

DEPARTMENT OF BIOTECHNOLOGY

B.TECH BIOCHEMISTRY LABORATORY (17BTCC81)



HOD

LABORATORY RULES AND REGULATIONS

- 1. Always wear lab Coat while performing the experiment
- 2. Be careful with hot plates, Bunsen burner and other heat source
- **3.** Wear disposable gloves when handling blood and other body fluids, mucus membranes non-intact skin or items and surfaces soiled with blood or body fluids.
- 4. Highly flammable chemicals must be handled in the fume hood.
- 5. Wash hands with soap and water before leaving the laboratory and before eating or drinking
- 6. Use mechanical pipetting devices; mouth pipetting is prohibited.
- 7. Report all spills , accidents or injuries to the instructor immediately
- 8. Properly dispose the broken glassware and other sharp objects (syringe needles) immediately in designated container.
- 9. Wear breathing mask as and when appropriate
- 10. Thoroughly clean the laboratory work space at the end of the laboratory session
- 11. Do not taste or smell hazardous chemicals
- 12. Never add water to acid. Always add acid to water
- 13. Never point the open end of the glassware containing the solution to be heated toward anyone.
- 14. Avoid distracting or startling others when handling hazardous chemicals
- **15.** While heating a solution make sure not to overheat it; therefore, vigorously mix the solution by shaking or stirring.
- 16. Flasks with flat-bottoms or thin walls should not be dessicated
- 17. Do not use any machine that smoke or sparks or appear defective anyway.

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TITRIMETRIC EXPERIMENTS

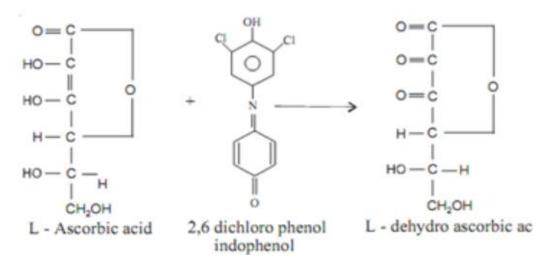
ESTIMATION OF ASCORBIC ACID

AIM:

To determine the amount of Ascorbic acid present in the given solution.

PRINCIPLE:-

Ascorbic acid (vitamin C) reduces 2.6 dichlorophenolindophenol, a coloured dye to colourless dyes. From in acidic medium .The vitamin gets oxidized to dehydroascorbic acid . Though the dye is blue in colour .The appearance of a pale colour indicates the end point of titration.



MATERIALS REQUIRED:-

- 1. Burette
- 2. Conical flask

REAGENTS REQUIRED:-

1. Standard Ascorbic acid stocks solution(1 mg /ml)

Weight 10 ml ascorbic acid and transform at carefully into a 100 ml volumetric flask dissolve vitamin and make up the volume 100 ml with oxalic acid solution.

2. Working standard (0.1mg/ml)

Dilute 10 ml of the stock solution to 100 ml with 4% oxalic acid in a 100 l volumetric flask.

3. Oxalic acid solution 4 %(w/v)

4. Dye solution:

Weigh 26mg of the dye and 21 mg of sodium bicarbonate and transfer them into a 100ml volumetric flask . Dissolve and make up the volume to 100 ml with distilled water filter the reagent before use.

PROCEDURE:

Titration I

Standardisation of Dye solution

Exactly 10 ml of the working standard solution was taken in a clean conical flask and it was titrared against 2.6 dichlorophenol indophenol taken in the burette. The appearance of pale pink colour which persists for few minutes indicates the end point of the titration .The titration is repeated for concordant values.

Titration II

Estimation of ascorbic acid:

The given solution was made up to 100ml with 4% oxalic acid solution, it was then shaken well for uniform concentration, exactly 10 ml of the given solution was taken in a clean conical flask and it was titrated against the dye solution .The end point was the appearance of the pale pink colour which persist for few minutes. The titration was repeated for concordant values, the volume the dye consumed was equivalent to the amount of ascorbic acid in the given solution.

RESULT:

The amount of Ascorbic acid present in the given solution is =

TITRATION I

Standardisation of 2.6 Dichlorophenol Indophenol using standard Ascorbic acid

Indication:-Self

S.N O	Volume of standard	Burett	e reading	Volume of 2.6 dichlorophenol	Concordant value (ml)
	Ascorbic acid (ml)	Initial(ml)	Final(ml)	indophenol consumed (ml)	
1	10	0	X	Х	
2	10	0	Х	Х	X
3	10	0	Х	Х	

End point:-

The appearance of pale pink colour which persist for few minutes

CALCULATION:

1ml of ascorbic acid solution contains 0.1 mg of ascorbic to.

10ml of ascorbic acid solution contains 1 mg of ascorbic acid

Therefore, X ml of 2.6 dichlorophenol indophenol is reduced by 1 mg of ascorbic acid

TITRATION II

Estimation of Ascorbic acid

Indicator :- self

S.N O	Volume of standard	Burett	e reading	Volume of 2.6 dichlorophenol	Concordant value (ml)
	Unknown Ascorbic acid (ml)	Initial(ml)	Final(ml)	indophenol consumed (ml)	
1	10	0	Y	Y	
2	10	0	Y	Y	Y
3	10	0	Y	Y	

CALCULATION:

X ml of 2,6 dichlorophenol indophenol is reduced 1mg of ascorbic acid Yml of 2,6 dichlorophenol indophenol is reduced by = $\underline{Y \times 1}$

= Z mg of Ascorbic acid

Х

 $10\ ml$ of the given unknown solution contains Z mg of Ascorbic acid.

Therefore, 100 ml of the given unknown solution contains = 100 x Z

10

= ____ mg of Ascorbic acid

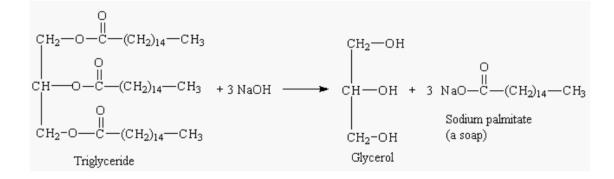
DETERMINATION OF SAPONIFICATION NUMBER OF EDIBLE OIL

AIM:

To determine the saponification number of an edible oil

PRINCIPLE:

Fats (triglycerides) upon alkaline hydrolysis (either with KOH or NAOH) yield glycerol and K+ or NA+ salts of fatty acids (soap). The saponification number is milligrams of KOH required to saponify the fatty acid liberated from 1g of fat.



MATERIALS REQUIRED:

- 1. Reflux condenser
- 2. Boiling water bath
- 3. Reflex condenser
- 4. Burette
- 5. Test compounds (Oil. Butter)

REAGENTS REQUIRED:

- 1. Fat solvent: A mixture of 95% ethanol and ether (1:1v/v)
- 2. 0 5 alcoholic KOH
- 3. 1% phenolphthalein solution in 95% alcohol
- 4. 0.5 N HCL

PROCEDURE: TITRATION: I Standardization of hydrochloric acid:

10 ml of standard potassium hydroxide was pipette out into a clean conical flask and titrated against hydrochloric acid using phenolphthalein as an indicator. The end point is the disappearance of pale permanent pink colour .The titration was repeated for concordant values .From the titre value, the strength of hydrochloric acid was calculated.

TITRATION: II Determination of saponification number:

- Ig of the fat solvent was weighed in a conical flask and dissolved in 3 ml of the fat solvent
- 25 ml of 0.5 N alcoholic KOH was added to it and attached to a reflex condenser .The content was then refluxed for 30 min .
- It was then cooled to room temperature and few drops of phenolphthalein was added to the flask
- > The contents of flaks were titrated with 0.5 N HCL till the pink colour disappear.
- Blank was refluxed with 26 ml of 0.5N alcoholic KOH without fat sample

TITRATION:-1

Standardization of hydrochloric acid :

Indicator :- phenolphthalein

S.NO	Volume of	Burette	reading	Volume of	Concordant
	Standard Potassium hydroxide(ml)	Initial(ml)	Final(ml)	hydrochloric acid consumed(ml)	value (ml)
1.	10	0	Х	Х	
2.	10	0	Х	Х	Х
3.	10	0	Х	Х	

End point:-Disappearance of pale pink colour.

CALCULATION:

Volume of standard potassium hydroxide V_1 = 10ml Normality of standard potassium hydroxide N_1 = 0.5N Volume of hydrochloric acid solution V_2 = Xml Normality of hydrochloric acid solution N_2 =?

$$V_1 N_1 = V_2 N_2$$
$$N_2 = \underbrace{V_1 N_1}_{V_2}$$

TITRATION:-2

Determination of saponification number of oil

S.NO	Volume of solution	Burette rea	ding	Volume of hydrochloric	Concordant value (ml)
	(ml)	Initial(ml)	Final(ml)	acid consumed (ml)	
1.	Blank solutions 3ml of fat solvent+25 ml of 0.5 alcoholic KOH				
2.	3ml of fat solvent +25 ml of 0.5 N alcoholic KOH				
1.	Test solution 1g of oil +3ml of fat solvent +25 ml of 0.5 N of Nalco.KOH				
2.	1g of oil +3ml of fat solvent +25 ml of 05. Alcoholic KOH				

CALCULATION:

Volume of hydrochloric acid $V_2 = X-Y ml$

Saponification values = $\underline{Volume of HCL \times Normality}$

Weight of sample

RESULT:

The saponification value of give edible oil is = g

DETERMINATION OF ACID VALUE OF FATS AND OILS

AIM:

To determination the acid number of the given fat.

PRINCIPLE:

Different fat samples may contain varying amounts of of fatty acids in addition the fats often become rancid during storage and this rancidity is caused by chemical on enzymatic hydrolysis of fats into free and glycerol the amount of free fatty acids can be determined volumetrically by titrating the sample with potassium hydroxide. The acidity of fats and oils in expressed as it's acid value or number which is defined as Mg KOH required to neutralize the free fatty acids present in 1g of fat and oil the amount of free fatty acids present or acid value of fat is a useful parameters which gives as an indicator about the age and extent of its deterioration.

MATERIAL REQUIRED:

- 1. Burette
- 2. Conical flask
- 3. Test compounds (olive oil,butter,coco coconut,oil,etc) fresh and the sample that have been stored at room temperature.

REAGENTS REQUIRED:

- 1. 1% percentage phenolphthalein solutions in 95 percentage of alcohol.
- 2. 0.1N KOH
- 3. Fat solvent (95% percentage ethanol, diethyl ether1:1 v/v)

PROCEDURE:

TITRATION I:

Standardization of potassium hydroxide:

10 ml of standard oxalic acid was pipette out into a clean conical flask and titrated against potassium hydroxide. Using phenolphthalein as a indicator the end point is the appearance of pale permanent pink colour the titration was repeated for concordant value from the titrate value the strength of potassium hydroxide is calculated.

TITRATION II:

Estimation of acid number:

- 5 grams of fat sample was taken in a conical flask 25 ml of fat solvent was added to it it was then Shaken well, few drops of phenolphthalein was added and mixed.
- > The sample was then titrated against 0.1KOH a faint pink colour persists for 20-30 sec.
- > The volume of KOH was used and then it was noted
- Step 1 to 4 with a blank without the fat sample.

RESULT :

The acid no of the given oil is = g.

TITRATION 1

STANDARDIZATION OF POTASSIUM HYDROXIDE

Indicator: phenolphthalein

S no	Volume of standard oxalic acid (ml)	Burette Initial. (ml)	reading Final (ml)	Volume of potassium hydroxide consumed (ml)	Concordant value(ml)
1	10	0	X	X	
2	10	0	X	X	X
3	10	0	X	X	

End point: Appearance of pink colour.

CALCULATION:

Volume of standard oxalic acid solution $V_1 = 10ml$

Normality of standard oxalic acid solution $N_1 = 0.1N$

Volume of potassium hydroxide solution $V_2 = X ml$

Normality of potassium hydroxide solution $N_2 = ?$

$$V_1 N_1 = V_2 N_2$$

$$N_2 = \underline{V_1 \ N_1}$$

TITRATION 2

ESTIMATION OF ACID NUMBER OF OIL

S.No	Volume of solutions (ml)	8		Vol of potassium	Concordant value (ml)
		Initial	Final	hydroxide consumed (ml)	value (III)
1.	Blank solutions 25 ml of fat solvent	0	X	X	X
2.	25 ml of fat Solvent	0	X	X	
3.	Test solution 1.5 g of oil + 25 ml of fat solvent	0	X	Y	Y
4.	2. 5 g oil + 25 ml of fat solvent	0	X	Y	

Calculation:

Volume of KOH solution V2 = Y-X ml

Acid value = vol of KOH xNormality of KOH x Equivalent weight of KOH

Weight of the sample (g)

= g

DETERMINATION OF IODINE NUMBER OF FAT SAMPLE

AIM:

To determine the iodine number of a given fat sample

PRINCIPLE:

Halogens such as iodine, bromine add across the double bonds of unsaturated oil to form saturated halo- products. The extent of halogenations is a measure of the degree of unsaturated in oil. Thus iodine numbers is defined as grams of iodine absorbed by 100 g of fat.

The reaction is conducted with iodine monochloride which reacts with the unsaturated fat .The unreacted iodine monochloride is determined after converting it into free iodine in the presence of potassium iodine .The liberated iodine is titrated against sodium thiosulphate.

R-CH=CH-R' + ICl (excess) \longrightarrow R-CHI-CHCl-R' + ICl (remaining) ICl(remaining) + KI \longrightarrow I₂ + KCl I₂ + 2NaS₂O₃ \longrightarrow Na₂S₂O₆ + 2NaI

MATERIAL REQUIRED:-

- 1. Stoppered bottles
- 2. Burette
- 3. Iodine flask
- 4. Test compounds 2% solution of corn oil ,butter in chloroform.

REAGENTS REQUIRED:-

- Wij's solution : Dissolve 8.5 g of iodine and 7.8 g of iodine trichloride separately in 450 ml of acetic acid and make upto 1L.
- 2. 0.1N sodium thiosulphate
- 3. 15% potassium iodine solution
- 4. 1% starch indicator

PROCEDURE:

TITRATION: I

Standardization of sodium thiosulphate:-

10 ml of standard potassium dichromate was pipetted out into a clean conical flask. About 10 ml of 10% potassium iodine and 5 ml of conc.Hcl was added and titrated against sodium thiosulphate solution till the yellow colour appears. The few drops of starch solution were added and the titrated was continued till the blue colour disappears .The titration was repeated for concordant values. From the titre value ,the strength of sodium thiosulphate acid was calculated.

TITRATION:-II

Determination of iodine number:-

- 10 ml of fat solution was taken into a stoppered bottle to watch 25 ml of wij's solution was added.
- > The stoppered bottle was shaken thoroughly and kept in dark for 1 hr.
- Similarly a blank solutions was prepared in which a fat solution was replaced by chloroform.
- The flask as then taken from the dark and 1 ml of 10% KI was added ,shaken well and titrated against sodium thiosulphate taken in the Burette till the yellow colour appears.
- Then few drops of starch solution were added and the titrated was continued till the blue colour disappears.

TITRATION:-

Standardization of Sodium thiosulphate:

S no	Volume of standard potassium dichromate (ml)	Burette Initial. (ml)	reading Final (ml)	Volume of sodium thiosulphate consumed (ml)	Concordant value(ml)
1	10	0	X	X	
2	10	0	X	X	Х
3	10	0	X	X	

End point :The disappearance of blue colour

CALCULATION:

Volume of standard potassium dichromate $V_1 = 10ml$

Normality of standard potassium dichromate $N_1 = 0.1N$

Volume of potassium sodium thiosulphate $V_2 = X ml$

Normality of sodium thiosulphate $N_2 = ?$

$$V_1 N_1 = V_2 N_2$$
$$N_2 = \frac{V_1 N_1}{V_2}$$

TITRATION:2

Determination of iodine numbers:

S.No	Volume of solutions (ml)	Burette reading		Vol of potassium	Concordant value (ml)
		Initial	Final	hydroxide consumed (ml)	value (IIII)
1.	Blank solutions 10 ml of chloroform+25 ml of solution +10 ml of KI	0	X	X	X
2.	10 ml of chloroform+25 ml of solution +10 ml of KI	0	X	X	
3.	Test solution 10 ml of chloroform+25 ml of solution+10 ml KI	0	X	Y	Y
4.	10 ml of chloroform+25 ml of wij's solution +10 ml KI	0	X	Y	

CALCULATION:

Volume of 0.1 N Sodium thiosulphate used for blank = X ml

Volume of 0.1 N sodium thiosulphate used for sample = Y ml

Iddine Number = $(X - Y) \times x12.7 \times x100$

1000 x Weight of the sample

RESULT:

The Iodine Number of the given fat sample = g

BIOCHEMICAL PREPARATIONS

ISOLATION OF CHLOROPLAST FROM SPINACH LEAVES

AIM: To isolate Chloroplast from spinach leaves MATERIALS REQUIRED:

- 1. Spinach leaves 30 g
- 2. Knife and scissors.
- 3. Cutting board.
- 4. Kitchen blender.
- 5. Muslin cloth.
- 6. Glass beaker.
- 7. 50 ml centrifuge tubes.
- 8. 1.5 ml centrifuge tubes.
- 9. .Micropipette.
- 10. Glass pipette.
- 11. Cooling centrifuges.
- 12. Spectrophotometer.

REAGENTS AND BUFFERS:

- 1x Chloroplast isolation buffer without BSA:- 0.33M sorbitol, 0.1M tris-Cl ph 7.8, 5mM MgCl2, 10mM NaCl, 2mM EDTA.
- 2. 1xChloroplast isolation buffer with BSA (0.1% w/v)
- 3. 40% percoll: 4ml percoll and 6 ml 1x CIB buffer with BSA to make 10 ml of 40% percoll. (Use 10 ml of 40% percoll for 6ml of chloroplast suspension).
- 4. 80% acetone.

PROCEDURE:

- ➤ Wash 30 gms of spinach leaves thoroughly first with tap water and then with distill water.
- Remove the midrib veins of the leaves and cut into small pieces.
- Add 120 ml of 1xCIB buffer with BSA to the cut leaves in a blender. Blend with 2-3 strokes.
- ▶ Filter the blended leaves through 6 layers of muslin cloth.
- > The filtrate is then evenly divided into four 50 ml centrifuge tubes.

- Centrifuge the tubes for 3 minutes at 200xg. A white pellet will be obtained.
- Transfer the supernatant into chilled 50 ml centrifuge tubes and centrifuge at 1000xg for 7 minutes. A green pellet will be obtained.
- > Discard the supernatant and break the green pellet gently by finger tapping.
- Resuspend the pellet in 2ml of 1x CIB buffer with BSA and mix gently by pipetting up and down.
- > Pool the suspended pellet into one centrifuge tube.
- ▶ Preparation of 40% percoll layer: Mix 4 ml percoll with 6 ml of 1x CIB buffer with BSA.
- ➤ Gently overlay 6ml of the chloroplast suspension over this 40% percoll layer.
- Centrifuge at 1700 xg for 6 minutes. The intact chloroplast will sediment to the bottom of the tube as a green pellet and the broken chloroplast will form the upper layer.
- Carefully remove the upper layer of the chloroplast suspension leaving only the pellet containing the intact chloroplast.
- ➤ Mix the pellet with 500 ul of 1x CIB buffer without BSA.

Estimation of chlorophyll concentration

- Add 10 ul of chloroplast suspension to 990ul of 80% acetone solution and mix gently.
- Centrifuge at 3000xg for 2 minutes.
- Take 100ul of the supernatant and transfer into a cuvette and measure the absorbance at 650 nm.
- ▶ Use 100 ul of 80% acetone as blank.
- ➤ Take duplicate OD 650 values.
- Take the average of the two values and estimate the mg/ml chlorophyll concentration using the following formula:

A 650 x 100/36 = mg/ml chlorophyll.

Where A 650 is the absorbance at 650 nm, 100 is the dilution factor and 36 is the extinction coefficient of chlorophyll.

RESULT:

The amount of Chloroplast present in the given leaves = mg

CHEESE PRODUCTION FROM MILK

AIM: To produce cheese from milk

PRINCIPLE:

Cheese is the most diverse group of dairy products and is, arguably, the most academically interesting and challenging. While many dairy products, if properly manufactured and stored, are biologically, biochemically and chemically very stable, cheeses are, in contrast, biologically and biochemically dynamic, and consequently, inherently unstable. Throughout manufacture and ripening, cheese production represents a finely orchestrated series of consecutive and concomitant biochemical events. These, if synchronized and balanced, lead to products with highly desirable flavors and body and texture, but when imbalanced, result in offflavors. Considering that the same raw material (milk) is subjected to a manufacturing protocol whose principles are common to most cheese varieties, it is fascinating that such a diverse range of products can be produced. No two batches of the same variety and indeed no two cheeses are identical. A further important facet of cheese is the range of scientific disciplines involved. Cheese manufacture and ripening involves the chemistry and biochemistry of milk constituents, fractionation and characterization of cheese constituents, microbiology, enzymology, molecular genetics, flavor chemistry, rheology and chemical engineering.

Cheese consists of a concentration of the constituents of milk, principally fat, casein and insoluble salts, together with water in which small amounts soluble salts, lactose and albumin are found. To retain these constituents in concentrated form, milk is coagulated either by means of lactic acid produced by bacteria or by the addition of rennet or by both. A portion of water is removed by cutting, cooking, stirring or draining the curd or by mechanical application of pressure. The cheese may or may not be ripened; the nature of the process depends upon the particular variety of cheese. Cheesemaking consists of the following five stages: 1. Curdling; 2. Pre-cheesing; 3. Heating, forming and saline bathing; 4. Fermenting and ripening; 5. Quality control.

Raw milk Clarification Standardization of fat ratio Pasteurization (10 minute at 72 °C) Cooling (to 35 °C) Addition of starter culture Addition of CaCl₂ (0.045 % in cheese milk) Renneting (at 35 °C, coagulation complete in 90 minute) Cutting (the coagulum is cut into 1-1.5 cm cubes and the curds rested for 5-10 minute) Draining (25-30 minute) Moulding and turning upside down (2 h each) Before adding salt wait to 70-75 °SH Salting (pieces of curd salted with 8 g NaCl/100 g of cheese at 38-40 °C for 2 h) Packaging (cheese blocks placed in tinned cans filled with brine, 8 g NaCl/100 g solution) Ripening and storage (at 5-7 °C for 90 days)

PROCEDURE:

1. Prepare rennet

A. Crush ¹/₄ tablet of rennet and dissolve in ¹/₄ cup of bottles water. OR

B. Liquid rennet is also available. The table can be replaced by an appropriate volume of liquid rennet.

C. Dissolve rennet in ¼ of bottle water

D. Set rennet solution aside to hydrate

E. Do not add rennet solution to milk until indicated.

2. In a pot large enough to heat 1 gallon of milk mix 1.5 tsp of citric acid to1cup of bottled water.

3. Stir until fully dissolved.

4. Pour 1 gallon of cold milk into the pot with the citric acid and mix well .

- 5. Heat this milk to 90°F on medium to medium high heat.
- 6. Once the milk has reached 90° F add rennet to the milk and stir in a top to bottom motion for 30---60 seconds then stop.
- 7. Turn off the heat.

8. Leave the milk aside for 5 - 10 min to form a curd. A longer set will result in firm curd.

9. Stir for 30 sec. At this point curd form into larger masses.

10. Turn off the heat and leave the pot for 10 -15 min.

11. Gently ladle the curds into a cheese cloth – layered colander and drain for 15 min to several hours.

12. Store in refrigerator.

PREPARATION OF CASEIN FROM MILK

AIM:

To prepare Casein from cow's milk.

PRINCIPLE:

Casein is the main protein found in milk and is present at a concentration of 35g in liter. It is actually a heterogeneous mixture of phosphorus containing protein and not a single compound. Most protein show minimum solubility at its isoelectric pH and this principle is used to isolate the casein from milk by adjusting to its isoelectric pH(4.6).Casein is also insoluble in ethanol and this property is used to remove unwanted fat from the preparation.

MATERIALS REQUIRED:

- 1. Milk
- 2. Acetic acid
- 3. Test tube
- 4. Beaker
- 5. Whatmann No 1 filter paper

PROCEDURE:

- ➤ Take one test tube of milk in 100mL beaker.
- Add equal volume of water & 2% acetic acid in drops till max precipitates occur.
- Stir well with glass rod without breaking the casein precipitate formed.
- Maximum precipitation of casein occurs at pH 4.6(isoelectric point).
- The precipitate is allowed to settle and the supernatant is filtered through Whatmann No 1 filter paper.
- > Pressing it between the filterpaper dries the precipitate.
- > Weigh the Casein and calculate the percentage yield of protein.

CALCULATION:

Weight of the filter paper (X)	=	8
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Weight of the paper with the substance $(Y) = ____g$

The amount of casein present in milk = Y-X g

RESULT:

The amount of casein present in the given sample = _____g

ISOLATION OF STARCH FROM POTATO

AIM: To isolate starch from potato

PRINCIPLE: Starch from potato is isolated by extraction of water followed by filtration.

REAGENTS REQUIRED:

Fresh cleaned potato.

PROCEDURE:

- 100g of peeled potatoes was weighed and cut into small pieces.
- It was then grounded using motor and paste with water.
- The homgerate was the transferred to a 500ml beaker.
- The pulverized materials was then allowed to settle, starch rapidly settle at the bottom.
- The supernatant was carefully decanded.
- The residue was washed twice with distilled water and finally with ethanol.
- It was the filtered muslin cloth and air dried.

CONFIRMATORY TEST FOR STARCH:

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1	Molisch's test to inolate	A purple ring appears at the	Presence
	starch solution and ml of	junction two layer	carbohydrates
	test		
2	Iodine test to isolate starch	Appearance of blue colour	Presence of
	solution acid 1ml iodine		starch
	solution		

CALCULATION:

Weight of potato $= \underline{g}$

Weight of watch glass = _____g

Weight of watch glass

+ Starch = _____g

Therefore weight of Starch = _____g

100g of potato contains = $\underline{\text{Amount of starch}}$ x 100

Weight of potato

<u>**RESULT</u>**: Amount of Starch present in the given sample = g</u>

FOOD ANALYSIS

DETERMINATION OF MOISTURE CONTENT

AIM:

To determine the moisture content of the given food sample.

PRINCIPLE:

Sample is dried at 125 degree Celsius for 3 hours in hot air ovenexample is dried at 125 degree Celsius for 3 hours in hot air oven the loss in the weight is reported as present moisture.

MATERIAL REQUIRED:

- **1.** Hot air oven with air circulation.
- 2. Analytical balance.
- 3. Wheat flour or cereals
- 4. Dessicator containing silicone gel or fused calcium chloride.
- 5. Moisture dish with lid.
- 6. Porcelain crucible.

PROCEDURE:

- 1. Food sample was weighted and transferred into a preweighed moisture dish, the sample was then spread evenly.
- 2. The sample was died in a hot air oven for 3 hours at 125 degree Celsius after drying the moisture dish was covered with the lid and the dish was then transferred into a dessicator to cool the content.
- 3. The moisture dish was weighted rapidly after 30 minutes of dessicator and the weight was recorded, the percentage of moisture content was calculated.

CALCULATION:

Percentage of moisture = $(B-C) \times 100$

А

Where,

A= Weight of the the sample in grams.

B= weight of the dish, plus sample prio prior to drying.

C= weight of dish, plus sample after drying.

(b-c) = drop in weight due to loss of moisture

RESULT:

The percentage of moisture in the given food sample =

=

DETERMINATION OF ASH CONTENT

AIM: TO determine the ash content of the give food sample

PRINCIPLE:

The ash content of the food stuff represent its inorganic constituents after the organic and volatile material have been oxidized completely during the process of incineration at 600°C in a muffle furnace.

MATERIALS REQUIRED:-

- 1. Corn flour
- 2. Muffle furnaces
- 3. Analytic balance
- 4. Desiccator
- 5. Silica or porcelain crucible

PROCEDURE:-

- ▶ Food sample was weighed into a crucible of know weighed.
- The temperature of the muffler furnace was food sample was placed into the muffled furnace after attaining the set of temperature using a metal long .The sample was unsaturated for 2 hours.
- > The crucible was transferred into a dedicatorand cooled at room temperature. The crucible was weighed along with ash to prevent moisture absorption . The process of incineration was repeated with a content weight is obtained.

CALCULATION:

% Ash content (B-C)/A×100

Where

- a= weight of the sample in grams
- b= (weight of the crucible)+(content weight of ash)
- c= weight of the empty crucible

(b-c)=weigh of ash

RESULT:-

The percentage of ash content in the give food sample =

