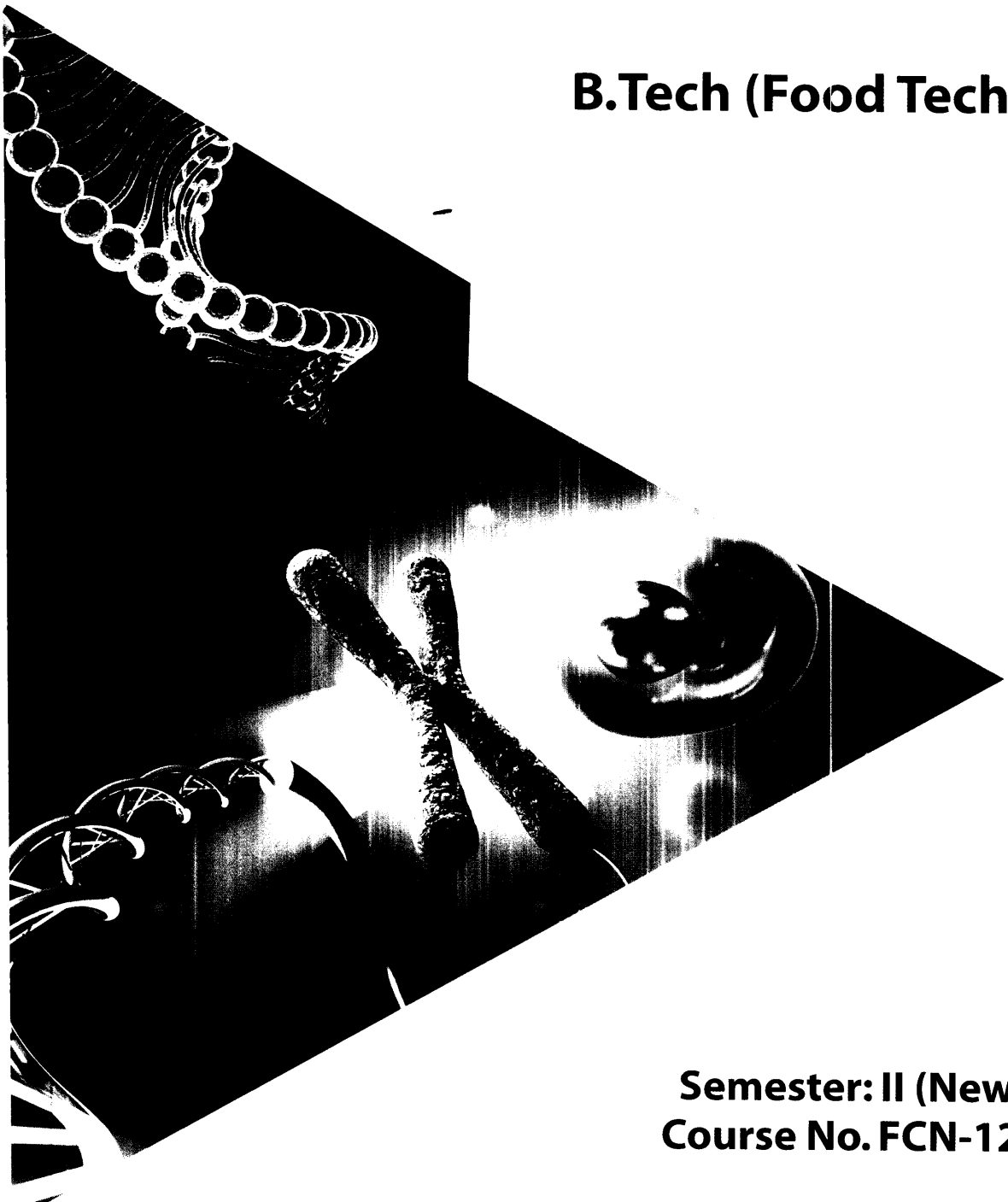
Practical Manual

Food Chemistry of Macronutrients

B.Tech (Food Technology)



Semester: II (New)
Course No. FCN-124

COLLEGE OF FOOD TECHNOLOGY
Vasantrao Naik Marathwada Krishi Vidyapeeth
Parbhani-431 402 (M.S.)

Prepared by

Dr. H.M. Syed (Head, Dept. of Food Chemistry & Nutrition)
Prof. P.U. Ghatge (Assistant Professor, Dept. of Food Chemistry & Nutrition)
Dr. B.A. Jadhav (Research Associate, Dept. of Food Chemistry & Nutrition)
Mr. R.V. Salve (Ph.D. Scholar, Dept. of Food Chemistry & Nutrition)
Mr. M.D. Sontakke (Ph.D. Scholar, Dept. of Food Chemistry & Nutrition)
Mr. K.A. Syed (Ph.D. Scholar, Dept. of Food Chemistry & Nutrition)

Published by

Associate Dean and Principal
College of Food Technology
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani

DEPARTMENT OF FOOD CHEMISTRY AND TECHNOLOGY
COLLEGE OF FOOD TECHNOLOGY
VNMKV, PARBHANI

Certificate

This is to certify that Shri/Ku. _____

Reg. No. _____ has completed the practical of Course No. FCN-124 (Food Chemistry of Macronutrients) as per the syllabus for B.Tech. (Food Technology) first year II semester as prescribed by MCAER, Pune.

Date: / / **Course Teacher**

INDEX

Expt. No.	Experiment	Page No.	Date	Signature
1	Determination of Moisture Content by Hot Air Oven Method	1		
2	Determination of Moisture Content of Liquid Foods by Karl Fischer Method	2		
3	Studies on Sorption Isotherm	4		
4	Preparation of different gel system	6		
5	Preparation of Emulsion and Determination of Emulsion Stability	7		
6	Isolation of Protein from different Food Sources	9		
7	Preparation of Protein Isolate/Concentrate	10		
8	Isolation of Starch of given sample	12		
9	Studies on different Properties of Starches	14		
10	Determination of Total Sugar in Food	16		
11	Estimation of Reducing Sugar in Food	18		
12	Determination of Physical Properties of Fat	20		
13	Determination of Acid Value of Oil	23		
14	Determination of Iodine Value of Oil	24		
15	Determination of Saponification Value of Oil	26		
16	Test for Detection of Different Oils (Baudouin Test, Halphens Test, Hexabromide Test)	28		

Determination of Moisture Content by Hot Air Oven Method

Objectives:

This test is used to determine the water content of a food materials by drying a food sample to constant mass at a specified temperature. The water content of a given food sample is defined as the ratio expressed as a percentage between Difference in the weight of sample after drying to the weight of food sample.

Principle: The sample is dried in an oven at 105°C temperature till the constant weight is obtained. Weight loss is calculate in per cent.

Glassware : Dishes (nickel, stainless steel, aluminum or porcelain), Glass Petri Plates, Desiccator
Apparatus : Hot Air Oven, weighing balance

Procedure:

1. Dry the empty dish lid in an oven for 15 minutes and transfer it to desiccator to cool. Weight the empty dish on weighing balance
2. Mix the prepared sample ground powdered thoroughly and transfer accurately 5 to 10 g to the dish.
3. Place the dish along with lid in the oven avoiding the contact of the dish with the walls of the oven. Dry the sample for four hours at 105°C temperature.
4. Remove the dish from the oven, replace the lid and cool it in a desiccator and weigh again after cooling.
5. Dry for a further hour to ensure that constant weight has been obtained.

Observation:

1. Wt. of empty dish with lid = A
2. Wt. of the dish, lid + sample = B
3. Wt. of the dish, lid + sample = C
   After drying
4. Loss in weight = (B-C)
5. weight of the sample taken = (B-A)

Calculation:

$$\text{Moisture percentage} = \frac{\text{Initial Weight (B)} - \text{Final Weight (C)}}{\text{Weight of sample (B-A)}} \times 100$$

Result :

EXPERIMENT - 2

Determination of Moisture Content of Liquid Foods by Karl Fischer Method

Objectives:

This is the method of choice for determination of water in many low moisture foods such as dried fruits and vegetables, roasted coffee, oils and fats, or any low-moisture food high in sugar or protein.

Principle:

The water in the sample is titrated by means of Karl Fischer reagent which consists of sulphur dioxide, pyridine and iodine in anhydrous methanol. This method is based on reduction of iodine by sulphur dioxide in the presence of water. Methanol and pyridine are used to dissolve iodine and sulphur dioxide. For each mole of water, one mole of iodine, one mole of sulphur dioxide, three moles of pyridine and one mole of methanol are required.

Reagents:

1. Dry methanol containing 11 % pyridine
2. Sodium acetate trihydrate
3. Karl Fischer reagent
4. Methylene blue

Apparatus: Karl Fischer apparatus, Titration vessel

Procedure:

1. Weigh one g of sample into a pre-dried round bottom 50 ml flask.
2. Add 40 ml of methanol into the flask quickly to disperse the sample.
3. Place it on the heating range and connect the reflux condenser.
4. Gently boil the contents of the flask under reflux for 15 min.
5. Remove the flask from heat and allow to drain for 15 min with condenser still attached.
6. Remove and stopper the flask.
7. Pipette 10ml aliquot of the extract into the titration vessel and titrate with Karl Fischer reagent till green colour appear.
8. Note the volume of Karl Fischer reagent used in titration.
9. Determine a blank titrate by taking 10 ml methanol.
10. Standardize Karl Fischer reagent against the water of crystallization of hydrated sodium acetate.

Standardization of Karl Fischer reagent:

1. Accurately measure the moisture per cent of hydrated sodium acetate by oven drying at 120°C for 4 hrs.
2. Weigh accurately 0.4 g of hydrated sodium acetate into a pre-dried 50 ml round bottom flask
3. Add 40 ml methanol into flask and stopper immediately.
4. Swirl the content.
5. Titrate 10 ml of this solution and 10 ml of methanol blank with Karl fischer reagent.

$$\textbf{Calculation Water equivalent of Karl Fischer reagent (F) (mg water/ml)} = \frac{W \times W_1 \times 2.5}{S - B}$$

Where

W =Weight of sodium acetate (g).

W_1 = Water per cent of sodium acetate.

S =Volume of reagent used for titration of standard (ml).

B =Volume of reagent used for titration of blank (ml).

$$\textbf{Moisture (\%)} = \frac{F \times (V_1 - V_2)}{W_1} \times 100$$

Where

F= Factor i.e. water equivalent of Karl Fischer reagent.

V_1= Volume of reagent used for sample (ml).

V_2 = Volume of reagent used for blank (ml).

W_1= Weight of sample (g)

Results:

Sample No.	Weight of sample =W_1	Volume of Karl Fischer reagent used		Factor	Moisture (%)
		For the sample V_1	For blank V_2		
1.					
2.					
3.					

Note: standardized Karl Fischer reagent daily by use of sodium acetate

Protect the reagents, titration flask, burette from atmospheric moisture

Studies on Sorption Isotherm

Objective:

It describes the thermodynamic relationship between water activity and the equilibrium of the moisture content of a food product at constant temperature and pressure.

Introductions:

Sorption isotherms is highly important in food science and technology for the design and optimization of drying equipment, design of packages, predictions of quality, stability, shelf-life and for calculating moisture changes that may occur during storage. Several preservation processes have been developed in order to prolong the shelf-life of food products by lowering the availability of water to micro-organisms and inhibiting some chemical reactions. The typical shape of an isotherm reflects the way in which the water binds the system. Weaker water molecule interactions generate a greater water activity, thus, the product becomes more unstable. Water activity depends on the composition, temperature and physical state of the compounds.

Measurement of sorption isotherms

For food products, the sorption isotherm can be measured by means of three different measuring techniques: gravimetric, manometric or hygrometric.

In the Gravimetric Methods, the weight of the sample is measured with a balance.

In the Manometric Methods, the vapour pressure of water is measured when it is in equilibrium with a sample at given moisture content.

In the Hygrometric Methods, the equilibrium relative humidity with a sample at a given moisture content is measured.

Two examples of more modern techniques to measure the concentration in a sample are the impedance spectroscopy technique and the light reflection and/or attenuation technique, such as the infrared spectroscopy.

Mathematical models of sorption isotherms

The most common equations that are used for describing sorption in food products are the Langmuir Equation and Oswin Model.

Langmuir Equation: Langmuir proposed the following physical adsorption model on the basis of unimolecular layers with identical and independent sorption sites, and which is expressed as it is shown in equation.

$$A_w \left[\frac{1}{M_w} - \frac{1}{M_o} \right] = \frac{1}{C M_o}$$

Observation:

Where,

M_w is the equilibrium moisture content (kg water/kg dry matter),

M_0 is the monolayer sorbate content (kg water/kg dry matter)

C is a constant.

Oswin Model:

It is an empirical model that consists in a series expansion for sigmoid shaped curves

The Oswin equation was used to relate the moisture content of fat free dry milk and freeze dried teaup to a water activity of 0.5

$$M_w = C \left[\frac{a_w}{1-a_w} \right]^n$$

Observations :

Results:

EXPERIMENT - 4

Preparation of different Gel System

Introductions:

Gelis an intermediate between a solid and liquid possessing both elastic (solid) and flow (liquid) characteristics. A sol (liquid) to gel (solid) transition takes place when gels are formed.A food gel can also be considered as a high moisture threedimensional polymeric network that resists flow under pressure and more or less retains their distinct structural shape.Gellification is the phenomenon involving the association or crosslinking of the polymer chains to form a three-dimensional network that traps or immobilizes water within it to form a rigid structure.

Starch Gel :

Materials: Corn starch, Whatman no. 1 filter papers, Distil water

Apparatus: Hot Water Bath

Procedure:

1. Take 10 g Starch in 100 ml of distilled water.
2. Samples were heated at specific temperatures (ranging from 35 to 90°C) for 30 min in 250 ml conical flasks in a water bath.
3. Samples were filtered through Whatman no. 1 filter papers, and the residue was freeze-dried.
4. Excess water was removed and clear starch gel was formed.

Observations :

1. weight of sample -

Results:

Preparation of Emulsion and Determination of Emulsion Stability

Introductions:

An emulsion is a dispersion in which the dispersed phase is composed of small globules of a liquid distributed throughout a liquid vehicle in which it is immiscible. In emulsion terminology, the dispersed phase is the *internal phase* and the dispersion medium is the *external* or *continuous phase*. Emulsions with an oleaginous internal phase and an aqueous external phase are oil-in-water (o/w) emulsions. Conversely, emulsions having an aqueous internal phase and an oleaginous external phase are termed water-in-oil (w/o) emulsions. Because the external phase of an emulsion is continuous, an oil-in-water emulsion may be diluted or extended with water or an aqueous solution. A water-in-oil emulsion can be diluted with an oil or an oil-miscible liquid.

A)Preparation of emulsions by manual processes (Using acacia Gum)

1.Formulation

Soybean oil	=	13 ml
Acacia (fine powder)	=	3.1 g
Distilled water	=	30-40 ml
Final volume	=	50 ml

2. The acacia powder is triturated with soybean oil in a perfectly dry porcelain mortar. After the oil and acacia have been thoroughly mixed, based on an oil:water:acacia ratio of 4:2:1, 6.5 ml of water is added and the mixture is titrated immediately in one direction until a creamy white primary emulsion is formed with a cracking sound generated by the movement of pestle. 40 ml of distilled water is then added to the primary emulsion and the emulsion is transferred to a graduate and made to the final volume of 50 ml with distilled water.

3) Examine the particle-size distribution of the emulsion under an optical microscope, record the particle size data, and determine the maximum and the average particle size.

B) Preparation of emulsion with the mechanical dispersion method (Using soy lecithin)

1. Formulation

Soybean oil	=	10 ml
Lecithin solution	=	25 ml
Distilled water	=	30-40 ml
Final volume	=	50 ml

2. Preparation of the soy lecithin solution: 1.1g of soy lecithin is thoroughly triturated with 1.8ml of glycerin in a mortar. The resultant mixture is further triturated with a small quantity of water and is subsequently diluted to 25 ml with distilled water.
3. Soybean oil, soy lecithin mixture and distilled water are combined in the mixing cup of a mechanical homogenizer. After homogenizing at 8000-12000 rpm for 1 min, hold the product for 1 min, and homogenize the product for an additional 1 min at the same speed.
4. Examine the morphology and particle-size distribution of the emulsion under an optical microscope, record the data, and calculate the average particle size.

Emulsion Stability:

Trial 1: Take 7 ml oil in the test tube. Add 3 ml of water to it. Stir it continuously on vertex mixer till get emulsion.

Trail 2: Take 7 ml oil in the test tube. Add emulsifier and mix it well .Add3 ml of water and stir continuously till get uniform emulsion and record the observation.

Trial 3: Observe 2 types of margarine products i.e. with emulsifier and without emulsifier and record the observation

Results :

Isolation of Protein from different Food Sources

Objective: To obtain pure form of protein compounds.

Introductions:

Protein is required for the growth, maintenance and repair of human body tissue, and is one of the essential building blocks of life. Groundnut is a rich source of protein. It is also a rich source of amino acids and vitamins. Biological value is 55%, protein efficiency ratio: 1.65%, net protein utilization: 43%. Groundnut has good digestibility in both raw and roasted formsIt supplies about 5.6 calories grain, when consumed raw and 5.8 calories grain when consumed roasted.

Material and Chemicals: Defatted groundnut oil cake, Sodium/Potassium Hydroxide, Sulphuric Acid

Apparatus: Centrifuge machine

Procedure:

Extraction of protein from groundnut oil cake:

1. The protein of defatted groundnut oil cake was obtained by alkaline extraction at room temperature by varying the pH from 6.8 to 10
2. For each extraction, take 50 g of different fractions of defatted groundnut cake and 1 lit.of water was used along with NaOH (0.2 M)/KoH (0.2 M) as appropriate for the various extraction.
3. The mixture was stirred at 1200 rpm for 1 hr. at 30°C and subsequently centrifuged at 3000 rpm for 20 min to remove the insoluble carbohydrate residues.
4. The supernatant was collected and the pH was adjusted to 4.5 with 1N H_2SO_4 to precipitate the proteins.
5. The precipitate was creamy white in color. Further, it was centrifuged at 5000 rpm for 15 min to recover the proteins
6. Washed repeatedly with water to free it from acid tinge.
7. Neutralized to pH 7 using sodium salt.
8. Air dried to obtain Proteins.

Observation: 1. Weight of sample :
2. Weight of protein obtained:

Result:

EXPERIMENT - 7

Preparation of Protein Isolate/Concentrate

Objective : To Prepare protein rich food product from soybean

Introductions:

Soya protein products have become increasingly popular because of their low price, high nutritional quality and versatile functional properties. Two important soybean protein products are soy protein concentrate (SPC) and soy protein isolate (SPI). SPC is defined as an edible protein product with a protein content of at least 65% on dry weight basis, whereas SPI is a product with at least 90% protein on dry weight basis. Currently, solvent-extracted white flakes (typically containing 50% protein) are generally the starting materials for SPC and SPI preparation. Other soybean meals or flours besides white flakes may also be used as starting materials provided that the final products meet protein content specifications and demonstrate desired functional properties.

Material & Chemicals :

Soyabean Flakes / Flour, Ethyal Alcohol, Sodium Hydroxide, Hydrochloric Acid

Apparatus : Vacuum Oven, Centrifuge Machine, Freezer, Refrigerator

Procedure: Procedure for soy protein concentrate (alcohol-wash method).

Soybean flakes/ flour

↓

Add 60% w/w aq. Ethyl alcohol(10:1) EA: SF

↓

Mix it throughly (40^0C for 40 min.)

↓

Centrifuge at 14000 rpm for 30 min 15^0C Supernatant

↓

Protein fiber

↓

Desolventize (40^0C,overneight in fume hood)

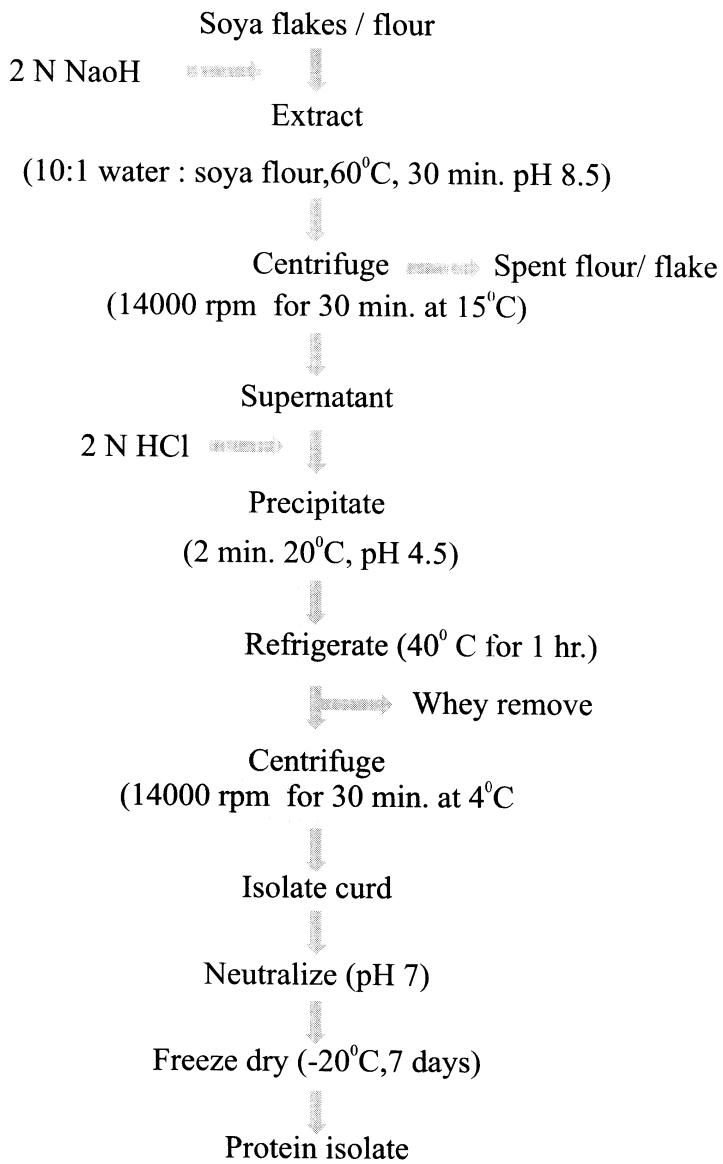
↓

Vacuum oven drying (50^0C for 8 hr.)

↓

Protein concentrate

Procedure for soy protein isolate :



Observations :

Results :

EXPERIMENT - 8

Isolation of starch of given sample

Objective: To obtain pure form of starch from mango kernals.

Introductions:

Starch is a carbohydrate consisting of large number of glucose units which are linked by glycosidic bond. It is a food reserve substance in plant and is widely used as a food additive usually as thickeners and stabilizers. It can also be used to produce gel and ethanol. Extensive research is done on the isolation of starch from variety of food sources like potato, maize, corn and rice and their application in food and non- food industry. One such source for the production of starch is mango (Mangiferaindica) seed kernel. The mango seed kernel is a promising seed because of its high level of starch and oil.

Material & Chemicals:

Mango kernel/stone, Sodium Hydrogen Sulphate, Muslin cloth, Thermometer (0 to 100°C), Crucible

Apparatus : Hot air oven, Centrifuge Machine, pH meter, Muffle Furnace

Procedure:

The mango seed is collected and sun dried for two days. Mango seed kernel is manually separated from the hull and cut into small pieces of 5-10mm size.

1. Take 10g of raw kernel sample is weighed.
2. 80ml of 0.16% of Sodium Hydrogen Sulphate solution is added.
3. The temperature of the process is maintained at 50^0C for 24hrs using hot air oven & thermometer
4. The content is agitated at an interval of 1hr. with the help of glass rod. This is called steeping process.
5. After the steeping process the solution is decanted to obtain the steeped kernel.
6. The steeped kernel is grounded in a mixer grinder by adding 50ml of distilled water to obtain brownish white color slurry.
7. This slurry is filtered using a muslin cloth and filtrate is obtained.
8. The filter cake is washed with distilled water until unless clear wash water is obtained.
9. The filter cake is disposed and the obtained filtrate is further centrifuged at 2800 rpm for 5 mints.
10. The upper non-white layer is disposed and the white layer was re-suspended in distilled water and again centrifuged.

11. This is done for 4 times
12. Finally the starch is collected and dried in an oven for 6 hrs. at 50° C.
13. A good quality starch was obtained.

Observation :

Result :

EXPERIMENT - 9

Studies on different properties of starches

1. Amylose content (%):

A starch sample (20 mg) was taken and 10 ml of 0.5 N KoH was added to it. The suspension was thoroughly mixed. The dispersed sample was transferred to a 100 ml volumetric flask and diluted to the mark with distilled water. An aliquot of test starch solution (10 ml) was pipetted into a 50 ml volumetric flask and 5 ml of 0.1 N HCl was added followed by 0.5 ml of iodine reagent. The volume was diluted to 50 ml and the absorbance was measured at 625 nm. The measurement of the amylose was determined from a standard curve developed using amylose and amylopectin blends.

2. Cold water-solubility :

Take 10 g starch was suspended in 40 g ethanol (40 %) at two different temperatures (25 and 35 °C) and stirred mechanically for 10 min. Aqueous suspensions (1 %) of native and alcoholic starches were taken and shaken at 1,000 rpm on a rotary shaker for 45 min at 25 or 35 °C. The suspensions were then centrifuged at 1200 rpm for 15 min and supernatant was dried at 105 °C for 6 h. The cold water-solubility of samples was calculated as:

$$\text{\% Solubility} = \frac{\text{Gm of solid in supernatant}}{\text{Gm of sample}} \times 100$$

3. Swelling property of starch:

1 g of starch in 10 mL distilled water is heated in a water bath at 50°C for 30 minutes. Then, the sample was centrifuged at 1600 rpm for 15 minutes. These steps were repeated three times for different heating temperatures (60°C, 70°C and 80°C). The precipitated was weighted and used to calculate swelling power with the following equation.

$$SP = \frac{\text{Weight of sedimental paste (g)}}{\text{Weight of sample (dry basis in gm.)}}$$

4. Viscosity (paste consistency):

Two concentrations of starch (10 g/400 mL and 32 g/400 mL) had been chosen that gave the highest viscosity values for native starches and their derivatives. The starch slurry was adjusted to pH 6.5 with a few drops of 5% HCl or 5% NaOH solution, pasted at a heating rate of 1.5 °C/min from 20 to 95 °C, held at 95 °C for 30 min, cooled at 1.5 °C/min from 95 to 50 °C, and finally held at 50 °C for 30 min.

5. Water binding capacity:

A suspension of 2.5 g sample in 20 ml of distilled water was agitated for 30 min in a shaker. The suspension was poured into pre-weighed centrifuge tube. Then 10 ml of distilled water used for rinsing starch from beaker, which was also added to centrifuge tube and centrifuged at 3000 rpm for 10 min. Supernatant was decanted and wet starch was weighed to determine water binding capacity.

Estimation of Total Soluble Sugars

Introductions:

The amount of total soluble sugars can be estimated using either anthrone or phenol-sulphuric acid method calorimetrically. Carbohydrates are important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides which cannot he split by hydrolysis intosimpler sugars. The carbohydrate content can be measured by hydrolyzing the simple sugars by acid hydrolysis and estimating the resultant monosaccharides.

Principal:

In hot acidic medium glucose is dehydrated to hydroxylmethyl furfural. This form an orange-yellow colored product with phenol and has absorption maximum at 490 nm

The basis of this reaction is the differential production of furfural when pentoses are heated with dilute acids and of hydroxyethyl furfural when hexoses are heated with dilute acids. When either product condenses with phenol, an orange to yellow product is formed.

Chemicals:

1. 5% Phenol: Dissolve 50g of redistilled (reagent grade) phenol in water and dilute to one litre.
2. 96% Sulphuric acid (reagent grade).
3. Standard glucose Stock - 100mg in 100 ml of water.

Working standard 10 ml of stock diluted to 100m1 with distilled water.

Procedure:

1. Method 100 mg of the sample into a boiling tube
2. Hydrolyzeby keeping it in a boiling water bath for 3h.with 5ml of 2.5N HCl and cool to room temperature.
3. Neutralize it with solid sodium carbonate until the effectiveness ceases.
4. Makeup the volume to 100 ml and centrifuge.
5. Pipette out 0.2, 0.4, 0.6, 0.8 and l ml of working standard into a series of test tubes.
6. Pipette out 0.1 and 0.2 ml of the sample solution in two separate test tubes. Make up the volume in each tube to l ml with water.
7. Set a blank with 1 ml of water.
8. Add 1 ml of phenol solution to each tube.
9. Add 5m1 of 96% sulphuric acid to each tube and shake well.
10. After 10 min shake the contents in the tubes and place in a water bath at 25-30°C for 20 min.

11. Read the colour at 490 N
12. Calculate the amount of total carbohydrate present in the sample solution using the standard graph.

Calculation :

$$\text{Total carbohydrate in: The sample} = \frac{\text{Sugar value from Graph (µg)}}{\text{Aliquot sample (1ml)}} \times \frac{\text{Total Vol.of Extract(100)}}{\text{Wt. of sample (100 mg)}} \times \frac{1}{1000}$$

Observations:

Result:

EXPERIMENT - 11

Estimation of Reducing Sugar

Introduction:

Sugars with potentially free aldehyde (CHO) or ketone (CO) groups are able to reduce metal ions under alkaline conditions. Such sugars are called as reducing sugars. Some of these are glucose, galactose, lactose and maltose.

DNS Method :

Several reagents have been employed which assay sugars by their reducing properties. One such compounds is 3, 5-dinitrosalicylic acid (DNS) which in alkaline solution is reduced to 3-amino-5-nitrosalicyclic acid.

Chemicals:

1. Dinitrosalicylic acid (DNS) reagent: Dissolve simultaneously 1 g of dinitrosalicylic acid, 200mg of crystalline phenol and 50mg of sodium sulphite in 100ml of 1% NaOH solution by stirring. Store the reagent in a stoppered bottle at 4°C. The reagent deteriorates, during storage due to atmospheric oxidation of the sulphite present. If required to be stored,prepare the reagent without sulphite and add it just before use.
2. 40% Rochelle salt solution (sodium-potassium tartrate solution).
3. Standard sugar solution:100mg in 100ml distilled water

Procedure:

1. Weight 100mg of the sample and extract the sugars with hot 80 % alcohol twice (5 ml each time)
2. Collect the supernatant and evaporate on the water bath
3. Add 10ml of thewater and dissolve the sugars.
4. Pipette out 0.5 to 3ml of alcohol-free extract into test tubes and makeup the volume to 3ml with water in all the tubes.
5. Add 3ml of DNS reagent and mix.
6. Heat for 5 min in a boiling water bath.
7. After the colour has developed, add 1ml of 40% Rochelle salt solution(When the contents are still warm) and mix.
8. Cool the tubes under running tap and measure the absorbance at 510 nm using reagent blank adjusted to zero absorbance.
9. Calculate the amount of reducing sugar in the sample, using a stand graph prepared from working standard glucose solution (0 to 500 (μg)in the same manner.

Calculation :

$$\text{Reducing sugars in: The sample} = \frac{\text{Sugar value from Graph (µg)}}{\text{Aliquot of alcohol Free extract (1ml)}} \times \frac{\text{Total Vol.of Alcohol free Extract (10ml)}}{\text{Wt. of sample (100 mg) 1000}} \times \frac{1}{1000}$$

Observations:

Result:

Determination of Physical properties of fat

1. Determination of Specific Gravity

Preparation of Sample: Filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is completely dry. Cool the sample to 30°C or ambient temperature desired for determination

Apparatus: a) Pycnometer fitted with a thermometer of suitable range with 0.1 or 0.2 subdivision or a density bottle b)Weight Balance c) Water bath maintain 20°C

Procedure: Fill the dry pycnometer or Specific Gravity bottle with the prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. Insert the stopper, immerse in water bath at 30° C and hold for 30 minutes. Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side arm and quickly weigh ensuring that the temperature does not fall below 30°C.

$$\text{Specific Gravity at } 30^{0}\text{C} = \frac{A-B}{C-B}$$

Where, A = weight in gm of specific gravity bottle with oil at 30°C
B = weight in gm of specific gravity bottle at 30°C
C = weight in gm of specific gravity bottle with water at 30°C

2. Determination of the Refractive Index

Definition:

The ratio of velocity of light in vacuum to the velocity of light in the oil or fat; more generally, it expresses the ratio between the sine of angle of incidence to the sine of angle of refraction when a ray of light of known wave length (usually 589.3 nm, the mean of D lines of Sodium) passes from air into the oil or fat. Refractive index varies with temperature and wavelength.

Principle:

Measurement of the refractive index of the sample is done by means of a suitable refractometer.

Apparatus: Refractometer - Abbe or Butyro Refractometer

Procedure: Melt the sample if it is not already liquid and filter through a filter paper to remove impurities and traces of moisture. Make sure sample is completely dry. Circulate stream of water

through the instrument. Adjust the temperature of the refractometer to the desired temperature. Ensure that the prisms are clean and dry. Place a few drops of the sample on the prism. Close the prisms and allow standing for 1-2 min. Adjust the instrument and lighting to obtain the most distinct reading possible and determining the refractive index or butyro-refractometer number as the case may be.

$$\mathbf{R = R1 + K1 (T^1 - T)}$$

Where, R = Reading of the refractometer reduced to the specified temperature T°C

R1 = Reading at T^1C

K = constant 0.000365 for fats and 0.000385 for oils (If Abbe Refractometer is used) or = 0.55 for fats and 0.58 for oils (if butyro-refractometer is used)

T^1 = Temperature at which the reading R1 is taken and

T = Specified temperature (generally 40°C)

Significance: Refractive index of oils increases with the increase in unsaturation and also chain length of fatty acids.

3. Determination of Flash point :

Principle: The method determines the temperature at which the sample will flash when a test flame is applied under the conditions specified for the test.

Outline of Method: The sample is heated in a test cup at a slow and constant rate with continual stirring. A small test flame is directed into the cup at regular intervals with simultaneous interruption of stirring. The flash point is taken as the lowest temperature at which the application of the test flame causes the vapour above the sample to ignite momentarily.

Apparatus: Pensky-Martens closed cup apparatus with thermometer.

Preparation of Sample: Samples containing dissolved or free water may be dehydrated with Calcium chloride or by filtering through a suitable filter paper or a loose plug of dry absorbent cotton. Warming the sample is permitted but it shall not be heated for prolonged periods or above the temperature of 16°C below its expected flash point.

Procedure: Thoroughly clean and dry all parts of the cup and its accessories before starting the test, being sure to remove any solvent which had been used to clean the apparatus. Support the tester on a level steady table. Fill the cup with the oil to be tested up to the level indicated by the filling mark. Place the lid on the cup and properly engage the heating devices. Insert the thermometer, light the test flame and adjust it to 4.0 mm in diameter. Heat the sample so that the temperature increase is about 5 to 6°C per min. During the heating, turn the stirring device from one to two revolutions per second. Apply the test flame when the temperature of the sample is a whole number not higher than 17°C below the flash point At every 5°C rise in temperature, discontinue stirring and apply the test flame by opening the device which controls the shutter and lowers the test flame into the shutter opening. Lower the test flame in for 0.5 second and quickly return to the raised position. Do not stir the sample while applying the test flame. As soon as the test flame has been returned to the raised position, resume stirring. The flash point is the temperature indicated by the thermometer at the time of the flame application that causes a distinct flash in the interior of the cup.

4. Determination of Colour

Principle: The method determines the colour of oils by comparison with Lovibond glasses of known colour characteristics. The colour is expressed as the sum total of the yellow and red slides used to match the colour of the oil in a cell of the specified size in the Lovibond Tintometer.

Apparatus:(a) Lovibond Tintometer (b) Glass cells (cell size 0.25 inch, 0.5 inch. 1.0 inch, 5.25 inch or 1.0 cm, 2.0 cm, 5.0 cm as required)

Procedure: Melt the sample if it is not already liquid and filter the oil through a filter paper to remove any impurities and traces of moisture. Make sure sample is absolutely clear and free from turbidity. Clean the glass cell of desired size with carbon tetrachloride and allow it to dry. Fill it with the oil and place the cell in position in the tintometer. Match the colour with sliding red, yellow and blue colours. Report the colour of the oil in terms of Lovibond units as follows :-

Colour reading = (a Y + 5 b R) or (a Y + 10 b R)

Where, a = sum total of the various yellow slides (Y) used

b = sum total of the various red (R) slides used

Y + 5R is the mode of expressing the colour of light coloured oils; and

Y + 10 R is for the dark-colored oils

Determination of Acid value

Introduction:

The acid value is the number of mg of KOH required to neutralize the free fatty acids present in lg of fat. The amount of free fatty acids gives e indication of the age and quality of the fat. A small quantity of free fatty acid is usually present in oils along with the triglycerides. It increases during storage The keeping quality of oil therefore relies upon the free fatty acid content.

Principal:

The free fatty acid in an oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required to neutralize the free fatty acids present in lg of sample. However, the free fatty acid content is expressed as oleic equivalents.

Reagents:

1. 1% Phenolphthalein in 95% ethanol
2. 0.1N Potassium hydroxide
3. Neutral solvent: Mix 25 ml ether, 25 ml 95% alcohol and 1 ml of 1% phenolphthalein solution and neutralize with N/10 alkali.

Procedure:

1. Dissolve 1-10g of oil or melted fat in 50m1 of the neutral solvent in a 250 ml conical flask.
2. Add a few drops of phenolphthalein.
3. Titrate the contents against 0.1N potassium hydroxide (KOH).
4. Shake constantly until a pink colour which persists for fifteen seconds is obtained.

Calculation:

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample (g)}}$$

(The free fatty aid is calculated as oleic acid using the equation: 1 ml N/10 KOH = 0.028 g oleic acid)

Observations:

Result:

Determination of iodine value of oil

Introduction:

The iodine value is a measure of the degree of unsaturation of an oil or fat It is constant for a particular oil or fat .It is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid

Principal:

The oils contain both saturated and unsaturated fatty acids. Halogens add across the double bonds of unsaturated fatty acids to form addition compounds. Iodine monochloride (ICI) is allowed to react with the fat in the dark. Iodine gets incorporated into the fatty acid chain wherever the double bonds exist. The amount of iodine consumed is then determined by titrating the iodine released (after adding KI) with standard thiosulphate and comparing with a blank in which the fat is omitted.Hence the measure of iodine absorbed by an oil or fat gives the degree of unsaturation. The iodine value or number is defined as the number of grams of iodine absorbed by 100 g of the oil/fat.

Chemicals:

1. Hanus iodine solution: Dissolve 6.8g of iodine in 500 ml of glacial acetic acid and heat to dissolve. Cool and add 1.5m1 of bromine.
2. 15% KI solution: Prepare in water.
3. Standard 0.1N sodium thiosulphate solution: Dissolve 6.2g of $Na_2S_20_3.$, $5H_2O$ in 250 ml of water. Standardize against standard $K_2Cr_20_7$ solution and dilute to get exactly 0.1N $Na_2S_20_3$ solution.
4. 1% Starch indicator: Take 1 gm starch & dissolve in 100Ml distil water.
5. Chloroform

Procedure:

1. Weigh out 0.2-0.3g of oil or fat into 500 ml conical flask.
2. Add 20 ml of chloroform and dissolve the oil completely.
3. Add 25m1 of Hanus iodine solution, mix well, stopper the flask and keep in dark for 30 min.
4. Add 20m1 of KI solution and mix well.
5. Titrate against standard 0.1N $Na_2S_20_3$ solution using starch as an indicator with vigorous shaking to extract the iodine from the chloroform layer.
6. Conduct blank similarly in the absence of oil.

Calculation:

$$\text{Iodine number of oil or fat} = \frac{A \times N \times 0.1269}{\text{Weight of oil (g)}} \times 100$$

Where, A = ml of $Na_2S_20_3$ (Blank - Test)

N = Normality of $Na_2S_20_3$ Solution

Note 1 ml of 1.0N $Na_2S_20_3$ = 0.1269 g of iodin

Observations:

Result:

EXPERIMENT - 15

Determination of Saponification Value of Oils

Introduction:

Saponification is the process by which the fatty acids in the glycerides of the oil/fat are hydrolyzed by an alkali. The resultant salts of fatty acids are calledas soaps.

When the oil or fat (triglyceride) is heated with KOH (or alkali) it is saponified (hydrolyzed) and releases fatty acids and glycerol. Each molecule of triglycerides uses 3 molecules of KOH for saponification .Saponification value is the number of milligram of KOH required to saponify the fatty acids resulting from the complete hydrolysis of 1 g of the fat or oil.This value gives an indication of the nature of fatty acids in the fat since the longer the carbon chain the less acid is liberated per gram of fat hydrolyzed .Thus value is useful for a comparative study of the fatty acid chain length in oils.

Principal:

A known quantity of oil is refluxed with an excess amount of alcoholic KOH. After saponification, remaining KOH is estimated by titrating it against a standard acid

Chemicals:

1. 0.5N Alcoholic KOH solution: Dissolve 28g of KOH in one lit. of 90% alcohol.
2. Standard 0.5N HCl: Prepare approximately 0.6N HCl solution by diluting 54 ml of cone. HCl to 1 lit. with water. Standardize this solutionagainst standard 0.5N sodium carbonate solution and dilute to get exactly 0.5N HCl.
3. 1% Phenolphthalein solution in alcohol.

Procedure:

1. Accurately weigh out 1-2g of oil into a 250 ml conical flask, add 25 ml of alcoholic KOH and dissolve the oil completely.
2. Connect air condenser to the flask and boil for about 30 min on a boiling water bath.
3. Cool to room temperature, add 2-3 drops of phenolphthalein indicator and mix.
4. Titrate against standard 0.5N HC1 until the pink colour disappears.
5. Treat the blank similarly in the absence of oil.

Calculation:

$$\text{Saponification value of an oil (mg KOH)} = \frac{(\text{Blank-Titre}) \times 28.06}{\text{Weight of oil (g)}}$$

Notes

1. 1ml of 0.5N HC1 = 28.06 mg KOH
2. If the sample is not liquid, melt it and filter through filter paper to remove any impurities and the last traces of moisture.
3. Alcohol should not get dried up during saponification. Effective cooling of alcohol vapour is essential.
4. Clarity and homogeneity of the test solution are indicators of complete saponification.

Observations:

Result:

Test for detection of different oils Boudouin Test, Halphens Test, Hexabromide Test

1. Baudouin Test

Principle:

The development of pink colour with furfural solution in the presence of hydrochloric acid indicates the presence of sesame oil. The color is produced on account of reaction with sesamolin present in sesame oil.

Apparatus: (a) Glass stopper test tubes / measuring cylinders

Chemicals:

1) Hydrochloric acid (concentrated)
2) Furfural solution (2 per cent furfural–freshly distilled in ethyl alcohol)

Procedure:

Take 5 ml of the oil or melted fat in a 25 ml measuring cylinder (or test tube) provided with a glass stopper, and add 5 ml of conc. hydrochloric acid and 0.4 ml of furfural solution. Insert the glass stopper and shake vigorously for two minutes.

Let it stand and allow the mixture to separate. The development of a pink or red colour in the lower acid layer indicates presence of sesame oil. Confirm by adding 5 ml of water and shaking again. If the colour in acid layer persists, sesame oil is present, if the colour disappears it is absent. (As furfural gives violet tint with HCl, it is necessary to use the dilute solution specified.)

Observations:

Result:

2. Halphen's Test

Principle:

The development of red colour on heating the oil with a solution of sulphur in carbon disulphide indicates the presence of cottonseed oil. The test is also given by Hempseed oil, Kapokseed oil / oils and fats containing cyclopropenoid fatty acids (such as sterculic and malvalic acid). Hydrogenation and deodorization wholly or partially destroy the cromogens and react with diminished intensity. A positive reaction is not given by an oil heated to 250°C or above. The fat of animals fed on cottonseed meal (butter, lard) or other cottonseed products may give faint positive reaction by this test.

Apparatus: (a) Test tubes (b) Water bath (c) Oil bath or Brine bath maintained at 110°C– 115°C

Chemicals:
(a) Sulphur solution: Prepare a one percent (w/v) solution of sulphur in carbon disulphide and then add an equal volume of amyl alcohol.

Procedure:

Take about 5 ml of the oil or melted fat in a test tube and add to it an equal volume of the sulphur solution. Mix thoroughly by shaking and heat gently on a water bath (70° to 80°C) for a few minutes with occasional shaking until the carbon disulphide has boiled off and the sample stops foaming. Place the tube in an oil bath or a saturated brine-bath maintained at 110-115°C and hold for 2.5 hours. A red colour at the end of this period indicates the presence of cottonseed oil. The test is sensitive to the extent of 0.5 % cottonseed oil in other oils.

Observations:

Result:

3. Hexabromide Test
Principle:
The formation of a precipitate of hexabromide when the oil in chloroform is treated with bromine and then with alcohol and ether in cold condition indicates the presence of linseed oil.

Apparatus: a) Boiling tubes b) Ice water bath
Chemicals:
i) Chloroform – A.R ii) Liquid bromine – A.R iii) Ethyl alcohol iv) Diethyl ether

Procedure:

Pipette one ml of the oil into a boiling tube (wide-mouthed 100 ml capacity). Add 5 ml of chloroform and about one millilitre of bromine drop-wise till the mixture becomes deep red in colour and cool the test-tube in an ice water-bath. Add about 1.5 ml of rectified spirit drop-wise while shaking the mixture until the precipitate which was first formed just dissolves and then add 10 ml of diethyl ether. Mix the contents and place the tube with in the ice water-bath for 20 minutes. Appearance of precipitate indicates the presence of linseed oil.
Note: 1. This test is not applicable for detecting linseed oil in mahua oil.
2. The use of safe and suitable pipette i.e Lunge-Ray pipette is suggested for the handling and addition of bromine.
3. The test is also given by fish oils and fats containing highly unsaturated fatty acids.

Observation:

Result: