water bath, allow it to cool and wipeany drops of water from its outer surface.

16- Determine the mass of the flask and the rinsed crushed chips.

17- Calculate the amount of fat in your sample of chips.

18- Share your results with your class.

19- Optional: You may repeat the procedure with another brand ofchips or French fries.

Result sheet:

Data and Calculations;

Brand of chips used:_____

Mass of clean, dry 250 mL Erlenmeyer flask :_____

Mass of a clean, dry 250 mL Erlenmeyer flask and crushed chips: _____

Mass of chips: _____

Mass of flask and the rinsed crushed chips: _____

Mass of fat in the chip sample: _____

Percent of fat in the chip sample: _____

5 - DETERMINATION OF TANNINS IN TEA

The word tannin is very old and reflects a traditional technology. "Tanning" (waterproofing and preserving) was the word used to describe the process of transforming animal hides into leather by using plant extracts from different plant parts of different plant species.

Tannins are complex phenolic compounds responsible for the sensation of astringency and are active in tanning of hide. Many food products contain tannins in their consumable forms e.g.:

• Tea, Cocoa.
• Unripe fruits (apples, cherries, strawberries, bananas)
• Walnuts
• Plant parts containing tannins include bark, wood, fruit, fruit pods, leaves, roots, and plant galls.
• Examples of plant species used to obtain tannins for tanning purposes are wattle (Acacia sp.), oak (Quercus sp.), eucalyptus (Eucalyptus sp.), birch (Betula sp.), willow (Salix caprea), pine (Pinus sp.), quebracho (Scinopsis balansae).

One of the most satisfactory definitions of tannins was given by Horvath (1981):
"Any phenolic compound of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups (i.e. carboxyl's) to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied".

Tannins can complex with:
• Proteins.
• Starch.
• Cellulose.
• Minerals.

Tannins are phenolic compounds that precipitate proteins. They arecomposed of a verydiverse group of oligomers and polymer. There is some confusion about the terminology used to identify or classify a substance as tannin, in fact.

Astringency is the contracting or drying taste, which results from coagulation of the proteins of saliva and the mucous epithelium of the mouth, causing a reduced lubricant action.

Tannins are water-soluble so they are extracted from tea by boiling with water.

Principle:

Ferric chloride reagent gives a color with tannins under acidic conditions. The color is measured spectrophotometrically and compared with the color obtained with a standard tannins solution.

Procedure:

1- Weigh accurately 0.5g of tea.
2- Add 75ml of water and boil for 30 min.
3- Filter in a 100ml-measuring flask and complete to volume with water.
4- Take 1ml of solution; add 1ml of ferric chloride reagent and 8ml of water.
5- The standard is prepared by adding in a tube 1ml of standard, 1ml of reagent and 8ml of water.
6- Read the absorbance of unknown and standard against blank at 540 nm.

Calculations:

Calculate the concentration of tannin in samples by using the equation:
$C_{unk} = A_{unk} \times C_{st} / A_{st}$

Result sheet:

6 - EXTRACTION OF TOTAL LIPIDS BY BLIGH AND DYER METHOD

Lipids form a heterogeneous group of hydrocarboncontainingorganic compounds which categorized by the fact that they are soluble in nonpolar solvents (such as alcohol, acetone, chloroform, benzene, ether and hexane) and are relatively insoluble in water. Lipid molecules have these properties because they consist largely of long hydrocarbon tails which are hydrophobic in nature.

Other than this hydrophobicity, no other common features can be attributed to their
structure. This property of specific solubility is made use of in extracting lipids from
tissues; free from any water-soluble matter but the subsequent analytical methods are
largely individualistic.

For general extraction of almost all lipids from biological samples, either a mixture
of ethanol and ethyl ether or a mixture of chloroform and methanol is used. The lipids
are generally bound to proteins in the biological samples and in that situation
(as lipoprotein) cannot be efficiently extracted by non-polar organic solvent s alone.
The inclusion of methanol or methanol helps in breaking the bonds between the lipids
and proteins.

Procedure:

1- Weigh 2g of sample.
2- Grind it with 10 ml of distilled water
3- Transfer in conical flask with 30 ml of chloroform and ethanol mixture (2 : 1 v/v)
and mixed well.
4- For complete extraction, it is advisable to keep this for 30 minutes at
room temperature in dark place.
5- At the end of this period centrifuge for 10-15 minutes at 2000-3000rpm.
6- Generally 3 layers are seen, a clear lower layer of chloroform containing the entire lipid.
7- The methanol layer is discarded and the lower layer is carefully collected free of inter phase either by sucking out with a fin capillary or by filtration through glass wool.

8- The organic layer "lower layer" is taken in a pre-weighed beaker (W1)and carefully evaporated by keep the sample in warm water bath. It is

also advisable to keep the sample covered with a dark paper to protectfrom light, because some lipids get polymerized or decomposed onexposure to light and heat.

9- When the solution is free of organic solvents, the weight is determined again (W2).

10- The difference in weight gives the weight of lipids (W1 – W2).

11-Express the results in terms of weight in milligrams of Total Lipid pergram of Fresh tissue.

Result sheet:

7 - DETERMINATION OF NICOTINIC ACID IN DRIED YEAST

Principle:

Nicotinic acid is pyridine-3-carboxylic acid, and can be determined by direct titration with carbonate-free 0.1 N sodium hydroxide using phenol red or phenolphthalein as indicator.

Procedure:

1- Weigh accurately about 2g of dried yeast.

2- Grind thoroughly in a mortar with about 10 ml of distilled water; centrifuge.
   Take supernatant liquid for titration or transfer quantitatively through muslin
into a conical flask using further suitable quantities of water (about 40 ml).

3- Add about drops of either phenol red or phenolphthalein.

4- Titrate with 0.1 N NaOH until the end point is reached.

Calculation:

1 ml 0.1 N NaOH = 0.01231g Nicotinic Acid

% Nicotinic Acid = e.p. of titration x 0.0123 x 100/2

Result sheet:

8 - QUALITATIVE ESTIMINATION OF FRUCTOSE IN BIOLOGICAL MATERIAL

Fructose is found mainly is fruits, from which it gets its name, or in honey. Honey
therefore is a form of sugar and cannot be used as a sugar substitute. The amount
of fructose in fruits depends on the degree of ripeness.
As the fruit ripens, some of the stored starch turns to sugar. Highfructose corn syrups
are increasingly being used in processed food products, and contribute a major source
of increases sugar intake. Fructose is the sweetest simplesugars.

Materials:

- Fructose standard solution: 2mg/ml, 2g/l, or 0.2g/l if it is so concentrated you
  can use 1mg/ml).
- Fructose tablets such as Canderel: 1 tablet/100ml.
- Honey: 0.4g/100ml.
- Fruit juice: Make decolorization by charcoal, filtrates, and then dilutes 1ml juice
  with 100ml-distilled water.
- Reagent (A) freshly prepared: 50mg resorcinol (0.05g) dissolved up to 50ml with
  95% ethanol. Store in a dark bottle at 4oC.
- Reagent (B): 50ml conc. HCl is added carefully to 10ml water.
  Spectrophotometer and tubes.

Method:

1- In each tube place the following:
    (2 ml sample - 2 ml reagent A - 6 ml reagent B).

2- Use water as blank.

3- Let stand in a water bath for 8 min. at 80oC.Cool to room temperature and read
at 530 nm.

Calculation:

Fructose % (mg/ml) = Aun/As* Cs x 100

Result sheet:

9- Determination of Vitamin C in Foods

By Iodometric Assay

Definition:

Vit. C is a water soluble vitamin that is necessary for normal growth and development.

Alternative Names:

Ascorbic acid

Food Sources:

Vitamin C is found in green peppers, broccoli, citrus fruits, strawberries, tommatos and white potato, fish and milk contain small amounts.

Function:

1- Promote healthy teeth and gums
2- Helps for absorption of iron
3- Promote wound healing

It is important to consume vitamin C every day since it is water soluble therefore the body excretes it regularly and can not be stored for later use. Recommended daily allowance (RDAs) of vit. C is = 70mg/day.
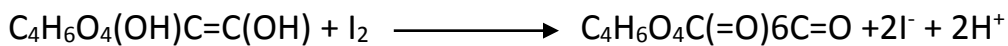
Side Effects:

Deficiency → Scurvy

Increased intake → Diarrhea

Principle:

Vit. C can be assayed by direct titration with iodine. The reaction taking place is the oxidation of vitamin C to yield an alpha diketone compound in the dehydro ascorbic acid compound.

$$C_4H_6O_4(OH)C=C(OH) + I_2 \longrightarrow C_4H_6O_4C(=O)6C=O + 2I^- + 2H^+$$

The vit. C can determined by using iodine as titration and starch as indicator.

Material:

1- Iodine 0.1 N.
2- Standard ascorbic acid 0.1 gm/100 ml.
3- Starch indicator 1%.

Procedure:

1- Measure 10 ml of juice
2- Add 10 drops of starch
3- Titer with 0.1 N iodine → blue color

Calculation:

1 L of 1 N iodine ≡ 88.06 ascorbic acid

1 ml of 0.1 N iodine = 88.06/ 10 x 1000= 0.008806

g% ascorbic acid ≡ Z x 0.008806 x 100/ 10ml (volume of juice)

Z = end point of titration

Result sheet:

Type of juice:

Concentration of vit. C=

10 – Determination of protein in milk using formaldehyde method

Principle:

By adding the formaldehyde to milk, the aldehyde group binds to the free amino group in milk. The media becomes acidic because of the production of carboxylic acidic group.

Protein $\xrightarrow{\text{formaldehde}}$ **acidic + H$_2$O**

When neutralizing the acidity, the amount of amine group can be estimated there for the protein content in milk can be determined.

Materials:

1- NaOH 0.1N
2- Phenol phethaine (indicator)
3- Formalin 40%
4- Milk

Procedure:

1- Pipette 10 ml of milk in a conical flask + drops of ph. Ph.

2- Titer with NaOH unitl pink color produced.

3- Add 2 ml of formalin then shake (pink color disappear)

4- Make blank 10 ml of water + ph.ph. Titer with NaOH to produce pink color.

Calculations:

T-B ×factor

Cow milk factor = 1.67gm%

Result sheet:

11- Estimation of water content in cow's milk

The quantities of the main milk constituents can vary considerably depending on the individual animal, its breed, stage of lactation, age and health status. The average composition of cow's milk is shown in table (1).

Table (1): composition of cow's milk

Main constituent	Range %
Water	85.5 – 89.5
Fat	2.5 – 6.0
Proteins	2.9 – 5.0
Lactose	3.6 – 5.5
Minerals	0.6 – 0.9

As shown, Water is the main constituent of milk. The water can be easily extracted from the sample, and therefore determined, by using acetone. This is because: acetone is miscible with water and both have lower boiling points which mean they will be evaporated before any other major components such as lipids, proteins, carbohydrates and minerals.

Matrials:

1. beakers
2. Milk
3. Acetone
4. Hot plates

Procedure:

1. Pipette 5 ml of milk into beaker and weight it =x1
2. Add 1 ml acetone and evaporate the milk to dryness on a hot plate
3. After complete evaporation of water, weigh the crucible again=x2

Weight of water= weight of crucible with milk (x1) – weight of crucible with dry milk (x2)

Calculations:

$$\text{Water \%} = \frac{(x1 - x2)}{5} \times 100$$

Result sheet:

12- Differences between starch in white and whole-wheat bread

-Both white and whole wheat bread are made from wheat flour.

- Wheat berries contain three parts:

1- barn ⟶ rich in nutrients and fiber

2- endosperm ⟶ contains starch and protiens

3- germ ⟶ rich in vitamins and minerals

White flour uses only the endosperm, while whole-wheat flour uses all the three parts. ⟹ We can conclude that white breads contains more starch than whole-wheat bread.

Principle:

Starch contains amylose and amylopectin which reacts with iodine and gives blue color.

Materials:
1-Iodine.
2-Part from whiten bread.
3-Part from brown bread.

Procedure:
1- Cut pieces of white and brown bread in 2 dishes.
2- 2- Add drop of iodine solution on the bread by droper.
3- Record your observation.

Result sheet: