

**Lipids:** Classification, general methods of analysis, refining of fats and oils; hydrogenation of vegetable oils, Determination of adulteration in fats and oils, Various methods used for measurement of spoilage of fats and fatty foods.

- Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules that can be extracted from tissues by nonpolar solvents, because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles or on albumin. Lipids are a major source of energy for the body, and they provide the hydrophobic barrier. Lipids serve additional functions in the body, for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis.

## Classification of lipids

1. **Simple lipids:** Esters of fatty acids with various alcohols.

a. *Fats:* Esters of fatty acids with glycerol. **Oils** are fats in the liquid state.

b. *Waxes:* Esters of fatty acids with higher molecular weight monohydric alcohols.

2. **Complex lipids:** Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

a. *Phospholipids:* Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, eg, in **glycerophospholipids** the alcohol is glycerol and in **sphingophospholipids** the alcohol is sphingosine.

b. *Glycolipids (glycosphingolipids):* Lipids containing a fatty acid, sphingosine, and carbohydrate.

c. *Other complex lipids:* Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.

3. **Precursor and derived lipids:** These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins and hormones.

- Fatty acids occur mainly as esters in natural fats and oils but do occur in the unesterified form as **free fatty acids**, a transport form found in the plasma. Fatty acids that occur in natural fats are usually straight-chain derivatives containing an even number of carbon atoms. The chain may be **saturated** (containing no double bonds) or **unsaturated** (containing one or more double bonds).

- Saturated Fatty Acids may base on acetic acid ( $\text{CH}_3\text{COOH}$ ) as the first member of the series in which  $-\text{CH}_2-$  is progressively added between the terminals  $-\text{CH}_3-$  and  $-\text{COOH}-$  groups.

- Unsaturated Fatty Acids contain one or more double bonds and it may be further subdivided as follows:

(1) Monounsaturated (monoethenoid, monoenoic) acids, containing one double bond.

(2) Polyunsaturated (polyethenoid, polyenoic) acids, containing two or more double bonds.

(3) Eicosanoids: These compounds, derived from eicosa- (20-carbon) polyenoic fatty acids, comprise the **prostanoids, leukotrienes (LTs), and lipoxins (LXs)**. Prostanoids include **prostaglandins (PGs), prostacyclins (PGIs), and thromboxanes (TXs)**.

- Saturated fatty acids.

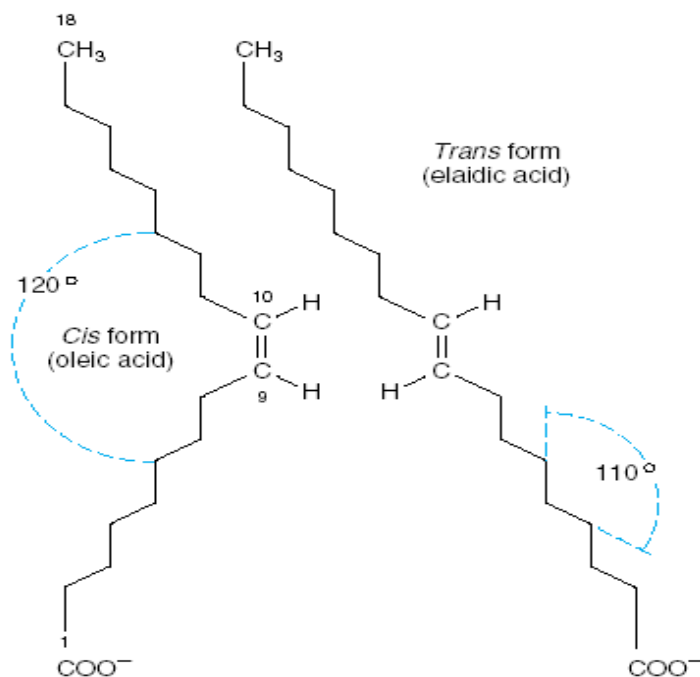
Common Name	Number of C Atoms	
Acetic	2	Major end product of carbohydrate fermentation by rumen organisms <sup>1</sup>
Propionic	3	An end product of carbohydrate fermentation by rumen organisms <sup>1</sup>
Butyric	4	In certain fats in small amounts (especially butter). An end product of carbohydrate fermentation by rumen organisms <sup>1</sup>
Valeric	5	
Caproic	6	
Lauric	12	Spermaceti, cinnamon, palm kernel, coconut oils, laurels, butter
Myristic	14	Nutmeg, palm kernel, coconut oils, myrtles, butter
Palmitic	16	Common in all animal and plant fats
Stearic	18	

- Unsaturated Fatty Acids

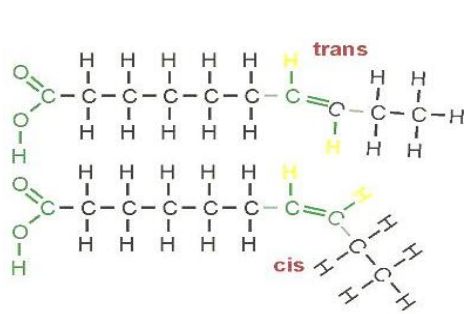
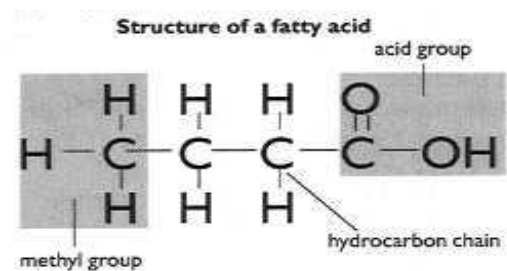
Number of C Atoms and Number and Position of Double Bonds	Common Name	Occurrence
<b>Monoenoic acids (one double bond)</b>		
16:1;9	Palmitoleic	In nearly all fats.
18:1;9	Oleic	Possibly the most common fatty acid in natural fats.
18:1;9	Elaidic	Hydrogenated and ruminant fats.
<b>Dienoic acids (two double bonds)</b>		
18:2;9,12	Linoleic	Corn, peanut, cottonseed, soybean, and many plant oils.
<b>Trienoic acids (three double bonds)</b>		
18:3;6,9,12	$\gamma$ -Linolenic	Some plants, eg, oil of evening primrose, borage oil; minor fatty acid in animals.
18:3;9,12,15	$\alpha$ -Linolenic	Frequently found with linoleic acid but particularly in linseed oil.
<b>Tetraenoic acids (four double bonds)</b>		
20:4;5,8,11,14	Arachidonic	Found in animal fats and in peanut oil; important component of phospholipids in animals.

### Chemistry of Fatty acids

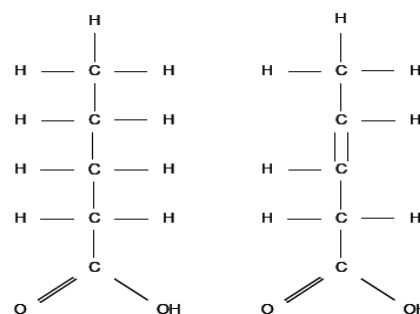
- The carbon chains of saturated fatty acids form a zigzag pattern when extended, as at low temperatures. At higher temperatures, some bonds rotate, causing chain shortening,
- A type of **geometric isomerism** occurs in unsaturated fatty acids, depending on the orientation of atoms or groups around the axes of double bonds, which do not allow rotation. If the acyl chains are on the same side of the bond, it is *cis*-, as in **oleic acid**; if on opposite sides, it is *trans*-, as in **elaidic acid**, the *trans* isomer of **oleic acid**.
- Naturally occurring unsaturated long-chain fatty acids are nearly all of the *cis* configuration, the molecules being "bent" 120 degrees at the double bond. Thus, **oleic acid** has an **L shape**, whereas **elaidic acid** remains "straight."
- Increase in the number of *cis* double bonds in a fatty acid leads to a variety of possible like **arachidonic acid**, with four *cis* double bonds, has "kinks" or a **U shape**.
- *Trans* double bonds alter these spatial relationships.
- The melting points of even-numbered-carbon fatty acids increase with chain length and decrease according to unsaturation.



Geometric isomerism of fatty acids (oleic and elaidic acids)

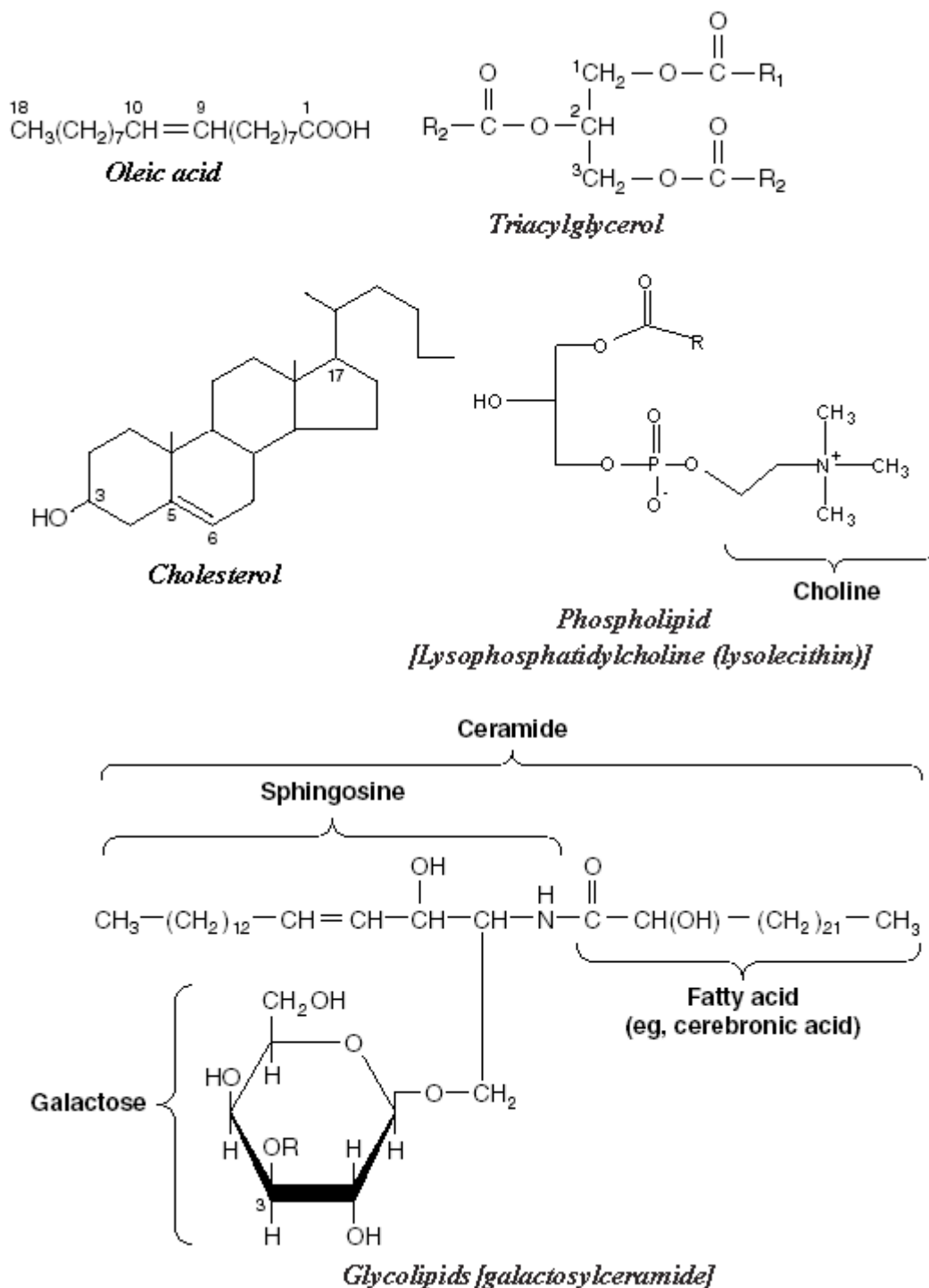


Trans and Cis- Fatty acid



Saturated Fatty acid Unsaturated Fatty acid

▪ Structures of some common classes of lipids



**Note By:** ► Naturally, occurring unsaturated vegetable oils have almost all **Cis** bonds, but using oil for frying causes some of the **Cis** bonds to convert to **Trans** bonds.

► Fatty acids with **Tran's** bonds are **carcinogenic**.

## Sources of Fats and Oils

- Fats and oil may be of vegetable, animal, and marine origin. Vegetable fats include the solid fat cocoa butter and the oils such as corn oil, sunflower oil, soybean oil, cotton oil, peanut oil, olive oil, canola oil, pumpkin seed oil, safflower oil, grape seed oil, sesame oil bran oil, argan oil, palm oil, linseed oil, coconut oil.
- Typically, common vegetable oils including soybean. Sunflower, safflower, mustard, olive, rice bran, sesame is low in saturated fats. While palm oil, palm kernel oil, coconut oil, tallow and butter fat are high in saturated fats.

Common saturated fatty acids in food fats and oils		Some common <i>cis</i> -Monounsaturated fatty acids in fats and oils	
Name	Sources	Name	Sources
Butyric	dairy fat	Palmitoleic	marine oils, macadamia oil, most animal and vegetable oils
Caproic	dairy fat		
Caprylic	dairy fat, coconut and palm kernel oils	Oleic	all fats and oils, especially olive oil, canola oil and high-oleic sunflower & safflower oil
Capric	dairy fat, coconut and palm kernel oils		
Lauric	coconut oil, palm kernel oil	<i>cis</i> -Vaccenic	most vegetable oils
Myristic	dairy fat, coconut oil, palm kernel oil	Gadoleic	marine oils
Palmitic	most fats and oils	Erucic acid	mustard seed oil, high erucic rapeseed oil
Stearic	most fats and oils	Nervonic	marine oils
Arachidic	peanut oil		
Behenic	peanut oil		
Lignoceric	peanut oil		

## Nutritionally important Polyunsaturated fatty acids

Name	Sources
Linoleic acid	most vegetable oils
$\gamma$ -Linolenic acid	borage and black currant seed oils
Homo- $\gamma$ -linolenic acid	very minor component in animal tissues
Arachidonic acid	animal fats, liver, egg lipids, fish
$\alpha$ -Linolenic ( $\omega$ -3 fatty acid)	flaxseed oil, perilla oil, canola oil, soybean oil
Stearidonic acid ( $\omega$ -3 fatty acid)	fish oils, genetically enhanced soybean oil, blackcurrant seed oil, hemp oil and very minor component in animal tissues
Eicosapentaenoic acid (omega-3 fatty acid)	fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
<b><i>Omega-3 fatty acid</i>: An unsaturated fatty acid of a kind occurring chiefly in fish oils, with double bonds between the carbon atoms that are third and second from the end of the hydrocarbon chain.</b>	

## General Methods of Analysis of Fats and Oils

- Fats and oils are triesters of glycerol and higher fatty acids. At ordinary temperatures, oils are liquids while fats are solids. Fats are present in gums, oils and milk foods. They are insoluble in water sparingly soluble in alcohol and soluble in chloroform. They serve as an excellent source of energy providers to the body.
- Fats and oils are greasy in nature and on hydrolysis with aqueous or alcoholic sodium or potassium hydroxide liberate glycerol. Fats and oils are of vegetable or animal origin.
- The following are the tests to identify the presence of fats and oils.
  - 1) Solubility test
  - 2) Translucent spot test
  - 3) Acrolein test
  - 4) Baudouin test
  - 5) Huble's test

### (a) Solubility Test

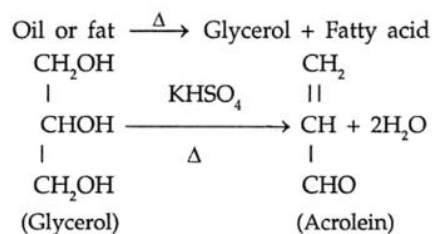
- Fats are soluble in organic solvents like chloroform, alcohol, etc. It is insoluble in water. So, if the given sample forms an oily layer above the surface of the water then fat is present. Partially soluble in alcohol and fully soluble in chloroform than the presence of fat is confirmed.
- *Note: If the sample is miscible with chloroform and immiscible with water the fat presence is confirmed.*

### (b) Translucent Spot Test

- In this test the given sample to be tested is rubbed between the folds of filter paper. The appearance of translucent spot confirms the presence of fats in the given sample.
- *Note: If there is presence of translucent spot then the presence of fats is confirmed.*

### (c) Acrolein Test

- Fats and oils when heated with some crystals of potassium bisulphate (KHSO<sub>4</sub>) in a test tube. A pungent irritating odour or smell of acrolein confirms the presence of fat or oil.



- *Note: If there is a pungent irritating odor then the presence of fats or oil is confirmed.*

### (d) Baudouin Test

- Baudouin test is applied to distinguish between desi ghee and vanaspati ghee. Vanaspati ghee contains 5% sesame oil. Pure ghee does not contain sesame oil. Fats and oils are treated with 5ml of concentrated hydrochloric acid and 2% furfural solution in alcohol. After 5-10 minutes rose-red colour appearance shows the presence of sesame oil in the given sample.
- *Note: This test is used to find out whether the given sample of desi ghee contains vanaspati or not.*

### (e) Huble's Test

- This test is used to know the degree of unsaturation in the given sample. Oils on reaction with Huble's reagent fads the violet colour of iodine then it is unsaturated and if the colour persists then the given fat or oil is saturated.
- *Note: In this test various oils can be compared on the basis of unsaturation.*

### ❖ Important Parameters for Analysis of Edible Oils and Fats

- Hence, several standards and norms describe the calculation of the most crucial quality control parameters.
  - 1) Transparency
  - 2) Colour
  - 3) Odour
  - 4) Taste
  - 5) Relative density
  - 6) Refractive Index
  - 7) Melting point
  - 8) Freezing point
  - 9) Smoke point
  - 1) Hydroxyl value according
  - 2) Iodine value
  - 3) Peroxide value
  - 4) Water content
  - 5) Saponification value
  - 6) Oxidation stability
  - 7) Acid value and free fatty acids value
  - 8) Ester Value
  - 9) Reichert Meissl (RM) Value
  - 10) Acetyl Value
  - 11) Iodine Value
  - 12) Nickel traces using polarography
  - 13) Anisidine value (in oils) test

*Essential Composition and Quality Factors of Crude Coconut Oil*

<i>Parameter</i>	<i>Specification</i>
<i>Moisture &amp; Impurities (%)</i>	Max 0.1
<i>Volatile Matters at 120°C (%)</i>	Max 0.2
<i>Free Fatty Acid (%)</i>	Max 0.2
<i>Peroxide Value (meq/kg)</i>	Max 3
<i>Relative density</i>	0.915 – 0.920
<i>Refractive index at 40°C</i>	1.4480 – 1.4492
<i>Insoluble impurities per cent by mass</i>	Max 0.05
<i>Saponification Value (Mg KOH/g oil)</i>	250 – 260 min
<i>Iodine Value</i>	4.1 -11
<i>Unsaponifiable matter % by mass, max</i>	0.2 - 0.5
<i>Specific gravity at 30 deg./30°C</i>	0.915 – 0.920
<i>Polenske Value, min</i>	13
<i>Total Plate Count</i>	< 0.5
<i>Colour</i>	Water Clear
<i>Odor and Taste</i>	Natural fresh coconut scent, free of sedi-ment, free from rancid odor and taste

### ✓ *Transparency, Colour, Odour and Taste*

- In general, pure fats and oils are colourless, odourless, and tasteless. The characteristic colours, odours, and flavours that we associate with some of them are imparted by foreign substances that are lipid soluble and have been absorbed by these lipids. For example, the yellow colour of butter is due to the presence of the pigment carotene; the taste of butter comes from two compounds – **diacetyl** and **3-hydroxy-2-butanone** – produced by bacteria in the ripening cream from which the butter is made.

### ✓ *Relative Density*

- In general, the relative density of most oils, both mineral and vegetable, is between 0,840 and 0,960. A simple and general oil definition could be oils are fatty substances that are in liquid state at room temperature. Oils are usually classified by its origin.
- The density of the oils varies with each type and temperature. The range is from 0.91 to 0.93 g/cm<sup>3</sup> between the temperatures of 15 °C and 25 °C. Comparing to water, whose density is 1.00 g/ml, cooking oil is less dense.
- **PYCNOMETER** is used to measuring the volume and the density of Oils and Fats

### ✓ *Refractive Index*

- The **Refractive Index** is measured under different temperature conditions applied such as 20°C for oils, 40°C for solid fats which are fully molten at that temperature, 60°C for hydrogenated fats and 80°C for waxes. Refractive index is very specific for a particular oil or fat. Each oil has a specific refractive index measured at a certain temperature, so we can differentiate them. Some of the refractive indices of important oils are described below at 20°C.

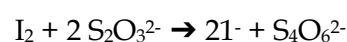
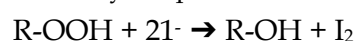
<i>Oil</i>	<i>Refractive Index</i>
<i>Peanut Oil</i>	1.460 - 1.465
<i>Maize Oil</i>	1.465 - 1.468
<i>Mustard Oil</i>	1.461 - 1.469
<i>Sesame Oil</i>	1.465 - 1.469
<i>Soybean Oil</i>	1.466 - 1.470
<i>Sunflower Oil</i>	1.461 - 1.468
<i>Palm Oil</i>	1.458 - 1.460 (at 40 °C)

### ✓ *Water Content*

- Water is an inevitable contaminant in both fresh oil (trace levels) and in frying oil (at high levels) being introduced by food. According to Food Standards Committee, the moisture content of the oil is of great importance for many scientific, technical and economic reasons. Low moisture content is a requirement for long storage life.
- The maximum limit of moisture in edible oils according to APCC (Association of Professional Compliance Consultants) should be 0.1%-0.5%.
- To determine the exact content of water **Karl Fischer titration** is used.
- **Reaction:**  $\text{CH}_3\text{OH} + \text{SO}_2 + 3\text{RN} + \text{I}_2 + \text{H}_2\text{O} \rightarrow (\text{RNH})\text{SO}_4\text{CH}_3 + 2(\text{RNH})\text{I}$

### ✓ *Determination of Peroxide Value (POV) in fats and oils*

- The Peroxide number (POV) is indicator for the state of unsaturated oils and fats. Unsaturated oils and fats become rancid by oxidation, forming peroxides.
- The determination of the POV is done by titration with sodium thiosulfate after reaction of the sample with potassium iodide, wherein the iodide is oxidized by the peroxides to iodine.



- The solvent used for the sample is a mixture of glacial acetic acid and chloroform. Depending on the sample, it is also possible to use decanol or hexanol instead of chloroform. The POV is calculated as mmol<sub>peroxide</sub>/kg.



### ✓ Thermo-Physical Properties (Melting point and Freezing point) of Fats and Oils

- Freezing point is the temperature at which a liquid becomes a solid at normal atmospheric pressure. Alternatively, a melting point is the temperature at which a solid becomes a liquid at normal atmospheric pressure.
- Melting point of fats is used to characterize oils and fats and is related to their physical properties, such as hardness and thermal behaviour.
- The melting point of lipids can be determined by introducing a tiny amount into a small capillary tube, attaching this to the stem of a thermometer centred in a heating bath, heating the bath slowly, and observing the temperatures at which melting begins and is complete.
- The melting point demonstration shows us that the lipids from animals (lard, butter) are more saturated than the lipids from plants (olive oil, safflower oil). Also, while the oils appear to have similar melting points, the lard has a much higher melting point than the butter.

Fat	Melt/Freeze Point Temperature	Monounsaturated Fat %	Polyunsaturated Fat %	Saturated Fat %
Cottonseed Oil	-55° F (48° C)	18	54	28
Flax Seed Oil	-11° F (-24° C)	21	71	8
Almond Oil	0° F (-18° C)	73	19	8
Sunflower Oil	1° F (-17° C)	20	69	11
Soybean Oil	3° F (-16° C)	24	61	15
Rice Bran Oil	14-23° F (-5 to -10° C)	38	37	25
Olive Oil	21° F (-6° C)	79	8	11
Peanut Oil	37° F (3° C)	48	34	18
Palm Kernel Oil	75° F (24° C)	12	2	86
Coconut Oil	77° F (25° C)	6	2	92
Cocoa Butter	93-100° F (34 to 38° C)	20 to 43	0 to 5	57 to 64
Palm Oil	95° F (35° C)	38	10	52

### ✓ Smoke Point

- The smoke point, also referred to as the burning point, is the temperature at which an oil or fat begins to produce a continuous bluish smoke that becomes clearly visible, dependent upon specific and defined conditions.
- Smoke point values can vary greatly, depending on factors such as the volume of oil utilized, the size of the container, the presence of air currents, the type and source of light as well as the quality of the oil and its acidity content, otherwise known as free fatty acid (FFA) content.
- The more free fatty acids (FFA) an oil contains, the quicker it will break down and start smoking. The lower in FFA, the higher the smoke point.
- It is essential to know and pay attention to an oil's smoke point because using burned oil to cook does more than give your food an unpleasant flavour.
- A high smoke point is considered 205° C and higher, and oils with a high smoke point are best used for frying. These include avocado oil, canola oil, corn oil, and peanut oil.

Fat	Smoke point	
Almond oil	221 °C	430 °F
Avocado oil	270 °C	520 °F
Beef tallow	250 °C	480 °F
Coconut oil (Refined, dry)	204 °C	400 °F
Coconut oil (Unrefined, dry expeller pressed, virgin)	177 °C	350 °F
Mustard oil	250 °C	480 °F
Olive oil (Refined)	199-243 °C	390-470 °F
Olive oil (Virgin)	210 °C	410 °F

### ✓ Acid Value

- In chemistry, acid value (or neutralization number or acid number or acidity) is the mass of potassium hydroxide (KOH) or sodium hydroxide (NaOH) in milligrams that is required to neutralize the free fatty acids in 1 g of the fat.
- The acid number is a measure of the number of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds.
- The acid number is used to quantify the acidity of a substance. It is the quantity of base, expressed in milligrams of potassium hydroxide or sodium hydroxide, which is required to neutralize the acidic constituents in 1 g of sample.
- **Method and procedure:**

#### (a) Reagents

- o *Phenolphthalein indicator:* Weigh 1 g of phenolphthalein and dissolve in 100 mL of ethanol.
- o *Sodium hydroxide titrant:* Weigh accurately 4.0 g of sodium hydroxide and place it in a 1000-mL volumetric flask. Make up to the mark with water.
- o *Ethanol-ether solution:* Prepare a mixture of ethanol and diethyl ether (1:1, v/v). Neutralize with sodium hydroxide titrant and add 1.0 mL of phenolphthalein indicator until pink coloration is observed. Freshly prepare the solution.

#### (b) Standardization of sodium hydroxide titrant

- o Weigh accurately 0.6 g of potassium hydrogen phthalate, previously dried to constant weight at 105°C, and place it in a 250-mL conical flask, then add 50 mL of water. Shake it well.
- o Then add 2 drops of phenolphthalein indicator.
- o Titrate the solution with the sodium hydroxide titrant until pink colouration can be observed.
- o Towards the end of titration, potassium hydrogen phthalate should be completely dissolved.
- o Calculate the concentration of the sodium hydroxide titrant according to the following equation:

$$C_{\text{NaOH}} = \frac{W_{\text{C}_8\text{H}_5\text{KO}_4} \times P_{\text{C}_8\text{H}_5\text{KO}_4} \times 1000}{V_{\text{NaOH}} \times \text{Mw}_{\text{C}_8\text{H}_5\text{KO}_4}}$$

where

- $C_{\text{NaOH}}$  = Molarity of sodium hydroxide titrant (mol/L)
- $V_{\text{NaOH}}$  = Volume of sodium hydroxide titrant used (mL)
- $\text{Mw}_{\text{C}_8\text{H}_5\text{KO}_4}$  = Molecular weight of potassium hydrogen phthalate (204.22 g)
- $W_{\text{C}_8\text{H}_5\text{KO}_4}$  = Weight of potassium hydrogen phthalate used (g)
- $P_{\text{C}_8\text{H}_5\text{KO}_4}$  = Purity of potassium hydrogen phthalate (%)

#### (c) Titration of test solution

- o Weigh accurately a quantity of the fatty oil and place it in a 250-mL conical flask, then add 50 mL of ethanol-ether solution.
- o Shake it well. If necessary, reflux the mixture gently until the substance is completely dissolved.
- o Titrate the solution with sodium hydroxide titrant until pink colouration can be observed which persists for 30 second.
- o Measure the volume of sodium hydroxide titrant used and calculate the acid value according to the following equation:

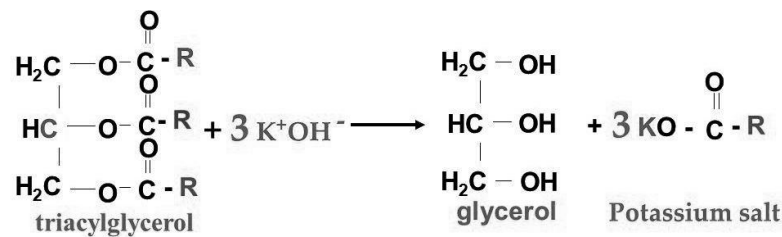
$$\text{Free fatty acid (\%)} = \frac{\text{Titre volume} \times \text{normality of NaOH} \times 28.2}{\text{Weight of sample}}$$

$$\text{Acid Value} = \% \text{ FFA} \times 1.99$$

### ✓ Saponification value

- Fats and oils are the principle stored forms of energy in many organisms. They are highly reduced compounds and are derivatives of fatty acids.
- Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons; they can be saturated or unsaturated. The simplest lipids constructed from fatty acids are triacylglycerol's or triglycerides.

- Triacylglycerol's are composed of three fatty acids each in ester linkage with a single glycerol. Since the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages, triacylglycerol's are non-polar, hydrophobic molecules, which are insoluble in water.
- Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid.



- **Definition:** Saponification value of oil, fat or of an ester is defined as the number of milligrams of potassium hydroxide (KOH) required to completely neutralizing the free acids to saponify the esters present in 1gm of the substance. (i.e. to neutralize the fatty acid resulting from the complete hydrolysis of 1gm of the oil or fat.)
- **Significance of Saponification Value:**
  - o The magnitude of saponification value gives an idea about the average molecular weight of the fat or oil.
  - o Higher the molecular weight of the fat, the smaller is its saponification value.
  - o Saponification Value also indicates the length of carbon chain of the acid present in that particular oil or fat.
  - o Higher the saponification value, greater is the percentage of the short chain acids present in the glycerides of the oil or fats.
- **Reagents Required:**
  - Ethanolic KOH (95% ethanol, v/v)
  - Potassium hydroxide [0.5N]
  - Fat solvent
  - Hydrochloric acid [0.5N]
  - Phenolphthalein indicator (1% in ethanol)
- **Procedure:**
  - Weigh 1g of fat in a tared beaker and dissolve in about 3ml of the fat solvent [ethanol / ether mixture].
  - Quantitatively transfer the contents of the beaker three times with a further 7ml of the solvent.
  - Add 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser.
  - Set up another reflux condenser as the blank with all other reagents present except the fat.
  - Place both the flasks in a boiling water bath for 30 minutes.
  - Cool the flasks to room temperature.
  - Now add phenolphthalein indicator to both the flasks and titrate with 0.5N HCl.
  - Note down the endpoint of blank and test.
  - The difference between the blank and test reading gives the number of millilitres of 0.5N KOH required to saponify 1g of fat.
- **Calculate the saponification value using the formula:**

$$\text{Saponification value} = \frac{28.05 (\text{Titrate value of Blank} - \text{Titrate value of Test Sample})}{\text{Weight of sample (1g)}}$$

#### ✓ Ester Value

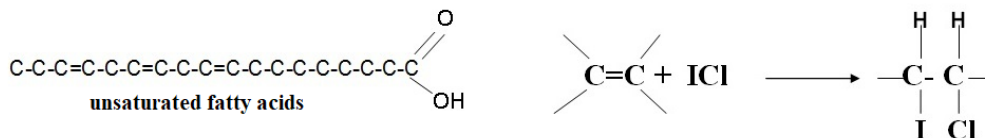
- The ester value is defined as the mg of KOH required to react with glycerine (glycerol/or glycerine) after saponify one gram of fat. It is calculated from the saponification Value (SV) and the acid Value (AV):

$$\text{Ester Value (EV)} = \text{Saponification Value (SV)} - \text{Acid Value (AV)}$$

$$\% \text{ glycerin} = \text{Ester Value} \times 0.054664$$

✓ **Iodine Value**

- The iodine value (IV) gives a measure of the average degree of unsaturation of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid.
- Iodine value (I.V.) is directly proportional to the degree of unsaturation (No of double bonds.) and inversely proportional to the melting point (M.P.) of lipid.
- An increase in I.V. indicates high susceptibility of lipid to oxidative rancidity due to high degree of unsaturation.
- Fatty acids react with a halogen [iodine] resulting in the addition of the halogen at the C=C double bond site. In this reaction, iodine monochloride reacts with the unsaturated bonds to produce a di-halogenated single bond, of which one carbon has bound an atom of iodine.



- After the reaction is complete, the amount of iodine that has reacted is determined by adding a solution of potassium iodide to the reaction product.:  $\text{ICl} + \text{KI} \leftrightarrow \text{KCl} + \text{I}_2$
- This causes the remaining unreacted ICl to form molecular iodine. The liberated  $\text{I}_2$  is then titrated with a standard solution of 0.1N sodium thiosulfate.  $\text{I}_2 + 2 \text{Na}_2\text{S}_2\text{O}_3 \rightleftharpoons 2 \text{NaI} + \text{Na}_2\text{S}_2\text{O}_4$
- Saturated fatty acids will not give the halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is oil. Starch is used as the indicator for this reaction so that the liberated iodine will react with starch to give purple coloured product and thus the endpoint can be observed.

- **Materials Required:**

- o Iodine Monochloride Reagent
- o Potassium Iodide
- o Standardized 0.1 N Sodium thiosulphate
- o 1% Starch indicator solution
- o Chloroform
- o Fat sample in chloroform
- o Iodination flask
- o Burette and burette stand with magnetic stirrer
- o Glass pipette
- o Measuring cylinder
- o Distilled water

- **Method:**

- o Arrange all the reagent solutions prepared and the requirements on the table.
- o Pipette out 10ml of fat sample dissolved in chloroform to an iodination flask labelled as "TEST".
- o Add 20ml of Iodine Monochloride reagent in to the flask. Mix the contents in the flask thoroughly.
- o Then the flask is allowed to stand for a half an hour incubation in dark.
- o Set up a BLANK in another iodination flask by adding 10ml Chloroform to the flask.
- o Add to the BLANK, 20ml of Iodine Monochloride reagent and mix the contents in the flask thoroughly.
- o Incubate the BLANK in dark for 30 minutes.
- o Meanwhile, Take out the TEST from incubation after 30 minutes and add 10 ml of potassium iodide solution into the flask.
- o Rinse the stopper and the sides of the flask using 50 ml distilled water.
- o Titrate the "TEST" against standardized sodium thiosulphate solution until a pale straw colour is observed.
- o Add about 1ml starch indicator into the contents in the flask, a purple colour is observed.
- o Continue the titration until the color of the solution in the flask turns colourless.
- o The disappearance of the blue colour is recorded as the end point of the titration.
- o Similarly, the procedure is repeated for the flask labelled 'Blank'.

- Record the endpoint values of the BLANK.
- Calculate the iodine number using the equation below:  
**Volume of Sodium thiosulphate used = [Blank- Test] ml**

$$\text{Iodine No. of fat} = \frac{\text{Equivalent Wt. of Iodine} \times \text{Volume of Na}_2\text{S}_2\text{O}_3 \text{ used} \times \text{Normality of Na}_2\text{S}_2\text{O}_3 \times 100 \times 10^{-3}}{\text{Weight of fat sample used for analysis (g)}}$$

**Equivalent Weight of Iodine = 127**

**Normality of sodium thiosulphate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) = 0.1**

**Or**

$$\text{Iodine Value} = \frac{\text{Volume of Sodium thiosulphate used} \times \text{Normality of Sodium thiosulphate} \times 0.127 \text{ g/meq weight of Iodine} \times 100}{\text{Weight of the fat sample used for analysis}}$$

### ✓ *Acetyl Value*

- Acetyl value measure of the free hydroxyl groups in a substance (as a fat or oil) as determined by acetylation, being the number of milligrams of potassium hydroxide required for neutralization of the acetic acid formed by hydrolysis of one gram of the acetylated substance.
- Some fatty acids contain hydroxyl groups. In order to determine the proportion of these, they are acetylated by means of acetic anhydride.
- This results in the introduction of acetyl groups in the place of free hydroxyl groups.
- The acetic acid in combination with fat can be determined by titration of the liberated acetic acid from acetylated fat or oil with standard alkali.
- Acetyl number is thus a measure of the number of hydroxyl groups present in fat or oil
- **Methods:**
  - Determination of the saponification value of the substance under examination. Acetylate the substance under examination by the following method. 10 g with 20 ml of acetic anhydride in a long-necked, round-bottomed 200-ml flask attached to a reflux air condenser. Support the flask on a sheet of resistant material in which a hole of about 4 cm in diameter has been cut and heat it with a small, naked flame, not more than 25 mm in height and which does not impinge on the bottom of the flask.
  - Boil gently for 2 hours, allow to cool, pour into 600 ml of water contained in a large beaker, add 0.2 g of pumice powder and boil for 30 minutes.
  - Cool and transfer to a separator and discard the lower layer. Wash the acetylated product with three or more quantities, each of 50 ml of a warmed saturated solution of sodium chloride until the washings are no longer acid to litmus paper.
  - Finally, shake with 20 ml of warm water and remove the aqueous layer completely as possible. Pour the acetylated substance into a small dish, add 1 g of powdered anhydrous sodium sulfate, stir thoroughly and filter through dry pleated filter.
  - Determine the saponification value of the acetylated substance.
- **Calculate the Acetyl value from the expression**

$$\text{Acetyl value} = 1335(b - a) / (1335 - a)$$

**Where, a = saponification value of the substance; b = saponification value of the acetylated substance.**

**✓ Reichert Meissl (RM) Value**

- The *Reichert value* (The *Reichert-Meissl-Wollny Value* or *Reichert-Meissl-Wollny Number*) is a value determined when examining fat.
- The *Reichert value* is an indicator of how much volatile fatty acid can be extracted from fat through saponification. It is equal to the number of **millilitres of 0.1 normal hydroxide** solution necessary for the neutralization of the **water-soluble volatile fatty acids** distilled and filtered from 5 grams of a given saponified fat. (The hydroxide solution used in such a titration is typically made from sodium hydroxide, potassium hydroxide, or barium hydroxide.)
- The material is saponified by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulfuric acid. The volatile acids are immediately steam distilled. The soluble volatile acid in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution.
- These determinations have been used principally for analysis of butter and margarines. Butter fat contains mainly butyric acid glycerides. Butyric acid is volatile and soluble in water.
- Butter fat contains mainly butyric acid glycerides. Butyric acid is volatile and soluble in water. No other fat contains butyric acid glycerides, and therefore, the *Reichert Meissl value* of the butter fat is higher than that for any other fat.
- **Reagents:**
  - (a) Glycerine
  - (b) Concentrated sodium hydroxide solution: 50 % (w / w) Dissolve
  - (c) Dilute sulfuric acid solution: Approximately 1.0 N
  - (d) Sodium hydroxide solution: 0.1N solution in water, accurately standardised
  - (e) Phenolphthalein indicator: Dissolve 0.1 g of phenolphthalein in 100 ml of ethyl alcohol.
  - (f) Ethyl alcohol: 90% by volume and neutral to phenolphthalein.
- **Procedure**
  - o Weigh accurately 5 ±0.1g of filtered oil or fat sample into a clean, dry, 300 ml distilling flask. Add 20 ml of glycerine and 2 ml of concentrated sodium hydroxide solution, and heat with swirling over a flame until completely saponified, as shown by the mixture becoming perfectly clear. Cool the contents slightly and add 90 ml of boiling distilled water, which has been vigorously boiled for about 15 min. After thorough mixing the solution should remain clear. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating over-heating), repeat the saponification with a fresh sample of the oil or fat. If the sample is old, the solution may sometimes be dark and not clear.
  - o Add about 0.6 - 0.7 gm of pumice stone grains, and 50 ml of dilute sulfuric acid solution. Immediately connect the flask to the distillation apparatus. Place the flask on asbestos board so that it fits snugly into the aperture. This will prevent the flame from impinging on the surface of the flask above the level of the liquid and avoid super heating. Heat very gently until the liberated fatty acids melt and separate. Then set the flame so that 110 ml of distillate shall be collected within 19 to 21 min. The beginning of the distillation is to be taken as the moment when the first drop of the distillate falls from the condenser in the receiving flask. Keeps the water in the condenser flowing at a sufficient speed to maintain the temperature of the outgoing water from the condenser between 15 and 20°C. Collect the distillate in a graduated flask.
  - o When the distillate exactly reaches the 110 ml mark on the flask, remove the flame and quickly replace the flask by a 25 ml measuring cylinder. Stopper the graduated flask and without mixing place it in a water bath maintained at 15°C for 10 min so that the 110 ml graduation mark is 1 cm below the water level in the bath. Swirl round the contents of the flask from time to time. Remove the graduated flask from the cold-water bath, dry the outside and mix the content gently by inverting the flask 4 to 5 times without shaking. Avoid wetting the stopper with the insoluble acids. Filter the liquid through a dry, 9 cm Whatmann No. 4 filter paper. Reject the first 2-3 ml of the filtrate and collect the rest in a dry flask. The filtrate should be clear. Pipette 100 ml of the filtrate and add 5 drops of the phenolphthalein solution and titrate against standard 0.1N sodium hydroxide solution.
  - o Run a Blank Test without the fat but using the same quantities of the reagents.

## - Calculation

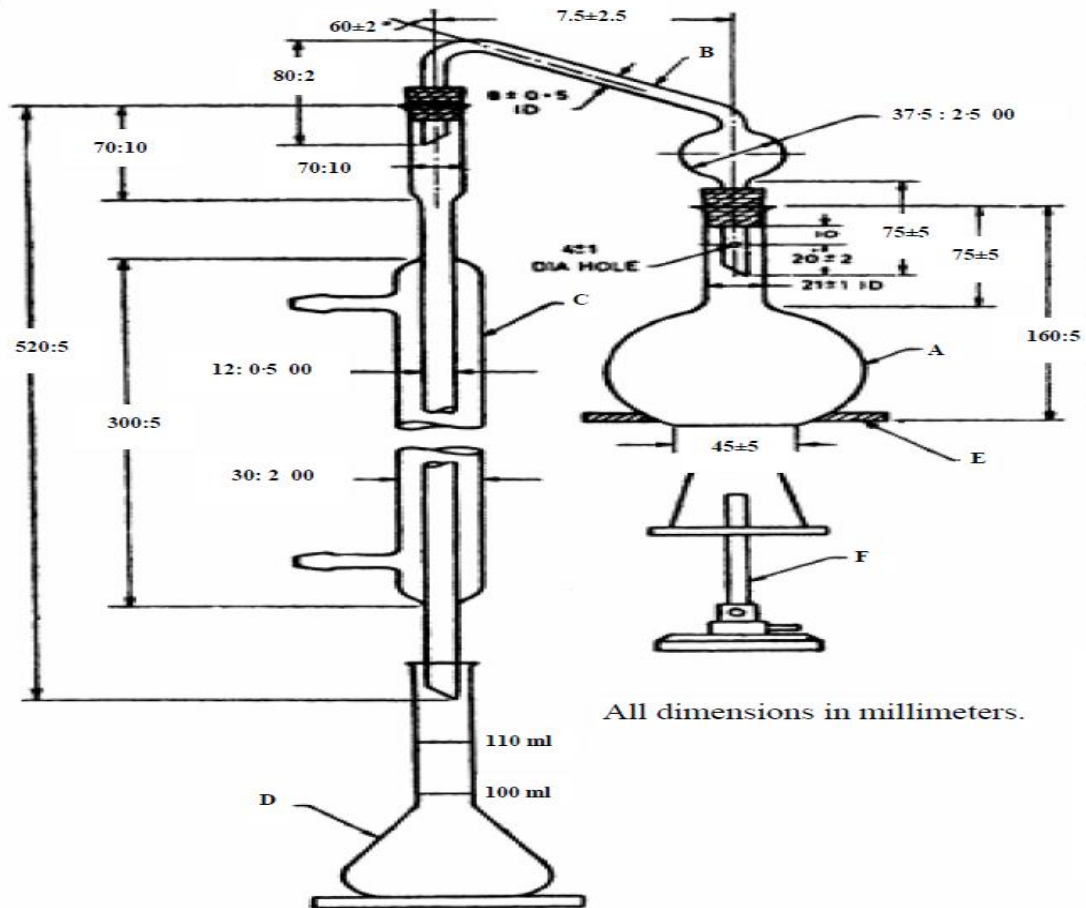
$$\text{Reichert-Meissl Value} = (A - B) \times N \times 11$$

where,

**A** = Volume in ml of standard sodium hydroxide solution required for the test

**B** = Volume in ml in standard sodium hydroxide solution required for the blank

**N** = Normality of standard sodium hydroxide solution.



All dimensions in millimeters.

**Reichert-Meissl Distillation Apparatus**

- The **Polenske value** and **Kirschner value** are related numbers based on similar tests. Values of reichert-meissel in milk are 28.5-33.
- The **Polenske value** (also known as the Polenske number) is a value determined when examining fat. It is an indicator of how much volatile fatty acid can be extracted from fat through saponification. It is equal to the number of millilitres of 0.1 normal alkali solution necessary for the neutralization of the water-insoluble volatile fatty acids distilled and filtered from 5 grams of a given saponified fat.

$$\text{Polenske value} = 10 \times (A-B) \times N$$

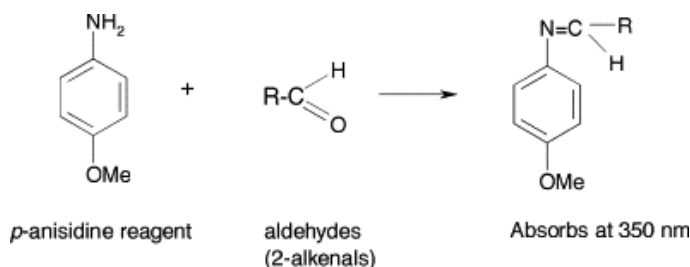
where, **A** = volume, in ml, of NaOH solution required for the test, **B** = volume, in ml, of NaOH solution required for blank, and **N** = normality of NaOH solution.

- The **Kirschner value** (also known as the Kirschner number) is a value determined when examining fat. The Kirschner value is an indicator of how much volatile fatty acid can be extracted from fat through saponification. It consists of the number of millilitres of 0.1 normal sodium hydroxide necessary for the neutralization of water-soluble silver salts made from the water-soluble volatile fatty acids distilled from 5 grams of a given fat.

### ✓ Anisidine Value (In Oils) Test

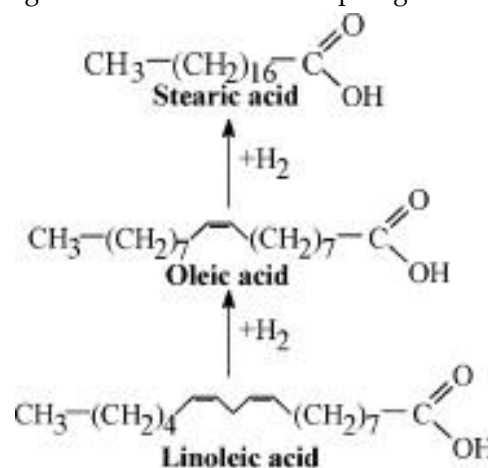
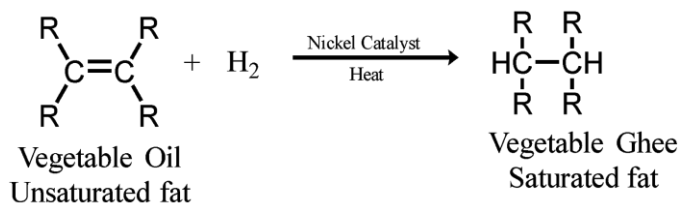
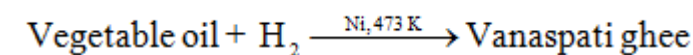
- The p-Anisidine Value analysis (AV) deals with fats and oils and with the oxidation processes occurring in them, the undesirable series of chemical reactions involving oxygen that degrades their quality.
- Oxidation generates a sequence of breakdown products, starting with primary oxidation products (peroxide value, dienes, free fatty acids) then secondary products (carbonyls, aldehydes, trienes) and then tertiary products.
- Food industries generally need to control the quality of the oil they use in the food processing to avoid the presence of a bad smell or rancid flavour in the final product. It allows also to check and control the freshness and the quality standards of the oil, especially in relation to its shelf-life.
- The chemical analysis method for p-Anisidine Value determines the number of aldehydes (principally 2-alkenals and 2,4-dienals) in animal and vegetable oils and fats by reaction of these compounds with the p-Anisidine. This reaction highlights the concentration of the quantity of aldehydes and ketons, giving the dimension of the secondary oxidation of the fat matrices.
- The official method, a spectrophotometric analysis method measuring the absorbance at 350 nm.
- P-Anisidine Value analysis allows to control the quality, the smell and the state of conservation of the matter detecting the deeper stages of the oxidation. The lower the p-Anisidine Value, the better the quality of fats and oils analyzed.

#### - Reaction:



## Hydrogenation of Vegetable Oils

- Vegetable oils also known as vanaspati oil can undergo addition reactions due to the presence of long chains and double bonds in them. They are unsaturated compounds. Thus, heating of vegetable oils in the presence of nickel catalyst converts the unsaturated fat to saturated fats called vegetable ghee also known as vanaspati ghee. This process is called the hydrogenation of vegetable oils.



- The vegetable oil is a viscous liquid whereas the vegetable ghee is semi solid/solid in nature. Here, hydrogens are added across the double bond hence this reaction is also known as addition reaction. The use of catalysts such as Nickel are used for quick and easy absorption since this metal has the capability to absorb hydrogen. The mechanism involved in this process is simple absorption of atomic hydrogen and unsaturated fat on the metal catalyst. It must be noted that hydrogen gas must be applied under pressure.
- The process of hydrogenation increases the melting point of the oil or fat. Besides, resistance to oxidation and flavour deterioration is also maintained.
- The hydrogenation of vegetable oils can also be done by platinum (Pt) catalyst in addition to Ni catalyst. Platinum catalysts can also absorb hydrogen with great ease making the hydrogenation process quick.



- **Hydrogenation definition**
- Hydrogenation is a chemical process where hydrogen reacts to an organic compound.
- It refers to the saturation of unsaturated liquid oils with hydrogen atoms.
- Molecules in unsaturated fatty acids are bent (have kinks in them), not allowing them to pack together closely.
- This limits the intramolecular attractive forces and makes their melting point lower, which means that they are liquid at room temperature.
- The hydrogenation process removes those kinks and makes the fatty acids straight, either by making them fully saturated or altering them to **Tran's** fatty acids.
- Both saturated and **Tran's** fatty acid chains are straight. Therefore, they can stack up closely together. This increases their melting point.
- ✓ **The main purposes of hydrogenation oils to solid or semi-solid fats are:**
- They are a cheap substitute of saturated fats from animal origins, such as lard or butter;
- Hydrogenated oils are more stable than unsaturated oils. The process of hydrogenation transforms fatty acids that are prone to oxidative rancidity, especially in high temperatures during cooking;
- Hydrogenated oils are also more stable than saturated products, such as butter.
- Although butter is considered as a saturated fat food, it contains some short chain fatty acids and unsaturated fats, both of which are more prone to rancidity than long chain saturated fatty acids present in hydrogenated products.
- ✓ **Full hydrogenation (full saturation)**
- The full hydrogenation process saturates all carbon atoms with the maximum possible number of hydrogen atoms, making the oil fully saturated (no more hydrogen atoms can be attached and also no Trans fatty acids exist).
- The process of full saturation transforms unsaturated into fully saturated fatty acids. All of the kinks/bends are removed from the structure.
- The shape of the chains becomes straight. Therefore, when they stack up together, they form a solid fat in room temperature.
- ✓ **What is partial hydrogenation (partial saturation)**
- Partial hydrogenation is an incomplete process. This means that the hydrogenation process stopped before completing a full saturation of carbons with hydrogen atoms.
- As a result, there are still some double bonds present. Some of these double bonds are of "cis" configuration (normal configuration characteristic for unsaturated fatty acids) and some have been damaged in the process forming a "trans" configuration, where one of the hydrogens at the carbon double bond has been removed and another placed on the opposite side of the chain.
- The purpose of partial hydrogenation of oils is to make their properties optimal for food preparation. Partial hydrogenation results in a product that is more suitable for culinary purposes than fully hydrogenated products.
- ✓ **Why are oils only partially hydrogenated?**
- Partially hydrogenated oils can give a wide range of consistencies of the finished fat product.
- Fully hydrogenating oils would make the oils hard at room temperature and not as versatile in food preparation.
- They increase the stability and the shelf life of fats in processed foods.
- They are extremely cost efficient and preferred for their usability

## Determination of adulteration in fats and oils

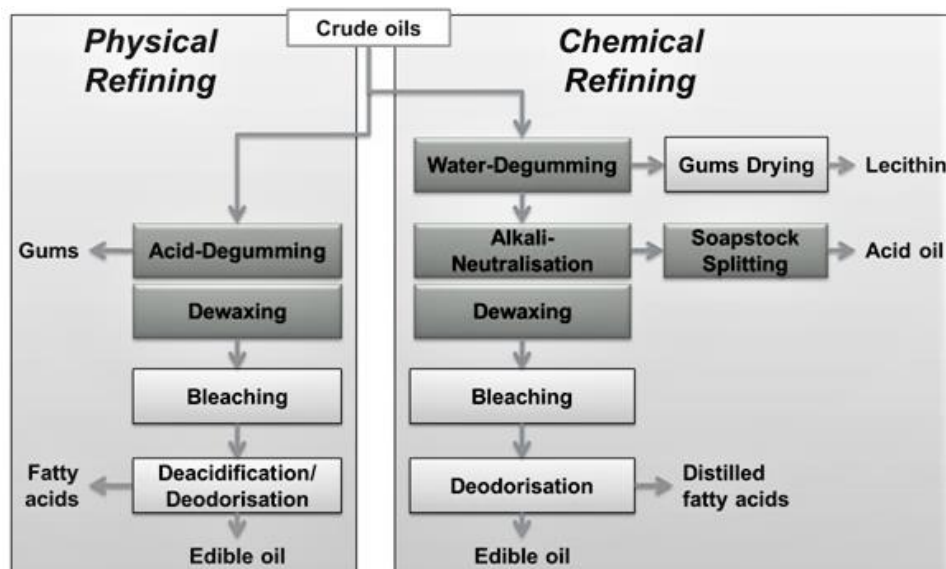
- Vegetable oils and fats (VOFs) have a big contribution in our diet as cooking or frying oil, salad oil or in food products formulation.
- Today, adulterations are more sophisticated. Therefore, it is necessary to use advanced and suitable methods to
- detect adulteration. Generally, using physical properties like refractive index, viscosity, melting point, saponification and iodine value are not anymore practical to detect adulteration. These properties are well arranged in adulterated Vegetable oils and fats to mask the adulteration.
- There are two major adulterations in edible oils and fats namely
  - 1) Admixing cold press oil with refined one and
  - 2) Replacement of more expensive oils and fats with cheaper one.
- However, detection of adulterant on basis of development of the colour, turbidity or precipitate is possible:
  - **Detection of sesame oil as adulterant:**
    - To 2 ml oil, add 1 ml hydrochloric acid containing 1 per cent sucrose and keep aside for 5 minutes.
    - The presence of pink colour in the acid layer indicates presence of sesame oil.
  - **Detection of cotton seed oil as adulterant by Halphen's test:**
    - To 2 ml oil, add 2.5ml alcohol and 2.5 ml solution of sulphur in carbon disulphide (1 per cent).
    - Heat the mixture Formation of pink or red colour indicates presence of cotton seed oil.
  - **Detection of karanja oil as adulterant:**
    - Take 5 ml of sample oil in a test tube; add 10 ml ethyl alcohol to it followed by addition of 5 drops of ferric chloride solution.
    - Shake vigorously two minutes and allow to stand for two hours.
    - The development of light blue to dark colour in the alcoholic layer indicates the presence of karanja oil or any other oil containing phenolic group.
  - **Detection of castor oil as adulterant:**
    - Take 1 ml of the sample to be tested in a clean test-tube and to it add acidified petroleum ether (60- 80°).
    - Shake it thoroughly.
    - Then to it add a drop ammonium molybdate solution.
    - Development of white turbidity indicates the presence of castor oil.
  - **Detection of argemone oil as adulterant:**
    - It is an adulterant to mustard oil.
    - Ferric chloride reacts with argemone oil in hydrochloric acid medium to produce small needle shaped reddish brown crystals.
    - However, if content of argemone oil is less than 1 per cent, needle-shaped crystal can be observed under microscope.
  - **Detection of linseed oil as adulterant:**
    - About 1 ml sample is dissolved in 5 ml chloroform in a dry test-tube and one ml liquid bromine is added.
    - The tube is stoppered and cooled in ice bath and to it added 1.5 ml rectified spirit and 10 ml ether.
    - It is then shaken thoroughly again for 30minutes.
    - The appearance of precipitate (due to formation of hexabromide) indicates presence of linseed oil.
  - **Detection of mineral oil as adulterant:**
    - To 1 ml oil or fat under test, add 25 ml alcoholic caustic potash.
    - Boil in water bath, till it becomes quite clear.
    - Transfer the contents of the flask to a wide mouth test tube and to it, add 25 ml distilled water along the side of the test tube, keeping the tube shaken during addition of water.
    - The presence of turbidity indicates presence of mineral oil.

## Refining of Fats and Oils

- Many refined oils and fats are used for frying and the ideal oil composition may be different depending on technical or nutritional considerations.
- A refining process is carried out following extraction of crude edible oils by means of screw presses and/or solvent extraction.
- In refining, physical and chemical processes are combined to remove undesirable natural as well as environmental-related components from the crude oil.
- **Refining Of Crude Oil:** Depending on the requirements, the following basic processes are implemented:
  - o Degumming for removal of phosphatides,
  - o Neutralization for removal of free fatty acids,
  - o Bleaching for removal of colour,
  - o Deodorization to distil odours and favors as well as free fatty acids and
  - o Winterization for separation of waxes.

<i>Basic steps of the refining process</i>		
Alkali or chemical refining	Main groups of compounds removed	Physical Refining
Degumming	Phospholipids	Degumming
Neutralization	Free fatty acids	-
Bleaching	Pigments/metals/soaps	Bleaching
Winterization	Waxes/saturated triacylglycerols	Winterization
Deodorization	Volatiles/free fatty acids	Deodorization/ deacidification

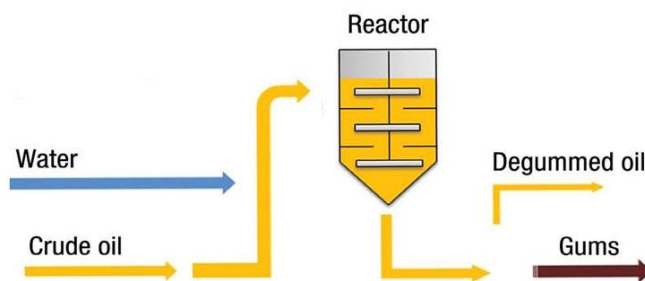
- **PHYSICAL REFINING:** it is a process making use of the lower boiling point of the free fatty acid (FFA) compared to the boiling point of the triglyceride oil. In chemical or alkaline refining, an alkali is used to neutralise the FFA.
- **CHEMICAL REFINING:** It is the traditional method used in past centuries. The main purpose of chemical refining is to saponify the FFA by an alkaline solution and dilute the resulting soaps in a water phase. These soaps are removed by separators. For small-scale batch processes static separation is used but for continuous processing and large-scale processes, centrifugal separation is used. The neutral oils are subsequently bleached and deodorised. This chemical refining can be used for reliably refining virtually all crude oils, including oils of low quality, with the exception of castor oil.



## • Process of Refining of Fats and Oils:

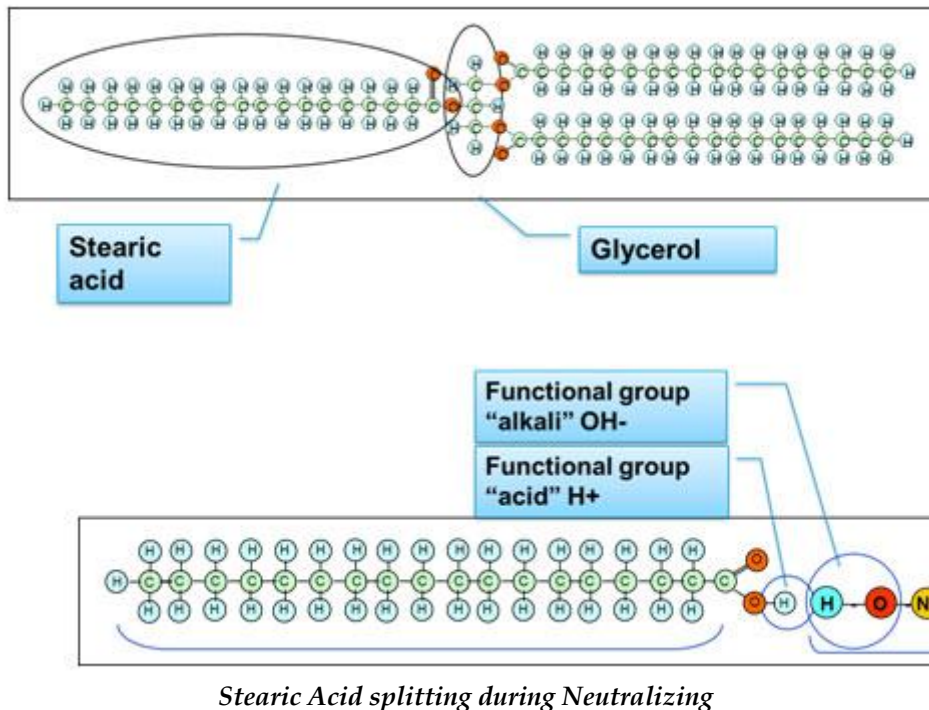
### ✓ *Degumming Process*

- Degumming is the first process in the vegetable oil refining which commences with the heating of crude oil.
- The main purpose of degumming is to remove the Phospholipids / Gums from the crude vegetable oils. There are generally two different types of phospholipids present in the crude oil depending upon their hydration levels:
  - (i) *Hydratable or Water Degumming:*
    - In this method, the crude oil is hydrated by mixing calculated amount of water. Since hydrated gums are insoluble in oil, hence it gets precipitated. These hydrated gums are then separated from oil. In this stage, only hydratable gums are removed.
  - (ii) *Non-Hydratable or Acid Degumming:*
    - For removal of non-hydratable gums, water degumming is not suitable and the oil is conditioned by acid media such as phosphoric acid.
    - The content of **phosphoric acid** addition depends on the amounts of gums present as well as the type of oil being refined.
    - Acid Degumming is perfectly suitable for High FFA Crude Oils where acid washing is followed by Water Washing and ensures total degumming.
    - Due to the variable content of phospholipids in crude oils, analysis of phosphorus prior to acid treatment is necessary to ensure that the acid dosage is correct.
- Importance of Degumming Process:
  - Elimination of Phosphatides from the crude oils to improve the quality.
  - To avoid high refining losses.
  - To avoid their decomposition so that oil does not darken due to their thermal instability.



### ✓ *Neutralization Process*

- Neutralization or simply Neutralizing is the second process in the vegetable oil refining which commences after degumming completion.
- The feedstock is taken into the Neutralizers where the heating temperature range is kept at **55° C to 70° C** depending on the quality of crude oil. The oil is under constant agitation to maintain the uniformity.
- The main purpose of neutralization is to remove the Free Fatty Acids present inside the crude vegetable oils. In chemical refining, an alkali is used to neutralize the free fatty acids (FFA) and to remove the oil acidity.
- The main purpose of Neutralization Process is to saponify the FFA by an alkaline solution and dilute the resulting soaps in a water phase.
- In the edible oil industry mostly, caustic soda is used for the treatment with FFA's.
- Occasionally potassium hydroxide might also be used by some processors. The FFA is a stearic acid that splits from a triglyceride molecule leaving a diglyceride in the oil.
- The acidity of the free fatty acid comes from the **H<sup>+</sup>** of the **carboxyl group**. This **H<sup>+</sup>** of the functional group of the **stearic acid** reacts with the **OH<sup>-</sup> group** of the **caustic soda** (NaOH) to produce soap and water.



- Following undesirable non-glyceride materials are also removed by Neutralizing Step:
  - o Some Phospholipids
  - o Oxidized Products
  - o Metal Ions
  - o Color Pigments (e.g., gossypol)
  - o Insoluble Impurities (e.g., meal fines)

✓ **Bleaching Process**

- Bleaching Process is the third step in vegetable oil refining which commences after completing of effective neutralization process.
- Bleacher Pressure Vessel is used to carry out the Bleaching Process.
- The purpose of Bleaching is to remove the colour pigments contained inside Vegetable Oils.
- The neutralized oil is heated at additional temperature through thermic boilers to ultimately raise the temperature of oil upto 120° C to 130° C.
- The oil is then treated with Bleaching Clays that adsorb the colour pigments.
- The addition amount for bleaching clay varies from oil to oil.
- It also depends on the colour content of the crude oil and the desired quality oil colour index.

<i>Following parameters are crucial while performing the Bleaching Process:</i>	<i>The following are the important stages in bleaching process</i>
<b>Selection of Clay Type</b>	Temperature Raise to Limit Level
<b>PH and Free Moisture Content in the Clay</b>	Process Control of Clay Dosing
<b>Activated or Non-Activated</b>	Continuous Agitation for Uniformity
<b>Surface Area and Porosity</b>	Understanding Desired Colour and Chlorophyll
<b>Surface Acidity</b>	Total Contact Time
<b>Particle Size Distribution</b>	Vacuum Pressure
<b>The Dosage Quantity</b>	Final Filtration Method
<b>Bleach-ability Index</b>	Oil colour index

### ✓ Deodorization Process

- Deodorization Process is the fourth step in vegetable oil refining which commences after finishing the bleaching.
- The purpose of Deodorizing Vegetable Oils is to remove odor substances.
- The temperature of the bleached vegetable oil is further raised from 120 C to 200 C. The oil is subjected to high temperature heating under vacuum.
- The oil is subjected to steam distillation under high temperature and vacuum to evaporate all odor substances.
- The resulting deodorized oil is almost bland and tasteless.
- Each crude oil responds differently to the deodorization parameters. Soyabean Oil will differ from Cottonseed Oil. Sunflower Oil will differ from Palm Oil. So is with Groundnut Oil or Rapeseed Oil or Coconut Oil.
- The thermal effect of the deodorizer will destroy the oil flavour and the heat sensitive colour pigments. In palm oil, the thermal breakdown of carotene leads to colour reduction or the heat bleaching.
- *The deodorization process is fully determined by four process parameters:*
  - Temperature
  - Time
  - Pressure
  - Amount of stripping steam

### ✓ Dewaxing Process

- Dewaxing may be the fifth process considered for the edible oil refining. It is not a compulsory process, and it is not required for every type of oil. Dewaxing is performed only for high wax containing oils such as **Sunflower Oil, Rice Bran Oil, Corn Oil** etc.
- Some oils are dewaxed before packing so as to remove waxes, which are dissolved in the oil. Most of the oils do not need dewaxing as they contain little or no waxes.
- Mainly **Sunflower Oil, Rice Bran Oil** and **Corn Oil** contain appreciable quantities of wax in them. This gives a hazy appearance to the oil during winter season. The reason for hazy appearance is due to the precipitation of dissolved waxes. The presence of wax makes the oil appear cloudy at room temperature. The oil normally becomes cloudy in **5-6 h** but with proper dewaxing the oil remains clear after **24h** of storage at **0° C**.
- *Steps for Dewaxing of Vegetable Oils*
  - Crude oil is refined and bleached to low phosphorus (<1ppm) and low moisture content (<0.1%).
  - The oil is heated to 55°C to make sure the oil is fully liquid. This is already achieved in the Cooler Vessel while reducing the deodorization temperature.
  - The oil is then slowly cooled 10-15°C. Chiller helps for the same.
  - The chilled oil is stored into the Insulated Crystallizers with a special slow-speed mechanical agitator.
  - Preferably, the oil is held for several hours at this temperature.
  - Crystal formation in the oil defines the completion of cooling.
  - Special transfer pump will slowly feed the slurry to the Filter press.
  - Filtration yields clear liquid oil and the by-product waxes as output.

### ✓ Dry Fractionation Process

- Apart from all the regular refining processes, there is one additional process that needs to be carried out for refining palm oil - **Fractionation Process**.
- For Palm Oil Refinery Plant, Fractionation Process is required in which **palm olein** and **palm stearin** are separated by scientifically cooling the refined palm oil by **chilling plant** and then **filtering the oil**.



*"Money doesn't grow on trees, but many oils do"*