Name of Unit	Fats and Oils
Subject /Course Name	Pharmaceutical Organic Chemistry-II
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Module No.	3
Class: B.Pharm.Semester	3rd
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Learning Outcome of Module-3

LO	Particular	Course Outcome Code
LO1.	Students are able to understand about Fats and oils.	BP301.2
LO2.	To gain knowledge aboutImportanceforlivingorganisms.	BP301.2
LO3.	To understand about Physical and Chemical reactions of Fats and oils.	BP301.2
LO4.	To understand aboutAnalysis of fats and oils.	BP301.6

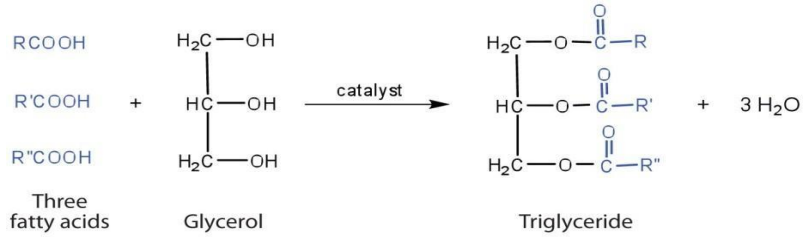
Module Content Table

No.	Topic
1.	Introduction of Fats and Oils.
2.	Reactions of Fats and Oils (Hydrolysis, Hydrogenation, Saponification and Rancidity of oils).
3.	Introduction of Analytical Constants of Fats and Oils
4.	Acid value.
5.	Saponification value.
6.	Ester value
7.	Iodine value
8.	Acetyl value
9.	Reichert Meissl (RM) value

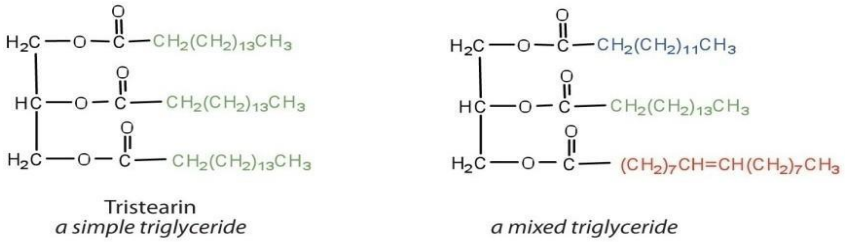
INTRODUCTION OF FATS ANDOILS: -

Fats and oils are composed of molecules known as triglycerides, which are esters composed of three fatty acid units linked to glycerol.

Fats and oils are called triglycerides because they are esters composed of three fatty acid units joined to glycerol, a trihydroxy alcohol:



If all three OH groups on the glycerol molecule are esterified with the same fatty acid, the resulting ester is called a simple triglyceride. Although simple triglycerides have been synthesized in the laboratory, they rarely occur in nature. Instead, a typical triglyceride obtained from naturally occurring fats and oils contains two or three different fatty acid components and is thus termed a mixed triglyceride.



A triglyceride is called a fat if it is a solid at 25°C; it is called oil if it is a liquid at that temperature. These differences in melting points reflect differences in the degree of unsaturation and number of carbon atoms in the constituent fatty acids. Triglycerides obtained from animal sources are usually solids, while those of plant origin are generally oils. Therefore, we commonly speak of animal fats and vegetable oils.

Saturated fats can stack themselves in a closely packed arrangement, so they can solidify easily and are typically solid at room temperature. For example, animal fats tallow and lard are high in saturated fatty acid content and are solids. Olive and linseed oils on the other hand are

unsaturated and liquid. Fats serve both as energy sources for the body, and as stores for energy in excess of what the body needs immediately. Fats are broken down in the healthy body to release their constituents, glycerol and fatty acids. Glycerol itself can be converted to glucose by the liver and so become a source of energy.

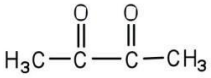
Differentiate between fats and oils: -

	Fats	Oils
1	Fats are solids or semisolids at room temperature	Oils are liquids at room temperature
2	Fats contains large amount of saturated fatty acids e.g. stearic and palmitic acids	Oils contains large amount of unsaturated fatty acids e.g. oleic acid
3	Fats melt at high temperature	Melt at low temperature
4	Fats do not contain double bonds	Oils have double bonds
5	Fats are more stable	Oils are less stable
6	Fats are animal fats	Oils are vegetable fats

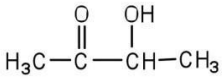
Physical Properties of Fats and Oils: -

Pure fats and oils are **colorless, odorless, and tasteless**. The characteristic colors, odors, and flavors 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 color 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.



Diacetyl



3-hydroxy-2-butanone

Fats and oils are **lighter than water,** having densities of about 0.8 g/cm^3. They are **poor conductors of heat and electricity** and therefore serve as excellent insulators for the body, slowing the loss of heat through the skin.

Chemical Structure: -

There are many different kinds of fats, but each is a variation on the same chemical structure. All fats are derivatives of fatty acids and glycerol. Most fats are glycerides, particularly triglycerides (triesters of glycerol). One chain of fatty acid is bonded to each of the three -OH groups of the glycerol by the reaction of the carboxyl end of the fatty acid (-COOH) with the alcohol; i.e, three chains per molecule. Water is eliminated and the carbons are linked by an -O- bond through dehydration synthesis. This process is called esterification and fats are therefore esters. As a simple visual illustration, if the kinks and angles of these chains were straightened out, the molecule would have the shape of a capital letter E. The fatty acids would each be a horizontal line; the glycerol "backbone" would be the vertical line that joins the horizontal lines. Fats therefore have "ester" bonds.

The properties of any specific fat molecule depend on the particular fatty acids that constitute it. Fatty acids form a family of compounds that are composed of increasing numbers of carbon atoms linked into a zig-zag chain (hydrogen atoms to the side). The more carbon atoms there are in any fatty acid, the longer its chain will be. Long chains are more susceptible to intermolecular forces of attraction (in this case, van der Waals forces), and so the longer ones melt at a higher temperature (melting point).

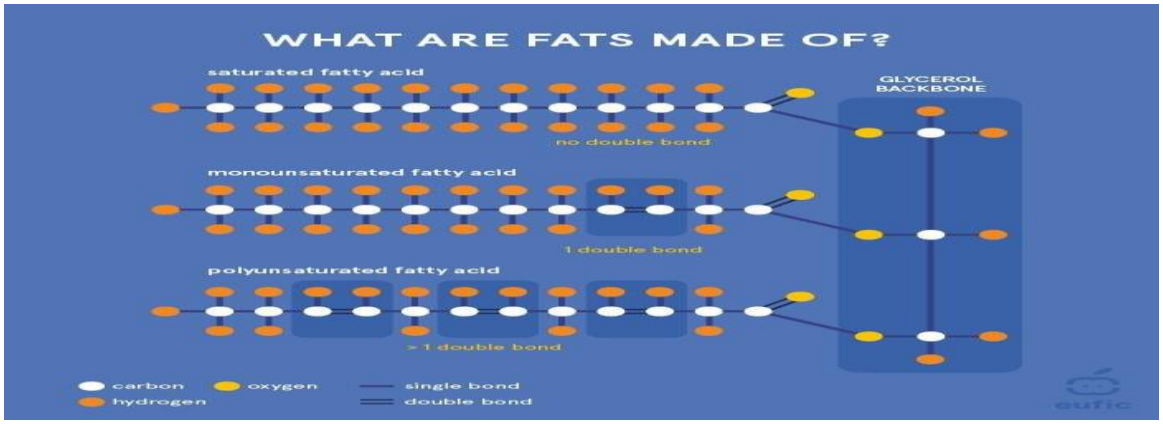


Fig. 1. Structure of a triglyceride and saturated, monounsaturated and polyunsaturated fatty acids.

Fatty acids are classified according to the presence and number of double bonds in their carbon chain. Saturated fatty acids (SFA) contain no double bonds, monounsaturated fatty acids (MUFA) contain one, and polyunsaturated fatty acids (PUFA) contain more than one double bond. Both length and saturation of fatty acids affect the arrangement of the membrane

in our body cells and thereby its fluidity. Shorter chain fatty acids and ones with greater unsaturation are less stiff and less viscous, making the membranes more flexible. This influences a range of important biological functions.

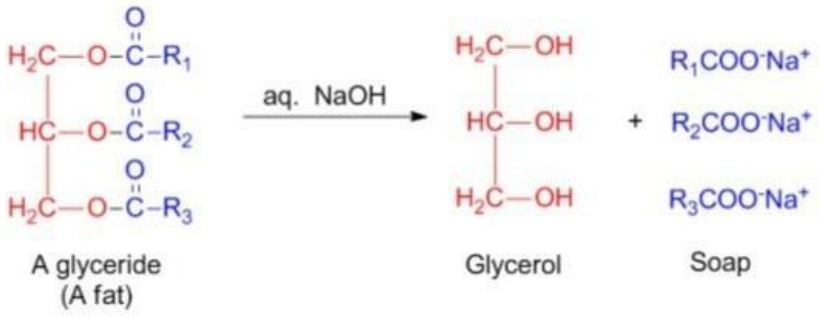
Importance for living organisms: -

Fats are also sources of essential fatty acids, an important dietary requirement. They provide energy as noted above. Vitamins A, D, E, and K are fat-soluble, meaning they can only be digested, absorbed, and transported in conjunction with fats. Fats play a vital role in maintaining healthy skin and hair, insulating body organs against shock, maintaining body temperature, and promoting healthy cell function. Fat also serves as a useful buffer against a host of diseases. When a particular substance, whether chemical or biotic, reaches unsafe levels in the bloodstream, the body can effectively dilute or at least maintain equilibrium of the offending substances by storing it in new fat tissue. This helps to protect vital organs, until such time as the offending substances can be metabolized or removed from the body by such means as excretion, urination, accidental or intentional bloodletting, sebum excretion, and hair growth.

Various chemical reactions of fats and oils: -

1. **Hydrolysis: -**

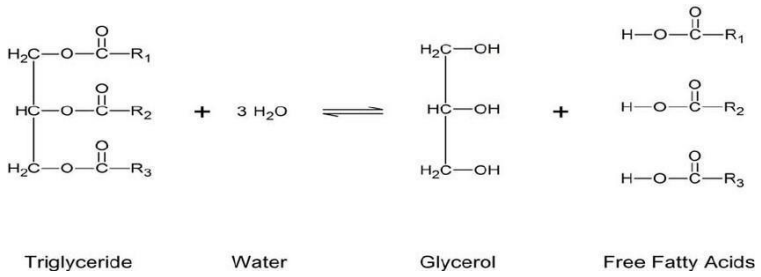
The hydrolysis of fats and oils in the presence of a base makes soap and is known as saponification. Double bonds present in unsaturated triglycerides can be hydrogenated to convert oils (liquid) into margarine (solid).



Hydrolysis can also be done by heating fat with water under pressure. Alkaline hydrolysis of fats produces salts of fatty acids called as soaps and hence this reaction is also known as saponification. Common soaps are the mixture of sodium salts of ‘C’ atoms (12 atoms) and higher fatty acids. Soap molecules have both lipophilic (lipid loving) and hydrophilic (water

loving) group. The lipophilic group dissolves oils while hydrophilic portion dissolves water. Soap molecules on dissolution in water forms micelle. Hydrolysis can be done in three ways-

A. **Hydrolysis by water: -** Fats undergoes hydrolysis in presence of water at 443K and 6-8 atmospheric pressure. Zinc oxide is used as catalyst.



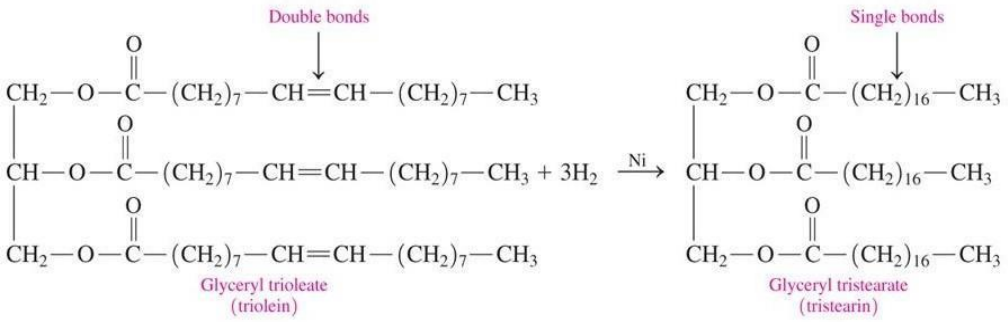
B. **Hydrolysis by Enzymes: -** Hydrolysis of fats and oils can be done by adding enzyme lipase to an emulsion of fat in water.

C. **Hydrolysis by acids: -** mineral acids cause hydrolysis of fats. For this mixture of sulphonic acids which are obtained by sulphonation of mixture of oleic acid and benzene.

The above three ways gives glycerol and fatty acids as a product of hydrolysis of fats while alkaline hydrolysis of fats gives glycerol and soap which are used as cleansing agent. The cleansing property of soap depends upon the ability to form emulsion with fat soluble materials.

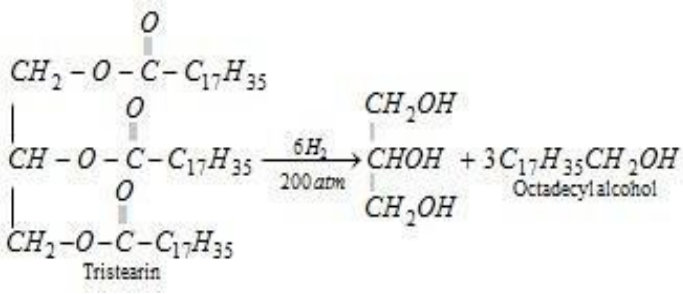
2. Hydrogenation: -

Oils have large amount of unsaturated portion in the form of glycerides. When hydrogen is passed through oils under pressure and by using catalyst at high temperature oils gets converted into solid fats. This process is known as **hardening of oils**. By hydrogenation, unsaturated acid part of oil gets reduced into saturated part and hence liquid oil gets converted into semi-solid fat. Hydrogenation is carried out in a closed container in the presence of finely powdered catalyst (0.05 - 0.2% of nickel) at temperature as high as 150-200^{o}C. The catalyst is usually removed by filtration. During hydrogenation process a proportion of the cis double bonds are isomerized to trans double bonds and there is also migration of double bonds. The hydrogenation process has made it possible to extend the food uses of a number of vegetable oils and marine oils whose melting points are too low.



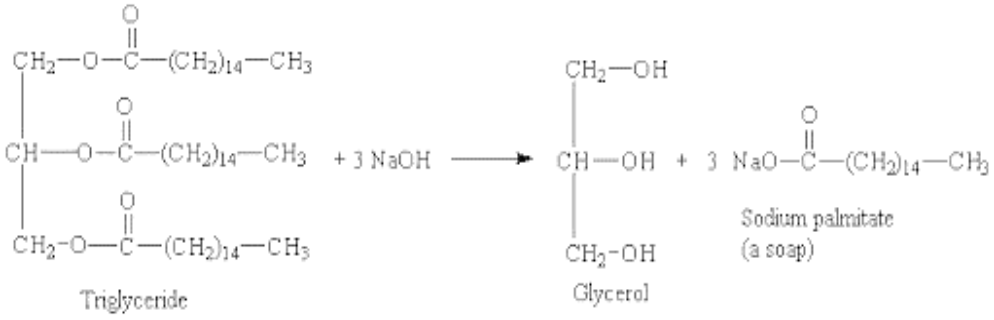
3. Hydrogenolysis: -

This is a cleavage reaction in which fat or oil molecule is treated with excess of hydrogen under pressure in presence of copper-chromium catalyst. In this reaction fat or oil gets splits up into glycerol and higher aliphatic alcohols.



4. Saponification: -

Saponification is a process that involves conversion of fat, oil or lipid into soap and glycerol by the action of heat in the presence of aqueous alkali (e.g. NaOH). Soaps are salts of fatty acids and fatty acids are monocarboxylic acids that have long carbon chains (at least 10) e.g. sodium palmitate.



5. Rancidification: -

Rancidification is the process of complete or incomplete oxidation or hydrolysis of fats and oils when exposed to air, light, or moisture or by bacterial action, resulting in unpleasant taste and odor. Rancidity reactions may be due to hydrolysis of ester bonds (hydrolytic rancidity) or due to oxidation of unsaturated fatty acids (oxidative rancidity). Rancidity occurs by the following ways-

A. Oxidation of unsaturated fatty acids: - in presence of light and moisture, small amount of unsaturated acids present in fats/oils gets oxidized by air to form peroxides which further break down into aldehydes having unpleasant smell and taste. Saturated fatty acids do not get rancid. This problem can be checked by adding small quantity of phenolic substances which act as antioxidant.

B. Enzymatic hydrolysis: - Due to presence of micro-organisms fats gets hydrolyzed by enzymes to produce fatty acids having sour taste and unpleasant odor. For example butter gets rancid due to production of butyric acid in this manner.

C. β-oxidation of saturated fatty acids: - fats having saturated fatty acids undergo ketone rancidity. Saturated acids undergo β-oxidation to form keto acids which gives carbon dioxide to form ketones having unpleasant odor.

Drying oils: -

A drying oil is the oil that hardens to a tough, solid film after a period of exposure to air. The oil hardens through a chemical reaction in which the components crosslink (and hence, polymerize) by the action of oxygen (not through the evaporation of water or other solvents). Drying oils are a key component of oil paint and some varnishes. Some commonly used drying oils include linseed oil, tung oil, poppy seed oil, perilla oil, and walnut oil. Drying oils (wild rose oil, linseed oil, wheat oil) contain more than 50% of polyunsaturated acids. They are quickly absorbed and leave no greasy layer on oily skin. Their light consistency makes them a good make-up primer.

The "drying", hardening, or, more properly, curing of oils is the result of autoxidation, the addition of oxygen to an organic compound and the subsequent crosslinking. This process begins with an oxygen molecule (O2) in the air inserting into carbon-hydrogen (C-H) bonds adjacent to one of the double bonds within the unsaturated fatty acid. The resulting hydroperoxides are susceptible to crosslinking reactions. Bonds form between neighboring

fatty acid chains, resulting in a polymer network, often visible by formation of a skin-like film on samples. This polymerization results in stable films that, while somewhat elastic, do not flow or deform readily. Diene-containing fatty acid derivatives, such as those derived from linoleic acid, are especially prone to this reaction because they generate pent dienyl radicals. Monounsaturated fatty acids, such as oleic acid, are slower to undergo drying because the allylic radical intermediates are less stable.

Oils depending upon their exposure to light and air can be classified as-

A. Non-drying oils: - These oils on exposure to light and long storage get rancid. Oils get decomposed into glycerol and fatty acids (saturated and unsaturated). The unsaturated acids gets oxidized into aldehydes and acids with lesser carbon atoms in the molecule. The saturated acids get decomposed by enzymes to form ketones. For example olive oil, almond oil, Babassu oil, Baobab oil, Coconut oil, Peanut oil and Tiger Nut Oil.

B. Drying oils: - They form a solid elastic film. A good drying oil dries within 4-5 hours. For example Linseed oil.

C. Semi-drying oils: - It is the oil which partially hardens when exposed to air. This is as opposed to a drying oil, which hardens completely, or a non-drying oil, which does not harden at all. Oils with an iodine number of 115-130 are considered semi-drying. Semi-drying oils contain 20%- 50% of polyunsaturated acids. They include: sweet almond oil, apricot seed oil, Cottonseed oil, Sesame oil and Grape seed oil.

Analysis of fats and oil: -

ACID VALUE: -

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

1.Phenolphthalein indicator: Weigh 1 g of phenolphthalein and dissolve in 100 mL of ethanol.

2.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.

3.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

Weigh accurately 0.6 g of potassium hydrogen phthalate, previously dried to constant weight at 105℃, and place it in a 250-mLconical flask, then add 50 mL of water. Shake it well. Then add 2 drops of phenolphthalein indicator.

Titrate the solution with the sodium hydroxide titrant until pink coloration can be observed.

Towards the end of titration, potassium hydrogen phthalate should be completely dissolved.

Calculate the concentration of the sodium hydroxide titrant according to the following equation:

$$C_{NaOH} = \frac{W_{C_8H_5KO_4} \times P_{C_8H_5KO_4} \times 1000}{V_{NaOH} \times Mw_{C_8H_5KO_4}}$$

where C_{NaOH} = Molarity of sodium hydroxide titrant (mol/L)

V_{NaOH} = Volume of sodium hydroxide titrant used (mL)

$Mw_{C_8H_5KO_4}$ = Molecular weight of potassium hydrogen phthalate (204.22 g)

$W_{C_8H_5KO_4}$ = Weight of potassium hydrogen phthalate used (g)

$P_{C_8H_5KO_4}$ = Purity of potassium hydrogen phthalate (%)

(c) Titration of test solution

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.

Shake it well. If necessary, reflux the mixture gently until the substance is completely dissolved.

Titrate the solution with sodium hydroxide titrant until pink coloration can be observed which persists for 30 second.

Measure the volume of sodium hydroxide titrant used and calculate the acid value according to the following equation:

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

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

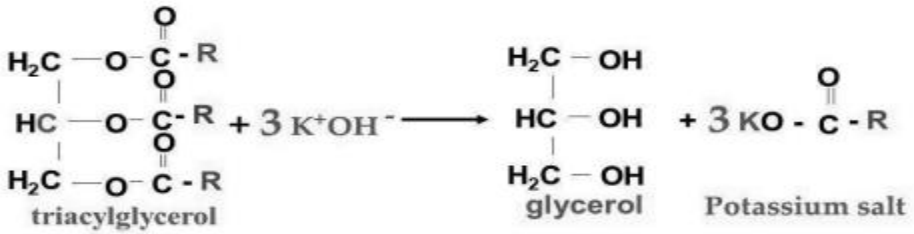
SAPONIFICATION VALUE: -

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.



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: - The magnitude of saponification value gives an idea about the average molecular weight of the fat or oil.

Higher the molecular weight of the fat, the smaller is its saponification value.

Saponification Value also indicates the length of carbon chain of the acid present in that particular oil or fat.

Higher the saponification value, greater is the percentage of the short chain acids present in the glycerides of the oil or fats.

Reagents Required:

1.Ethanolic KOH (95% ethanol, v/v)

2.Potassium hydroxide [0.5N]

3.Fat solvent

4.Hydrochloric acid [0.5N]

5. Phenolphthalein indicator (1%in ethanol)

Procedure:

1.Weigh 1g of fat in a tared beaker and dissolve in about 3ml of the fat solvent [ethanol /ether mixture].

2.Quantitatively transfer the contents of the beaker three times with a further 7ml of the solvent.

3.Add 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser.

4.Set up another reflux condenser as the blank with all other reagents present except the fat.

5. Place both the flasks in a boiling water bath for 30 minutes.

6.Cool the flasks to room temperature.

7.Now add phenolphthalein indicator to both the flasks and titrate with 0.5N HCl.

8.Note down the endpoint of blank and test.

9.The difference between the blank and test reading gives the number of milliliters of 0.5N KOH required to saponify 1g of fat.

Calculate the saponification value using the formula:

Saponification Value=28.05(Titrate Value of Blank-Titrate Value of Test sample)

Weight of sample(1g)

ESTER VALUE: -

The ester value is defined as the mg of KOH required to react with glycerin (glycerol / or glycerin) after saponify one gram of fat.

It is calculated from the saponification Value (SV) and the acid Value (AV):

Ester Value (EV) = Saponification Value (SV) – Acid Value (AV)

% glycerin □ Ester Value □ 0.054664

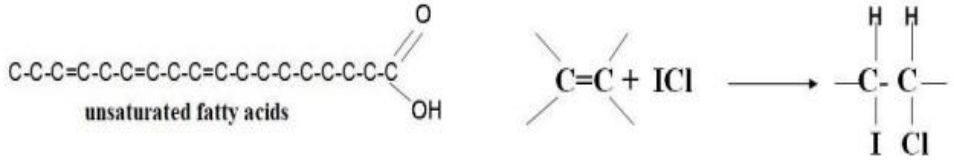
IODINE VALUE - The iodine value (IV) gives a measure of **the average degree of unsaturation of a lipid.**

The **higher the iodine value, the greater the number of C=C double bonds**.

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.

$$ICl + KI \leftrightarrow KCl + I_2$$

This causes the remaining unreacted ICl to form molecular iodine. The liberated I2 is then titrated with a standard solution of 0.1N sodium thiosulfate.

$$I_2 + 2\ Na_2S_2O_3 \rightleftharpoons 2\ NaI + Na_2S_2O_4$$

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 colored product and thus the endpoint can be observed.

Materials Required:

1.Iodine Monochloride Reagent

2.Potassium Iodide

3.Standardized 0.1 N Sodium thiosulphate

4.1% Starch indicator solution

5.Chloroform

6.Fat sample in chloroform

7.Iodination flask

8. Burette and burette stand with magnetic stirrer

9. Glass pipette

10.Measuring cylinder, distilled water

Method:

1.Arrange all the reagent solutions prepared and the requirements on the table.

2.Pipette out 10ml of fat sample dissolved in chloroform to an iodination flask labelled as "TEST".

3. Add 20ml of Iodine Monochloride reagent in to the flask. Mix the contents in the flask thoroughly. Then the flask is allowed to stand for a half an hour incubation in dark.

4.Set up a BLANK in another iodination flask by adding 10ml Chloroform to the flask.

5.Add to the BLANK, 20ml of Iodine Monochloride reagent and mix the contents in the flask thoroughly. Incubate the BLANK in dark for 30 minutes.

6.Meanwhile, Take out the TEST from incubation after 30 minutes and add 10 ml of potassium iodide solution into the flask.

7.Rinse the stopper and the sides of the flask using 50 ml distilled water.

8.Titrate the "TEST" against standardized sodium thiosulphate solution until a pale straw colour is observed.

9.Add about 1ml starch indicator into the contents in the flask, a purple color is observed.

10.Continue the titration until the color of the solution in the flask turns colorless.

11.The disappearance of the blue color is recorded as the end point of the titration.

12. Similarly, the procedure is repeated for the flask labelled „Blank'.

13. Record the endpoint values of the BLANK.

Calculate the iodine number using the equation below:

Volume of Sodium thiosulphate used = [Blank- Test] ml

$$Iodine\ No.of\ fat = \frac{Equivalent\ Wt.of\ Iodine \times Volume\ of\ Na_2S_2O_3\ used \times Normality\ of\ Na_2S_2O_3 \times 100 \times 10^{-3}}{Weight\ of\ fat\ sample\ used\ for\ analysis(g)}$$

Equivalent Weight of Iodine = 127

Normality of sodium thiosulphate ($Na_2S_2O_3$) = 0.1

Or

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

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:

1.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.

2.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.

3. 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.

4.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.

5.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 milliliters 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 Reagents any other fat

(a) Glycerin

(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 standardized

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

1. Weigh accurately 5 ±0.1g of filtered oil or fat sample into a clean, dry, 300 ml distilling flask. Add 20 ml of glycerin and 2 ml of concentrated sodium hydroxide solution, and heat with swirling over a flame until completely saponified, 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.

2. 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.

3.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 placed it in a water bath maintained at 15ºC 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 Whatman 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.

Run a Blank Test without the fat, but using the same quantities of the reagents.

Calculation

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

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.

IMPORTANT QUESTIONS

Questions carrying 2 marks.

1. What are fats and oils?

2. What are drying oils?

3. What is oxidative rancidification of oil and fats?

4. What is iodine value?

5. What is ester value?

6. What is R.M. value?

7. What is hardening of oils?

8. What is saponification value? What is its significance?

Questions carrying for 5 or 10 marks

1. Give detailed account of different analytical constants and their significance in the analysis of fats and oils.

2. Write a brief note on hydrolysis and hydrogenation of oils.

3. Discuss acid value, saponification value and ester value of fats and oil.

4. What is Reichert Meissl value? How is it calculated? Discuss its significance.

5. Describe hydrolytic and oxidative rancidification of fats and oil.