UNIT 12 PHYSICAL AND CHEMICAL ANALYSIS OF FOODS

Structure

12.0 OBJECTIVES

After reading this unit, we shall be able to:

- explain the physical properties of foods;
- analyze the food for its chemical composition;
- explain the importance of physico-chemical properties of foods;
- define the chemical constants of oils and fats; and
- discuss the advantages and disadvantages of a particular techniques used for food analysis.

12.1 INTRODUCTION

Analysis plays an important role in assessment and maintenance of food quality and safety, both in industry as well as for enforcement authorities at national and international levels. Earlier, food analysis was concerned with food adulteration only. Now-a-days there is an increasing tendency to examine food from a more positive and broader view point. Processed foods are produced within the limits of prescribed manufacturing formulations, set also to comply with legal and/or other requirements. In many food laboratories, most of the routine work is confined to proximate (e.g. moisture, protein, carbohydrate, lipid, fiber, ash) analysis and the analysis of additives and contaminants. This is done by analysis of the product at different stages of processing starting at the farm level. The regulatory requirements for the analysis of food additives and contaminants at very low level have necessitated the development of instrumental techniques suitable for rapid assessment. In case of proximate analysis, the methods may vary for different category of food products. Hence, the results obtained for a particular food constituent depends on the procedure adopted. However as long as the same standard procedure is applied to the same food each time, the results are usually repetitive and thus provide an adequate basis for interpretation.

In food industry, various food components and parameters are analyzed in both raw and processed products. Knowledge of chemical composition of food is important to health, well being and safety of the consumers. Knowledge of the chemical and biochemical composition of food is useful to manufacturers in understanding the importance of various nutritional constituents so that the amount of essential nutrients may be maintained or improved during and after processing. The knowledge of the principles of different food analysis techniques is useful for selection of appropriate technique for analyzing a particular food. The compilation of physical and chemical techniques in this unit would be helpful in understanding the basic principles of food analysis.

12.2 PHYSICAL PROPERTIES

The physical properties of food products have not received adequate attention from the food scientists, although a thorough understanding of the physical changes and principles involved in delivering food products from farm to consumers is essential. Important physical parameters are briefly described below:

12.2.1 Specific Gravity/Density

The specific gravity of a substance is the ratio of density of the material to the density of water at a specified temperature. Specific Gravity can be expressed as

$SG = \rho / \rho H_2O$

where

SG = specific gravity

ρ = density of fluid or substance (kg/m^3)

ρH_2O = density of water (kg/m^3)

It is common to use the density of water at 4 ^{o}C (39^{o}F) as reference - at this point the density of water is at the highest - 1000 kg/m^3 or 62.4 lb/ft^3. Water is the standard for solids and liquids, while hydrogen is the standard for gases.

The density of a liquid at a particular temperature is the mass (e.g. gram) of unit volume (e.g. 1 mL) of the liquid.

$$\rho = \frac{m}{V}$$

The density of solid indicates the weight of a substance held in a unit volume. Densities of liquids are generally measured either by weighing a definite volume of the liquid in a density bottle or pyknometer or by determining the buoyancy acting on a sinker immersed in a liquid. The same principal is used in lactometer for determining density of milk. When sufficient liquid is available, the density can be determined by means of hydrometers or more accurately by means of the Westphal balance.

12.2.2 Specific Heat Capacity

Specific heat capacity of a substance is the amount of heat required to raise the temperature of that substance by one degree centigrade. Specific heat of a substance helps in calculating the amount of heat required to raise the temperature of a substance by a certain amount. Unit of specific heat capacity is J g^{-1}K^{-1} or cal g^{-1}K^{-1}

For example, how much heat would be required to raise the temperature of 100 g of water from 30°C to 65°C?

Formula:

Units of heat (Calories) = $mc\Delta T$, where m is the mass in g, c is specific heat capacity and ΔT corresponds to change of temperature

$$= 100 \text{ g} \times 0.94 \times 35°\text{C}$$

$$= 3290 \text{ calories}$$

12.2.3 Surface Tension

Surface tension is defined as the force acting upon a line of unit (1cm) length on the surface of a liquid. Surface tension is a state of stress at the surface of a liquid, which occurs due to inward force of attraction on the surface molecules as result of which the upper surface of a liquid behaves like a stretched membrane. Surface tension is a manifestation of the forces of attraction that hold the molecules together in the liquid (or solid) state; thus liquid droplets tend to become spheres – the form of least surface area – because of the material cohesion of molecules. High surface tension is found with liquids that have strong cohesive forces and consequently high boiling points whereas volatile organic liquids have low surface tension. For a given liquid, the surface tension decreases with rise of temperature and becomes zero at critical point.

Surface tension may be measured by several ways:

A. *Capillary rise method:* If a capillary tube is placed in a liquid it is found that the liquid rises in the tube. To determine the height, to which the liquid

rises in the tube, it is measured by cathetometer or travelling microscope. Surface tension is determined by following equation:

$$\text{Surface tension } (\gamma) = \frac{r \times h \times d \times g}{2}$$

where,

r = radius of capillary tube

d = density of the liquid

h = height to which the liquid rises, and

g = acceleration due to gravity.

B. Drop-weight method: This is one of the simplest method. The size of drop issuing from a capillary orifice is governed by the surface tension of the liquid. The instrument employed is called a stalagmometer. The surface tension of a liquid food material like milk can be determined by comparing with water at same temperature. A tube of uniform bore is used and the number of drops falling per unit of time are counted and compared to that of water. Hence, a liquid showing 200 drops as compared to water with 100 drops would have a surface tension one-half as great as that of water.

C. Torsion balance method: A more accurate method is the platinum ring procedure. The surface tension can be measured by the force required to detach a horizontal ring of platinum wire from the surface of a liquid. The ring is connected to a delicate balance and the pull required to draw it out of a liquid is measured by a torsion balance.

12.2.4 Viscosity

Viscosity is a property of a liquid closely related to the resistance to flow. It is the frictional effect due to the passage of one layer of fluid (liquid/gas) over another. The coefficient of viscosity is defined as the force required per unit area to maintain unit difference of velocity between two parallel planes in the fluid at 1cm apart. The kinematic viscosity of a liquid is equal to the ratio of the dynamic viscosity and density of the liquid at the same temperature. The unit of kinematic viscosity is stokes (S) and the unit of dynamic viscosity is dynes/cm^2 (Poise). Viscosity of liquid may be determined by any method that will measure the resistance to shear offered by the liquid.

When a liquid flows through a capillary tube of radius r for a time t, under a constant pressure head p, the volume of liquid v issuing from the tube is given by :

$$v = \frac{\pi \times p \times t \times r^4}{8 \times l \times \eta}$$

Where,

η = Coefficient of viscosity, and

l = length of the tube.

It is difficult to determine the absolute coefficient of viscosity for a liquid, however; the relative viscosity of a liquid with respect to water may be determined. The simplest way to do this is to make use of Ostwald viscometer.

The viscosity may be determined by the method of the falling sphere, which depends on the time taken for a sphere to fall through a given distance. This is

used for the measurement of viscous liquids. The viscosity of liquid is calculated by the following equation.

$$\frac{\eta}{\eta_s} = \frac{(D-d) \times t}{(D-d) \times t_s}$$

Where,

η = Coefficient of viscosity of the liquid,

D = density of sphere,

d = density of liquid, and

t = time taken. The subscript s refers to a standard liquid, the viscosity of which is known.

A wide range of viscosity can also be measured by Brookfield viscometer using different spindle and speed. Brookfield viscometer is usually suitable for the measurement of highly viscous liquids.

12.2.5 Refractive Index

The measurement of refractive index of certain substances is helpful in identifying and establishing their purity, in determining the molecular structure of organic compounds and quantitative analysis of certain types of solutions. The Abbe refractometer is particularly used in food analysis and in the testing of oils. It covers a wide range of refractive indices and uses a very small amount of sample. In making a refractive index measurement, the beam of light is usually passed from air through the solid or liquid medium being measured and then through a glass prism and out into the air again. The refractive index may be calculated from the angle through which a telescope must be turned in order to pick up the emerging beam on a cross hair. The angle measured includes the refraction at the liquid-glass interface and at the glass-air interface.

12.2.6 Filth

Any foreign matter in product associated with objectionable conditions or practices in production, storage, or distribution included are filth, decomposed material and miscellaneous matter *viz.*, sand, soil, glass or other foreign substances excluding bacterial counts.

A. *Filth:* Any objectionable matter contributed by animal contamination of product such as rodent, insect, or bird matter; or any other objectionable matter contributed by unsanitary conditions.

B. *Heavy filth:* Heavier filth material is separated from food products by sedimentation techniques based on different densities of filth, food particles and immersion liquids such as chloroform. e.g., insect and rodent excreta pellets and pellets fragments, sand and soil. When chloroform is mixed vigorously with the plant material and allowed to separate, most of the plant tissues rise and the heavy extraneous matter settles to the bottom, which can be separated by draining on filter cloth.

C. *Light filth:* Lighter filth particles that are oleophillic and are separated from product by floating them in an oil-aqueous liquid mixture e.g., insect fragments, whole insects, rodent hairs and feather barbules. It is difficult to wet all the insect parts without creating frothy emulsion of the plant

material. Droplets of oil adhering to sides of trap flask may prevent insects from rising. To overcome it, the oil is worked thoroughly into the water-food mixture without incorporating air. If an emulsion is formed, capryl alcohol or ethanol may be used to break the emulsion.

D. Sieved filth: Filth particles of specific size ranges are separated quantitatively from the product by use of selected sieve mesh sizes.

12.2.7 Particle Size

Particle size analysis means the separation of a sample of material into fractions of different average diameters. Hand sieving is the most common method. The sieves are arranged in a nest, the outer casing of each sieve fitting the casing of the sieve below it and the nest provided with a top cover and bottom blank pan. The weighed sample is placed on the coarsest sieve and the whole nest is given a preliminary shaking after which each sieve is shaken separately to complete the separation. Both time and intensity of shaking must be kept as uniform as possible. A mechanical shaker is preferable to hand shaking since time and intensity of shaking can be exactly repeated from sample to sample. For separation finer than 200 mesh elutriation is used. Elutriation is the separation of material by the action of a rising current of fluid. Microscopic sizing analysis is a form of direct counting and is theoretically more accurate than either screening or elutriation. However, the method is only a guide, since the quantity of material visible in the microscopic field is very minute.

Check Your Progress Exercise 1

Note: a) Use the space below for your answers.
b) Check your answers with those given at the end of the unit.

1) What is filth? Briefly discuss the separation technique for the heavy filth?

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2) Correlate heat and specific heat?

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3) How can you measure the viscosity of liquid food product with low consistency?

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4) Differentiate the specific gravity and density of foods?

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5) Define surface tension? Suggest a simple method for its determination?

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12.3 CHEMICAL PROPERTIES

12.3.1 Moisture

Water, the simplest of all constituents of foods, is one of great concern to producer, consumer and chemist. The weight of food has little significance unless taken into consideration with the water content. The accurate determination of moisture poses many challenges. One of the problems is the difficulty of separating all of the water from the food sample, resulting in underestimation of moisture content. On the other hand, harsher conditions to remove all moisture from a food may simultaneously cause decomposition of the product, which may result in the production of water along with/or a loss in sample mass. Thus the accuracy of the method would be severely in question. Most of the methods for the estimation of water in foods depend on the loss in weight on heating. An exposure to the air of the drying oven causes the oxidation of certain oils and other constituents; a gain in weight of such constituents offsets the loss in weight due to moisture. To obviate the error, the drying should be performed in vacuum. The loss in weight on heating is not entirely because of water as other volatile substances evident to the sense of smell are present in most foods, although the amount is too small. Significant loss of volatile compounds from the food is another potential difficulty in the determination. Most of the spices however contain notable quantities of volatile oil that pass off with the water.

Analytical methods of moisture determination can be classified in two ways.

1) **Direct methods:** Moisture analysis normally involves removing water from the food samples by drying, distillation, extraction and its quantity is measured by weighing, titration and so forth, e.g., oven drying, vacuum drying, freeze drying, distillation method, Karl Fischer method, chemical desiccation, thermo-gravimetric analysis and gas chromatography.

2) **Indirect methods:** The indirect methods must be calibrated against standard moisture values that have been precisely determined using direct methods, e.g., refractometry, infrared absorption, near infrared reflectance spectroscopy, microwave absorption, dielectric capacitance, mass spectrometry, NMR spectroscopy, neutron scattering method, etc.

I) Air-Oven Drying Method

It is one of the most common and widely used methods for routine moisture determination. The ovens should be thermally regulated to ±0.5°C and have minimal temperature variations (<±3°C) within the oven. The main criterion of food for moisture determination by air-oven drying is that sample should be thermally stable and should not contain significant amount of volatile compounds.

II) Vacuum Oven Drying Method

It is the standard and most accurate drying method for moisture analysis of foods. The AOAC methods generally recommend that moisture content of food can be determined by heating foods at 98 to 102°C at a pressure of 25-100 mm Hg for 2-6 h. Lower temperatures (60-70°C) can be used for heat sensitive/ sugary food products in sugar to prevent decomposition used for products like jam, confectionery etc.

III) Distillation Method

Two types of distillation procedures exist for moisture determination; (a) Direct distillation and (b) Reflux distillation.

a) Direct Distillation

In this method, a food is heated in a liquid immiscible in water and has a high boiling point (e.g., mineral oil). The water in the food like spices and herbs distils directly from this liquid, condenses and collects in a graduated tubes; the volume of the water removed is then measured e.g., Spices, herbs.

b) Reflux Distillation

It makes use of the azeotropic properties of solvent mixtures. During heating, water and an immiscible solvent (toluene or xylene) distil off together at a constant ratio and frequently at a temperature lower than the boiling point of either component. For example, the boiling points of water and toluene are 100°C and 110.6°C, respectively, but the boiling point of the binary mixture is 85°C; the distillation ratio of the mixture is 20% water and 80% toluene. As water is denser than toluene, the water is again collected in a suitable measuring apparatus where it separates and has its volume measured. A representation of the reflux distillation apparatus is shown in Fig. 12.1.

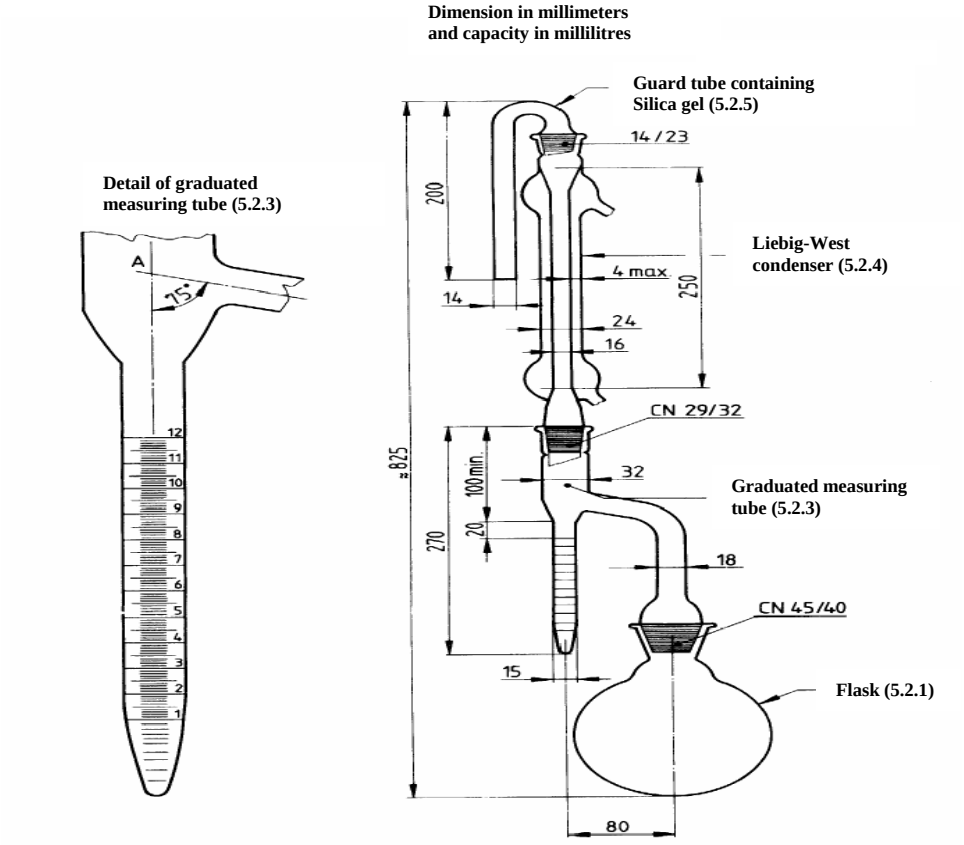


Fig. 12.1: Dean and Stark Apparatus for Moisture Determination

IV) Karl Fischer Titration Method

The Karl Fishcer (KF) titration has become a standard method for the moisture analysis in liquids and solids due to its selectivity, high precision and speed. The method is particularly suitable for food where heating methods give erratic results. This method has been approved for dried vegetables, oils and fats, cocoa products and liquid molasses. The KF titrimetric method is based upon the quantitative reaction of water with an anhydrous solution of sulphur dioxide and iodine dissolved in pyridine and an alcohol. The Karl Fischer reagent consists of iodine, pyridine, SO_2 and methanol. The titration is conducted either by volumetric method (where the end point is brown colour, determined visually) or by coulometric titration, where the end point is determined by a potentiometer. Food samples may be directly introduced into the reaction vessel if the water is easily accessible to the reagent. In solid foods where water is not accessible, the water is frequently extracted into anhydrous methanol and then estimated.

V) Refractometry

The refractive index is a ratio of the sine of the angle of incidence made by a ray in air to the sine of the angle of refraction made by the ray in the material being tested. The refractive index increases as the moisture content decreases. By measuring the refractive index of a solution, the moisture content can be rapidly determined using an appropriate calibration curve. Refractometry is best suited for high sugar products *viz.*, fruit products, syrups and honey. For solid or semi-solid foods, the sample can be homogenized with an anhydrous solvent (e.g., isopropanol) and then the refractive index of the solution can be

measured using a refractometer. The moisture content of the sample may be calculated using the calibration curve (produced by measuring refractive index of solutions containing the same solvents with known amounts of added water) and the mass of food homogenized in the solvent.

12.3.2 Water Activity

The water activity (a_w) of a food describes the energy status of the water in the food. It is the ratio of vapour pressure of water in a sample to saturation vapour pressure at sample temperature. The water activity is not influenced by the total quantity of water in a sample but only by that fraction which is least tightly bound. Temperature affects a_w due to changes in water binding, solubility of solution in water and the state of sample matrix. Most high moisture foods exhibit negligible change with temperature. The water activity controls all aspects of microbial growth by lengthening the lag phase of microbial growth. The water activity influences non-enzymatic browning, lipid oxidation, degradation of vitamins, enzymatic reactions, protein denaturation, starch gelatinization and starch retrogradation. The water activity is usually measured by using different hygrometers *viz.*, hair hygrometer, resistance sensor, capacitance sensor, dew point hygrometer, etc.

12.3.3 Protein

All natural foods contain protein, although trace amounts are found in honey and maple sugar. The quantification of total protein in food and food products can be performed directly or by determining total nitrogen from conversion of crude protein using a suitable conversion factor. The protein content is calculated from the total nitrogen determined by either Kjeldahl method or Dumas/Pregl-Dimas method. Amides (abundant in young shoots), ammonium salts, nitrates, lecithin, nucleic acid, purines of tea, coffee, cocoa and meat extracts in addition to protein contain nitrogen in varying proportions. Although small, these compounds thus add error to the calculated protein estimate. However, the protein calculated by factor is a valuable figure, not only because it represents approximately the true protein present but also because it is an index of the content of other groups. The protein content can also be determined directly by formal titration, UV spectrophotometry, Lowry method, Dye binding method, IR spectrophotometry, NMR spectroscopy, turbidimetry, refractometry, etc.

I) Direct Method

Since foods contain mixtures of proteins, the methods for the direct determination of proteins need to be calibrated against a reference standard for nitrogen, e.g. Kjeldahl method.

i) Formal Titration Method

When formaldehyde is added to neutralized aqueous solution containing protein, the $–NH_2$ group of protein converts to methylene-amino group ($-N=CH_2-$) with the release of proton. This may be titrated.

ii) Spectrophotometric Method

The Lowry method is based on the amplification of the biuret reaction (complex of cupric ions with protein) by subsequent reduction of the Folin phenol reagent (mixed acids of phosphomolybdic and phosphotungstic) by

tyrosine and tryptophan. This redox reaction is accompanied by the formation of a blue colour (λ_{abs} 745 – 750 nm), which is highly pH dependant (10-10.5).

II) Indirect Method

i) Kjeldahl Method

It has wide acceptance for the determination of protein in food products. The method follows three steps:

Digestion – Decomposition of organic matter by heating in the presence of concentrated sulphuric acid, the end product is ammonium sulphate solution.

Distillation – Ammonium sulphate is converted into gaseous ammonia by addition of an excess base, followed by boiling and condensation of the ammonia in a receiving solution (acid).

Titration – Quantification of the unreacted acid in the collecting vessel.

The rate of digestion and the completeness of the breakdown of nitrogenous compounds to ammonium sulphate mainly depends upon the heat input, amount of boiling point elevator of acid (alkali sulphate), addition of catalyst (mercury, cupper sulphate, titanium dioxide), oxidant (hydrogen peroxide), reflux rate of sulphuric acid and length of digestion.

Ammonia is liberated from the acid digestion mixture by distillation in the presence of alkali (50% NaOH). A total recovery of ammonia from the digest can be obtained within 5 to 20 min by direct distillation and about 10 min by steam distillation.

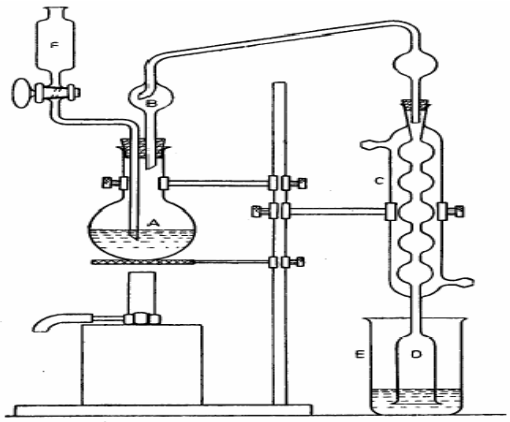


Fig. 12.2: Kjeldahl Nitrogen distillation assembly

ii) Dumas Combustion Method

The protein content of foods can be estimated by the determination of elemental nitrogen using instruments based on the Dumas principle. In these instruments, the nitrogen containing constituents of the sample are combusted at high temperature about 1000°C in the presence of oxygen to oxides of nitrogen (NOx) and then reduced over copper or tungsten to gaseous nitrogen which is measured by gas solid chromatograph using thermal conductivity detector. This method offers significant advantage over Kjeldahl method i.e., shorter analysis time (3-4 min), but these instruments appear to have limited usefulness for some food products because they can only deal with very less amount of sample.

12.3.4 Fat

The oils and fats from oilseeds and fruits as well as from animal fatty tissues correspond quite closely with those extracted by diethyl ether. Practically, all the sterols and phosphorus containing organic compounds notably the lecithins are extracted with the glycerides. Essential oil and resins are the chief constituents of the ether extract of certain spices. Similarly, pepper contains nitrogenous ether soluble substance, piperine (alkaloids). Other solvents *viz.*, chloroform, carbon tetrachloride, carbon disulphide and petroleum distillates of lower or higher boiling points dissolve fats and oils and can be used but the yield and composition of the extract differ somewhat with the solvent. Free fat can be extracted by the less polar solvents such as petroleum ether and diethyl ether, whereas the bound fat requires more polar solvents *viz.*, alcohols for their extraction. The bound fat may be broken down by hydrolysis or other chemical treatment to yield free fat. Hence, the amount of extracted fat found in food products will depend on the method of analysis used.

I) Direct Solvent Extraction Method

The free fat content can be conveniently determined in foods by extracting the dried and ground material with petroleum ether or diethyl ether in Soxhlet extraction apparatus (Fig. 12.3). Extraction in the presence of alcohols causes the release of lipoidal substances bound to proteins and carbohydrates *viz.*, phospholipids and glycolipids. Hence, maximum extraction is obtained by a mixture of polar and non-polar solvents. This procedure co-extracts water and water soluble substances. Hence, the residue after solvent removal and the addition of anhydrous sodium sulphate needs to be extracted with petroleum ether.

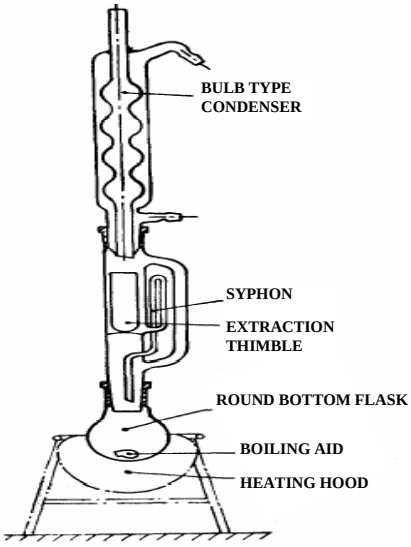


Fig. 12.3: Soxhlet extraction apparatus

II) Solubilization Extraction Method

Bound fat can be made free if the food sample is dissolved completely prior to extraction with polar solvents. Dissolution of the food can be achieved by acid or alkaline hydrolysis.

In acid hydrolysis method, the sample is heated on a steam bath with dilute HCl and boiled for 30 min. The sample solution is filtered through a wet filter paper and washed with hot water. The filter paper is then oven dried and placed directly into a Soxhlet apparatus and extracted with ethyl or petroleum ether or dichloromethane.

In alkali hydrolysis method (Rose Gottlieb method), the material is treated with ammonia and alcohol in cold and the fat is extracted with diethyl ether-petroleum ether mixture. The alcohol precipitates the protein, which dissolves in the ammonia; the fat can then be extracted with ether. Petroleum ether is then added as it reduces the proportion of water and hence all non-fatty substances.

(All dimensions in millimeters)

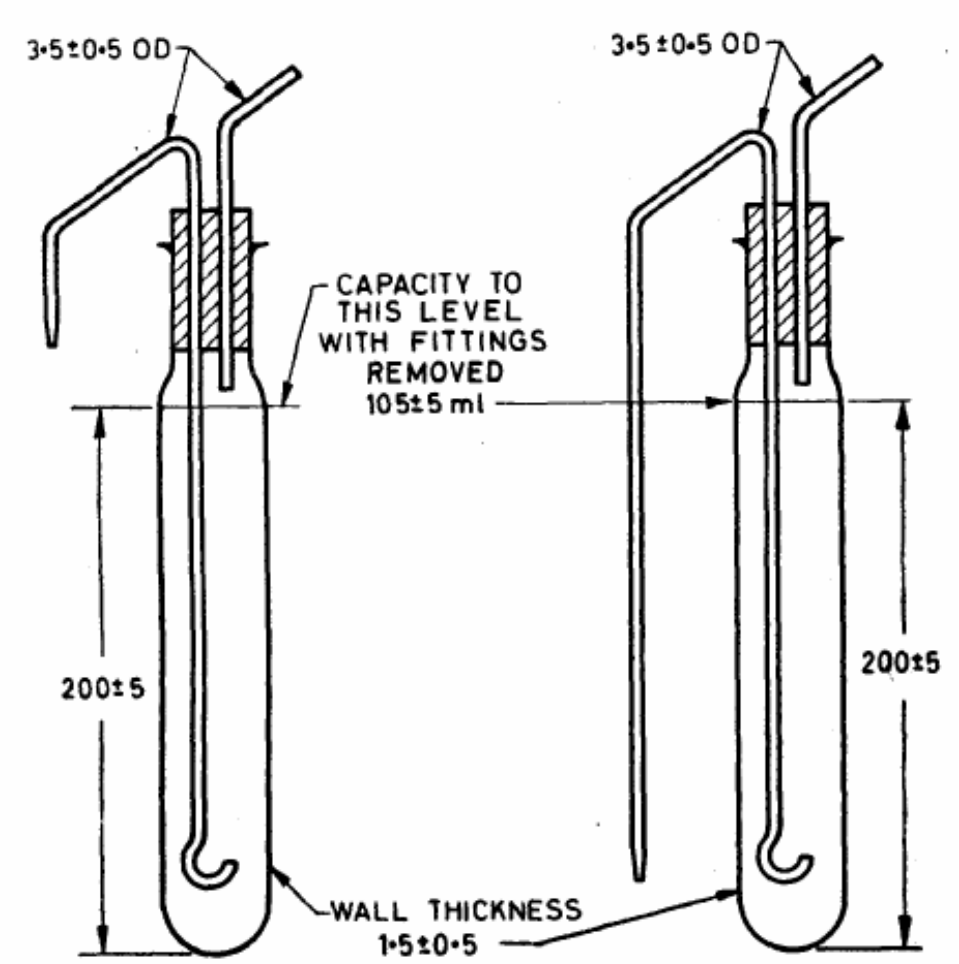


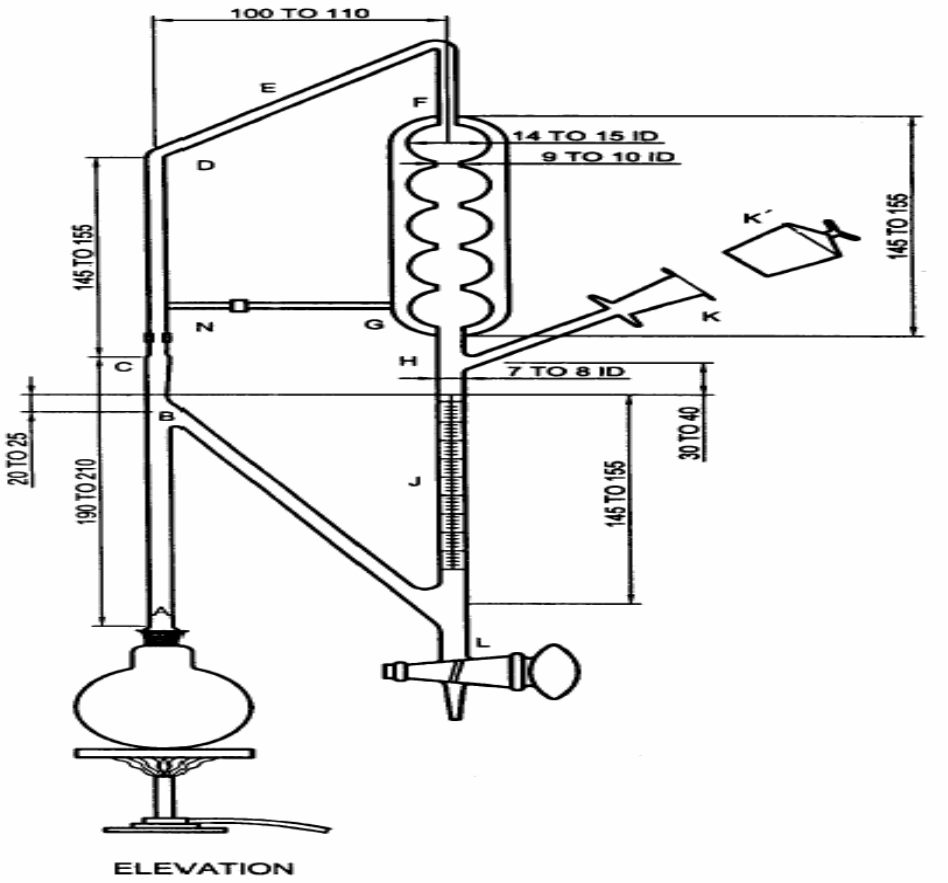
Fig. 12.4: Fat extraction Rose Gottlieb tubes with fitting

III) Volumetric Method

These involve dissolving the sample in sulphuric acid and centrifuging out the fat in specially calibrated glass vessels (butyrometers). The Gerber method is commonly employed for the routine determination of fat in milk and dairy products.

12.3.5 Volatile Oil

The method involves distilling the volatile oil over with boiling water, condensing and collecting the oil in a measured volume of xylene in a graduated tube. (Fig. 12.5).



All Dimension in millimeters.

Fig. 12.5: Apparatus for determination of volatile oil

12.3.6 Crude Fiber

The crude fibre representing the cell wall material left after boiling with dilute acid and alkali in the process, is a mixture of cellulose, lignin and pentosans, together with sand, silica and other mineral matter locked in the tissues and little nitrogenous matter after grinding and defatting, boiling with sulphuric acid solution, and separation and washing of the insoluble residue. This residue is boiled with sodium hydroxide solution, separated, washed, and dried and the insoluble residue is then weighed. The loss in mass on incineration is also noted.

12.3.7 Dietary Fiber

The current surge of interest in dietary fibre is attributed to its prophylactic and curative properties against colon cancer, coronary heart disease, obesity, gallstones, constipation, bowel irregularities and even hemorrhoids. The food industry is responding to the desires of today's consumers for fibre-rich products that can be used to foster their health, vitality and well-being. Dietary fiber refers to a macro-constituent of food, which includes the remnants of edible plant cells including polysaccharides (cellulose, hemicellulose, gums, mucilage, pectin), lignin, and associated substances *viz.*, oligosaccharides, waxes, cutin and suberin that are resistant to digestion in the alimentary tract of human being. Dietary fiber is separated from other constituents of food by means of enzymatic hydrolysis while crude fiber is separated by means of acid and alkali hydrolysis of other food constituents.

I) Total Dietary Fibre (TDF)

Duplicate test portions of dried foods (fat extracted if containing >10% at) are gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Four volumes of ethyl alcohol are added to precipitate soluble dietary fibre. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, residue is weighed. One duplicate is analyzed for protein and other is incinerated at 525°C and ash is determined.

$$\text{Total dietary fibre (\%)} = \frac{\text{Weight of residue–weight (protein + ash)}}{\text{Weight of sample}} \times 100$$

II) Soluble Dietary Fibre (SDF)

Duplicate test portions of dried foods, (fat extracted if containing >10%fat), are gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Insoluble dietary fibre is removed by filtering and washing residue with water. Soluble dietary fibre in filtrate is precipitated by adding 95% ethyl alcohol to filtrate. Precipitate is filtered and washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone, dried and weighed. One duplicate is analyzed for protein and second is incinerated at 525°C to determine ash. Soluble dietary fibre = Weight residue – weight (protein + ash).

III) Insoluble Dietary Fibre (IDF)

Duplicate test portions of dried foods, (fat extracted if containing >10%fat), are gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Enzyme digest is filtered and residue is washed with warm water, dried and weighed. Insoluble dietary fibre residue value is corrected for protein, ash and blank.

12.3.8 Total Ash

Ash refers to the inorganic residue remaining after total incineration of organic matter. The ash content is an indicator of product quality and the nutritional value of food products. When a high ash figure suggests the presence of an inorganic adulterant, it is advisable to determine the acid insoluble ash.

I) Dry Ashing

Dry ashing is the most standard method for determining the ash content of a food sample. The sample is commonly ignited at 550-600°C to oxidize all organic materials without flaming. The inorganic residue that does not volatilize at that temperature is called ash. The ash content is determined from the loss of weight, which occurs from complete oxidation of sample.

II) Wet Ashing

Wet ashing is usually used for the elemental analysis. Wet ashing commonly employs concentrated nitric acid and perchloric acid or nitric acid and sulphuric acid to oxidize the organic matter of the food sample. These acids are partially removed by volatilization and the soluble minerals remain dissolved in nitric acid. Any silica present is dehydrated and made insoluble. However, great care must be taken when using perchloric acid, because it can be explosive on contact with water.

12.3.9 Acid Insoluble Ash

The acid insoluble ash is a measure of the sandy matter and maxima are prescribed for herbs and spices. Acid insoluble ash is determined by dissolving ash in dilute hydrochloric acid (10% w/w), the liquid filtered through an ashless filter paper and thoroughly washed with hot water. The filter paper is then ignited in the original dish, cooled and weighed.

12.3.10 Sulphated Ash

This involves moistening the ash with concentrated sulphuric acid and igniting gently to constant weight. The sulphated ash gives a more reliable ash figure for sample containing varying amount of volatile inorganic substances that may be lost at the ignition temperature used.

12.3.11 Reducing and Non-Reducing Sugars

I) Lane and Eynon Volumetric Method

Reducing sugar and non-reducing sugar (after inversion) reduces the copper in Fehling's solution to the red precipitate cuprous oxide. The sugar content in a food sample is estimated by determining the volume of unknown sugar solution required to completely reduce a measured volume of Fehling's solution. The Fehling's solution is an alkaline solution of copper sulphate ($CuSO_4$. $5H_2O$, 6.9%) and Rochelle salt Sodium Potassium Tartrate ($KNaC_4H_4O_6.4H_2O$, 34.6%). In this method, methylene blue is used as an oxidation-reduction indicator of the end point. The methylene blue is added to the reaction mixture of sugar and Fehling's solution. Its use is based on the fact that it is reduced and completely decolourized by minute amounts of reducing sugar or invert sugar but not so long as any cupric salt is present. The reduction is carried out in a flask in which the liquid is kept boiling constantly to prevent reoxidation. In acid-base indicators, the change is often in the nature of colour i.e. in its position in the spectrum; but with oxidation-reduction indicators it is a change in intensity of colour.

II) Colorimetric Method

Food sample is clarified with the help of suitable clarifying agent and the filtrate has to be free from protein and fat. Phenol solution and concentrated sulphuric acid are added to an aliquot portion of the filtrate, thus producing a colour which is proportional to the amount of carbohydrate present, which is measured photometrically at a wavelength of 490 nm.

12.3.12 Starch

After the sugars present in the sample are leached out, starch is hydrolyzed using acid or enzyme and then the sugar is estimated.

I) Acid Hydrolysis Method

The sugar is leached out by precipitating starch with alcohol and the starch is hydrolyzed with conc. hydrochloric acid and the resulting reducing sugar is determined by titrimetric or by colorimetric method.

Starch (%) = % Reducing sugar × 0.90

II) Enzymatic Hydrolysis Method

Sugars are removed by leaching with alcohol. If much fat and proteinaceous materials are present, the sample is treated with hot ethanolic KOH and washed

with 80% ethanol. The starch in the residue is gelatinized and incubated with amyloglucosidase enzyme at pH 4.5 which converts the starch to glucose which is measured enzymatically using glucoxidase.

Let us answer a few questions before we move to the next section of physical and chemical testing of Fats and Carbohydrates. Microscopic field is very minute.

Check Your Progress Exercise 2

Note: a) Use the space below for your answers.
b) Check your answers with those given at the end of the unit.

1) What are the different techniques used for moisture determination in food products?

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2) Describe the principle of Kjeldahl method used for protein determination?

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3) Differentiate water content and water activity of food?

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4) Differentiate crude fibre and dietary fibre?

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5) What is the significance of ash content in foods?

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6) Explain the role of methylene blue indicator in the determination of sugar?

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12.4 PHYSICAL AND CHEMICAL PROPERTIES OF OILS AND FATS

Modern advances in oils and fats technology and the nutrition science have led to the need for greater awareness of the composition and structure of dietary lipids and many new advanced test methods and analytical procedures have been introduced. This section describes the general methods used to examine oils and fats for their physical and chemical properties and methods for assessing their quality criteria.

12.4.1 Acid Value and Free Fatty Acids

Acid value is the amount of KOH in milligram, required to neutralize the free fatty acids present in 1 g of the oil or fat. It is determined by directly titrating the material in an alcoholic medium with aqueous sodium or potassium hydroxide solution. Free fatty acid is calculated as oleic, lauric, ricinoleic or palmitic acids. Acid value when expressed, as mg of KOH/g of fat should be not more than 4.0 in virgin oils and 0.6 in non-virgin oils.

$$\text{Free fatty acid (\%) (as oleic acid)} = \frac{\text{Titre volume} \times \text{normality of NaOH} \times 28.2}{\text{Weight of sample}}$$

Acid Value = % FFA × 1.99

12.4.2 Unsaponifiable Matter

Unsaponifiable matter is that fraction of oils and fats which is not saponified by caustic alkali, but is soluble in ordinary fat solvents. The material is completely saponified with alcoholic potassium hydroxide solution and extracted with petroleum ether. The petroleum ether extract is washed with aqueous alcohol and then again with water. The washed ether extract is evaporated and the residue weighed. Unsaponifiable matter is this residue minus the fatty acid present in it, which is determined by titration with sodium hydroxide solution in alcoholic medium.

12.4.3 Melting Point

Oils and fats are chiefly mixtures of glycerides. They do not exhibit either a definite or a sharp melting point. Therefore, the term melting point does not imply the same characteristics that it does with pure crystalline substances. Fats pass through a stage of gradual softening before they become completely liquid. The melting point is, therefore, defined by the specific conditions of the method by which it is determined. The melting point is determined by taking the solid fat inside a small capillary tube and sample may be compared by measuring the temperature at which under specified conditions a column of fat fixed length rises in an open capillary tube under a definite pressure (slip point).

12.4.4 Solid-liquid Ratio

This provides information on the extent of saturation of triglycerides in fat, e.g. the extent of hydrogenation of oil or the suitability of fat for a particular use. The ratio can be measured by dilatometry. This is based on the measurement of isothermal expansion of the fat. The sample is melted, put in an enclosed calibrated glass tube known as a dilatometer and solidified under standardized conditions. The temperature of the solidified fat is then raised in 5°C stages and the volume of the fat measured each time until it is almost completely molten. By plotting the change in volume against temperature a melting dilation graph is obtained. However, determination of solid-liquid ratio in fat by Nuclear Magnetic Resonance (NMR) method is more suitable and accurate.

12.4.5 Specific Gravity

The specific gravity may be determined with a specific gravity bottle or pyknometer. The temperatures at which the specific gravity is determined shall be reported, namely, sp gr 30°C/30°C or sp gr 95°C/30°C.

12.4.6 Titre Value

When the molten fatty acids are cooled and begin to solidify, the latent heat of fusion is liberated and consequently a sudden rise in temperature can be observed. The sample is prepared from the fat by saponification and subsequent liberation of the fatty acids by dilute mineral acid. The washed and dried fatty acids are transferred to a test tube of specified dimensions in which they are cooled and agitated with a stirrer until solidification becomes noticeable. The highest/simplify temperature recorded, after mixing has been discontinued, is known as the titre value.

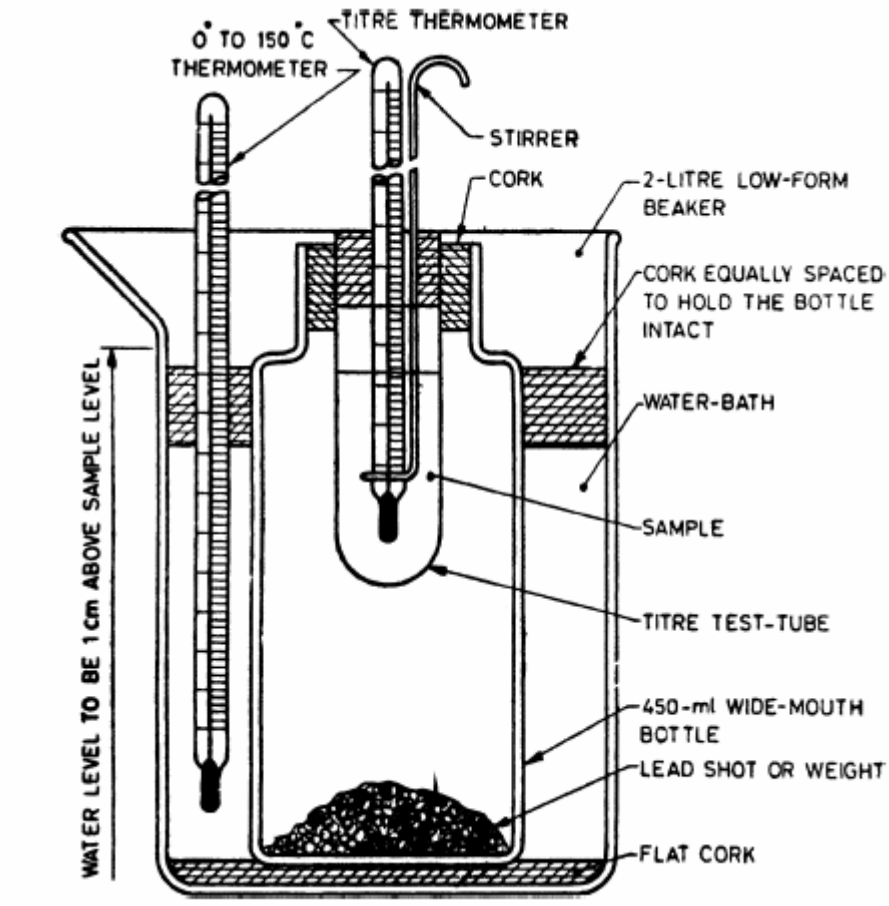


Fig. 12.6: Assembly of Apparatus for Titre Test

Determination of Titre — Fill the low-form beaker with water up to two-thirds of its capacity. Adjust the temperature of water between 15°C and 20°C below the expected titre point when it is not above 35°C, and at 20 ± 1°C when it is 35°C or higher. Fill the test-tube up to the mark with the fatty acid preparation at a temperature 10 to 12°C higher than the expected titre point. Insert the titre thermometer in the centre of the sample and adjust its height so that its immersion mark coincides with the top surface of the fatty acid layer. When the temperature of the fatty acid comes down to about 10°C higher than the titre point, set the stirrer moving in a vertical direction at a rate of about 60 complete up and down motions per minute. The temperature of the fatty acid gradually comes down and stirring is continued until the temperature remains constant for 30 seconds. The stirring is stopped when the temperature begins to rise and the stirrer is raised out of the sample. The highest temperature recorded by the thermometer during this rise is the titre point. Duplicate determinations should agree within 0.2°C.

12.4.7 Colour

This 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. The colour may also be measured in a spectrophotometer using carbon tetrachloride as blank at the wavelength of maximum absorption.

12.4.8 Iodine Value (Wij's)

Iodine value is a measure of level of unsaturation in fat. Iodine value is the amount of iodine in (g) absorbed per 100 g of the oil or fat. The material is treated, in carbon tetrachloride medium, with a known excess of iodine monochloride solution in glacial acetic acid (Wijs solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with standard sodium thiosulphate solution.

$$\text{Iodine value (mg/100g)} = \frac{\text{Titre volume (blank – sample)} \times \text{normality of } Na_2S_2O_3 \times 12.69}{\text{Weight of sample}}$$

$$H_2O + ICl \rightarrow IOH + HCl$$

$$KI + HOH \rightarrow KOH + HI$$

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$$

12.4.9 Saponification Value

When fat is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution, the triglycerides hydrolyze, while glycerol and soap are formed. The alkali consumed for this hydrolysis is a measure of the saponification value, which is determined by titrating the excess alkali with standard hydrochloric acid. Saponification value is defined as the amount of KOH in mg required in saponifying completely 1 g of oil or fat. It is also a measure of the mean molecular weight of the fatty acids originally bound as triglycerides.

$$\text{Saponification value} = \frac{\text{titre value (blank – sample)} \times \text{normality of HCL} \times 56.1}{\text{Weight of sample}}$$

12.4.10 Acetyl Value and Hydroxyl Value

The acetyl value of fats or oils defined as the amount of KOH in mg required for the neutralization of the acetic acid obtained by the saponification of 1 g of the acetylated product.

The process consists in acetylating the oil or fat with a measured quantity of acetic anhydride in pyridine decomposing the excess anhydride by boiling with water and then, after the addition of sufficient butyl alcohol to give a homogeneous solution, titrating with alcoholic alkali. A control test with the acetic anhydride and pyridine without the oil or fat provides a measure of the acetic anhydride available for acetylation; a similar test with the oil or fat and the pyridine without the acetic anhydride provides a measure of the free fatty acid present. From the figures obtained, the acetyl value or the hydroxyl value of the fat is calculated.

12.4.11 Reichert-Meissl (RM) Value

Reichert Meissl value is a measure of water soluble steam volatile fatty acids chiefly butyric and caproic acids. RM value is the number of ml of 0.1N NaOH solution required to neutralize the steam volatile water soluble fatty acids distilled from 5 g of oil or fat under the precise conditions. The material is saponified by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulphuric acid. The volatile acids are immediately steam distilled. The soluble volatile acids in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution. RM value is a unique test used for evaluation of adulteration of milk fat with other fat.

12.4.12 Polenske Value

Polenske value differs from the RM value in that it is a measure of ‘steam volatile’ but of water insoluble fatty acid like caprylic, capric and lauric acids present in oils and fats. The condenser, the 25-ml cylinder and the receiver used in the Reichert-Meissl value determination are washed into the filter paper through which the distillate was filtered for that determination. After rinsing, the residue on the filter paper is taken up with ethyl alcohol and titrated with standard sodium hydroxide solution.

12.4.13 Rancidity

The reactions that take place when a fat becomes rancid are only partly understood. It appears that the more common type of rancidity that results in the formation of a rancid odour and taste is due to oxidation at the double bonds. Apparently, peroxides are formed in the early stages of the process and these are later decomposed into aldehydes, ketones and acid fragments. The evaluation of rancidity can be done by determining different oxidation products.

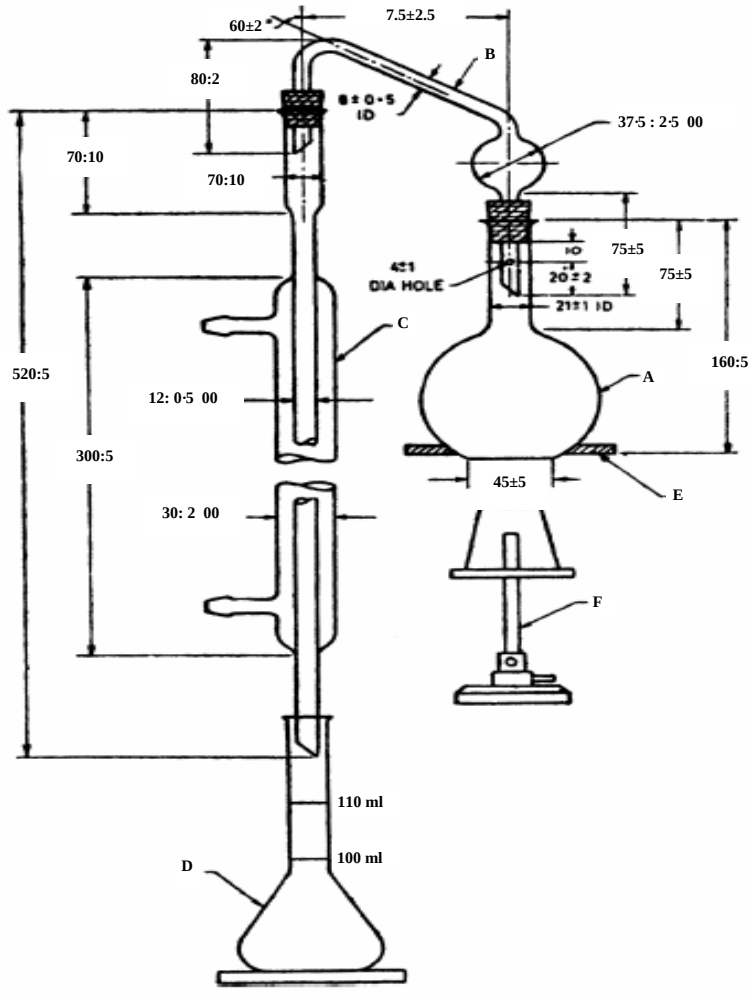
I) **Kreis Test:** The method employs phloroglucinol as a reagent in ether in the presence of concentrated hydrochloric acid. If epihydrinaldehyde is present, a reddish addition product is formed, indicating rancidity. Addition reaction of aldehydes with phloroglucinol can be determined using spectrophotometric detection at 540 nm.

II) Peroxide Value: The peroxide value is an indicator of oxidative rancidity in fats. However, the incipient stages of rancidity can be detected by this test before the spoilage can be detected organoleptically. The peroxide value is a measure of the peroxides contained in a sample of fat, expressed as milli-equivalents of peroxide oxygen per kg of the material. The material in an acetic acid-chloroform medium is treated with an aqueous solution of potassium iodide. The liberated iodine is titrated with standard sodium thiosulphate solution. The peroxide value of fresh oil is less than 10.

$$\text{Peroxide value (meq/1000g)} = \frac{\text{Normality of } Na_2S_2O_3 \times \text{titre volume} \times 1000}{\text{Weight of sample}}$$

$$ROO + KI \xrightarrow{H^+/\Delta} ROH + KOH + I_2$$

$$2Na_2S_2O_3 + I_2 + \text{starch} \rightarrow \text{starch} - I_2 + Na_2S_4O_6 + 2NaI$$



All dimensions in millimeters.

Fig. 12.7: Reichert-Meissl Distillation Apparatus

Check Your Progress Exercise 3

Note: a) Use the space below for your answers.
b) Check your answers with those given at the end of the unit.

1) What are the physical parameters used for the quality evaluation of oils and fats?

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2) Define the iodine value? What is the significance of iodine value?

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3) Define RM value? Briefly discuss its method of determination?

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4) Which test will you perform to evaluate the rancidity in oil?

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12.5 LET US SUM UP

The physical and chemical analysis techniques used for food analysis are enumerated in this unit. The principles and methods of determining different physical parameters of food products are discussed in this unit. The chemical analysis of foods includes proximate analysis and ultimate analysis. The proximate analysis consists of determining the moisture, fat, protein, sugar, starch, ash and crude fibre or dietary fibre. The suitability of different chemical analysis techniques for determining different food constituents is briefly discussed. However, the ultimate analysis that refers to the determination of a particular element or a compound present in the material is out of the scope. The physico-chemical properties of oils and fats are also included in this unit.

12.6 KEY WORDS

Dryi	**:**	An o

n g

p e r a t i o n

i n

w h i c h

w a t e r

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r e m o v e d

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Refluxing **:** An operation in which a part of the product str

e a m t h a t i s r e t u r n e d t o t h e c h e m i c a l p r o c e s s d u r i n g h

eating to assisting in giving increased conversion or re

Distillation : The process of producing a vapour from a liquid and recovery.

d by heating the liquid in a vessel and collecting an

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yzing the composition of a solution by adding known a

amounts of a standardized solution until a given reaction

ion (colour change, precipitation, or conductivity ch

Vapour Pressure : a range) is produced. For a liquid or solid, the pressure

of the vapour in equilibrium with the liquid or soli

d a t a p a r t i c u l a r t e m p e r a t u r e .

B i u r e t **:** A c o n d e n s a t i o n p r o d u c t

of urea, also known as carboomyl urea ($NH_2CONHCONH_2$).

Oxidation-reduction Indicator : A compound whose colour in the oxidized state differs

from that in the reduced state.

Volatile Oil : An oil with the tende

ncy to become vapour generally found by the distillat

ion of spices or condiments. Its components are mainl

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12.7 TERMINAL QUESTIONS

1) Describe the importance of filth in food products? Briefly discuss the method of determination of filth?

2) Discuss the method of gradation of cereals according to the size of grains?

3) Describe at least three different techniques for the measurement of moisture in food products?

4) Explain the different factors responsible for the error in protein determination by Kjeldahl method?

5) Discuss the different steps involved in the determination of total dietary fibre in foods?

6) Describe the effect of water activity on different physico-chemical properties of foods?

7) How can you determine starch in foods containing higher content of sugar?

8) Name the different physico-chemical parameters oil by which we can evaluate the purity of oil?

12.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include following points:

1) Filth is any objectionable matter contributed by animal contamination of product such as rodent, insect or bird matter or any other objectionable matter contributed by insanitary conditions. Heavy filth can be separated by sedimentation based on different densities of filth, after immersing food particles in solvents like chloroform.

2) Specific heat is the number of calories (heat) required to raise the temperature of one gram of substance by 1°C. Heat = Weight × specific heat × change of temperature.

3) Ostwald viscometer is suitable for measuring the viscosity of liquid food product with low consistency. Ostwald viscometer is a capillary viscometer which gives more accurate result than any other viscometer.

4) The specific gravity of a substance is the ratio of its weight to the weight of an equal volume of another substance taken as standard while density is the ratio of mass to a fixed volume of a material at a particular temperature.

5) The surface tension is defined as the force acting upon a line of unit (1cm) length in the surface of the liquid. The falling drop method is a simple method used to measure the surface tension of a liquid.

Check Your Progress Exercise 2

Your answer should include following points:

1) More frequently moisture in food products is determined by methods like oven drying, vacuum drying, distillation, Karl Fischer titration, etc.

2) The Kjeldahl method for protein determination in foods follows three basic steps: (a) Digestion – decomposition of organic matter by heating in the presence of concentrated sulphuric acid, the end result is ammonium sulphate solution. (b) Distillation – Ammonium sulphate is converted into gaseous ammonia by addition of an excess base, followed by boiling and condensation of the ammonia in a receiving solution. (c) Titration – quantification of the ammonia released from the digest.

3) The water activity is not determined by the total quantity of water in a sample but only by that fraction which is least tightly bound.

4) Dietary fiber is separated from other constituents of food by means of enzymatic hydrolysis while crude fiber is separated by means of acid and alkali hydrolysis of other food constituents.

5) The ash content is an indicator of product quality and the nutritional value of food products; e.g. milk powder with high ash content indicates adulteration with alkali neutralizer.

6) In Lane and Eynon titration method for sugar estimation, methylene blue is used as an oxidation-reduction indicator of the end point. Its use is based on the fact that it is reduced and completely decolourized by minute amounts of reducing sugar or invert sugar but not so long as any cupric salt is present.

Check Your Progress Exercise 3

Your answer should include following points:

1) The important physical parameters used for the quality evaluation of oils/fats are specific gravity, refractive index, melting point, colour, etc.

2) Iodine value is defined as the number of g of iodine absorbed per 100 g of the oil or fat under specified conditions. Iodine value indicates the level of unsaturation in fat.

3) RM value is defined as the number of ml of 0.1N NaOH solution required to neutralize the steam volatile water soluble fatty acids distilled from 5 g of oil or fat under the precise conditions. This is determined by saponifying fat by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulphuric acid. The volatile acids are immediately steam distilled. The soluble volatile acids in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution.

4) Definitely rancid fat proclaims itself by odour and taste but it is not so simple to determine the presence of rancidity in the early stages of development. The rancidity in fat or oil can be evaluated either by Kreis test or by determining peroxide value.

12.9 SOME USEFUL BOOKS

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