

DEFINITION OF LIPIDS

Lipids are organic substances relatively insoluble in water, soluble in organic solvents (alcohol, ether etc.) actually or potentially related to fatty acids and utilized by living cells.

Lipids includes fats and oils (triacylglycerides), phospholipids, steroids (cholesterol) and some other related compounds.

Lipids are better energy sources than sugars because they are less oxidized than carbohydrates.

Synthesized in adipose tissue, mammary gland and liver. Some lipids secreted unchanged from the blood stream.

SIGNIFICANCE OF MILK LIPIDS

Milk lipids are important:

- ✓ Source of essential fatty acids (linoleic acid) and serves as carrier of fat-soluble vitamins (A, D, E, K).
- ✓ For the rich-pleasing flavour and distinctive nutritional, textural and organoleptic properties in foods as well as dairy products (cream, butter, whole milk powder and cheese).
- ✓ Lipids also serve as precursors of compounds that cause off-flavour defects (hydrolytic and oxidative rancidity).
- ✓ In certain products and after certain processes, fatty acids serve as precursors of very flavourful compounds such as methyl ketones and lactones.
- ✓ Important as cellular metabolic regulators (steroid hormones and prostaglandins).

COMPOSITION OF MILK LIPIDS

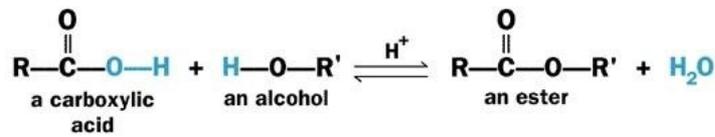
Constituents	Amount (%)	Location in milk
Triacylglycerides	98% of total milk fats	Fat globule
Diacylglycerides	0.28-0.59	-
Monoacylglycerides	0.01-0.03	-
Phospholipids	0.2-1.0	Fat globule membrane and milk serum
Cholesterol	0.5	Fat globule, fat globule membrane and milk serum
Glycolipids	0.006	-
Free fatty acids	0.1-0.4	Fat globule and milk serum
Hydrocarbons	Traces	-

Combinations of about 400 fatty acids are present in the triacylglycerols. These include short, medium and long chain saturated fatty acids as well as monounsaturated and polyunsaturated fatty acids which include cis and trans isomers.

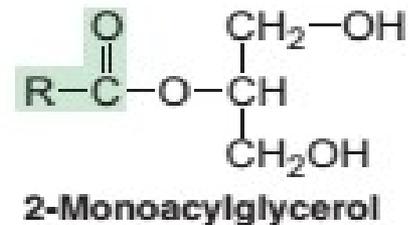
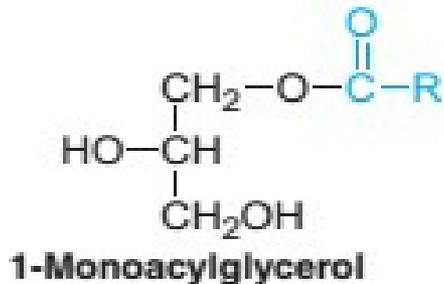
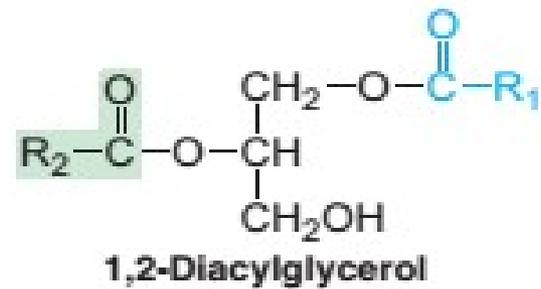
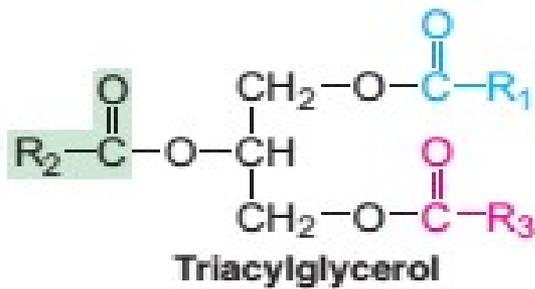
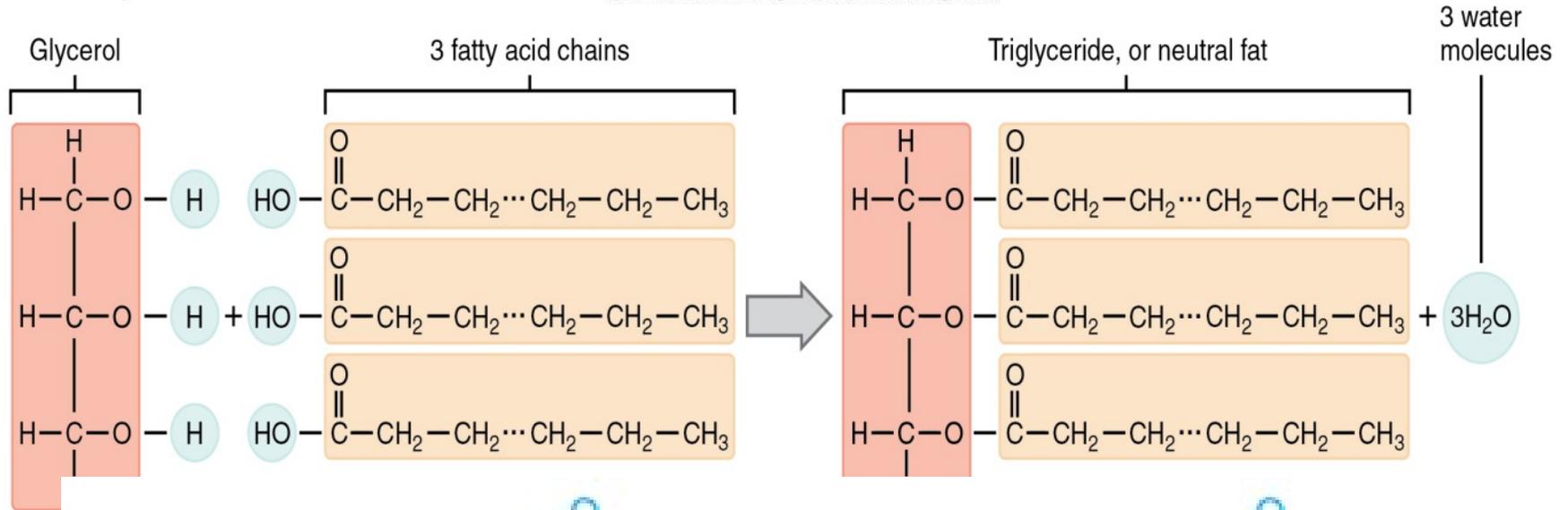
CLASSIFICATION OF LIPIDS

Simple Lipids: The lipids which yields only fatty acids and glycerol upon hydrolysis.

- a) **Fats and oils:** Found in adipose tissue, butterfat, fish oils, olive oil, corn oil, etc. This includes triacylglycerol (made from two kinds of molecules: glycerol, a trihydroxy alcohol and three molecules of fatty acids attached to it by ester linkage) diacylglycerol and monoacylglycerol. Oil is a liquid while fat is a solid at room temperature.
- b) **Waxes:** Composed of esters of fatty acids with long chain and alcohol other than glycerol. It has industrial and medicinal importance.



(Where R and R' are general hydrocarbon groups)



General structures of acylglycerols

Compound Lipids: Esters of fatty acids with alcohols containing additional groups (phosphate, nitrogenous base, carbohydrate and protein)

a) Phospholipids: Found chiefly in animal tissues. They contain alcohol, fatty acids, phosphoric acid bound in ester linkage to a nitrogenous base.

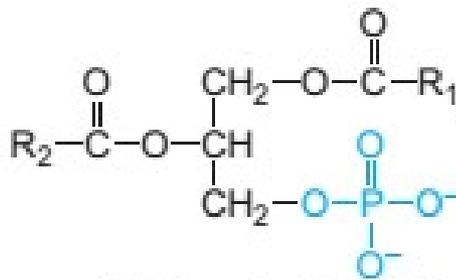
Glycerophospholipids: Glycerol as alcohol

i. Lecithin (Phosphatidyl choline) found in brain, egg yolk, and organ meats. where; phosphatide linked to choline (a lipotropic agent; important in fat metabolism and transport; used as emulsifying agent in the food industry.

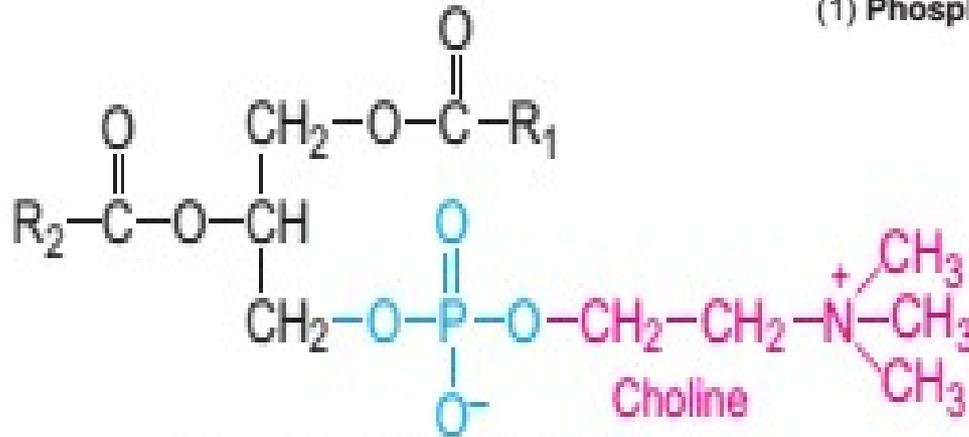
ii. **Cephalin (Phosphatidyl ethanolamine):** phosphatide linkage to ethanolamine; plays a role in blood clotting.

iii. **Plasmalogens:** When a fatty acid is attached by an ether linkage at C1 of glycerol in the glycerophospholipids, the compound is called plasmalogen.

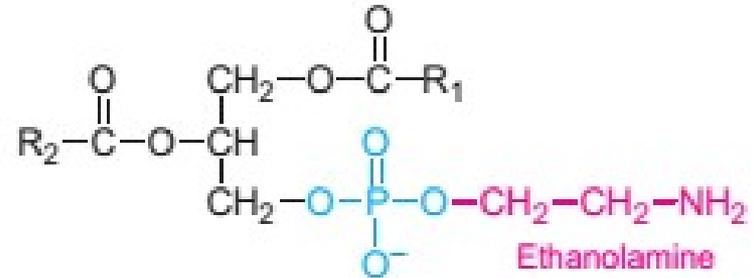
iv. **Phosphatidylinositol:** Important component of cell membranes, the stereoisomer myo-inositol (promotes proper utilization of hormone insulin) is attached to phosphatidic acid.



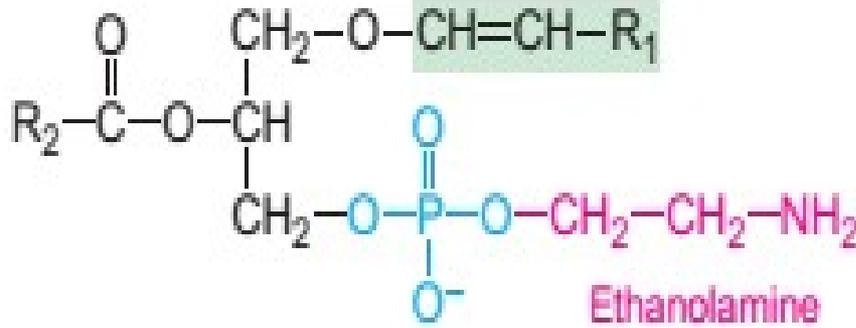
(1) Phosphatidic acid



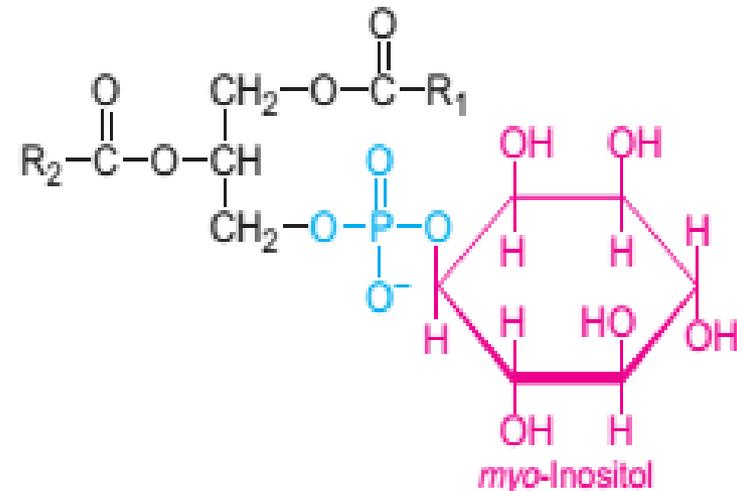
(2) Lecithin (phosphatidylcholine)



(3) Cephalin (phosphatidylethanolamine)



(6) Plasmalogen (phosphatidylethanolamine)



(4) Phosphatidylinositol

Sphingophospholipids: Sphingosine as alcohol

i. Sphingomyelin: Sphingosine is an amino alcohol present in sphingomyelins and do not contain glycerol. Sphingosine is attached by an amide linkage to a fatty acid to produce ceramide.

b) Glycolipids (glycosphingolipids): The lipid contain a fatty acid, carbohydrate and nitrogenous base. The alcohol is sphingosine. Glycerol and phosphate are absent eg: cerebrosides, gangliosides

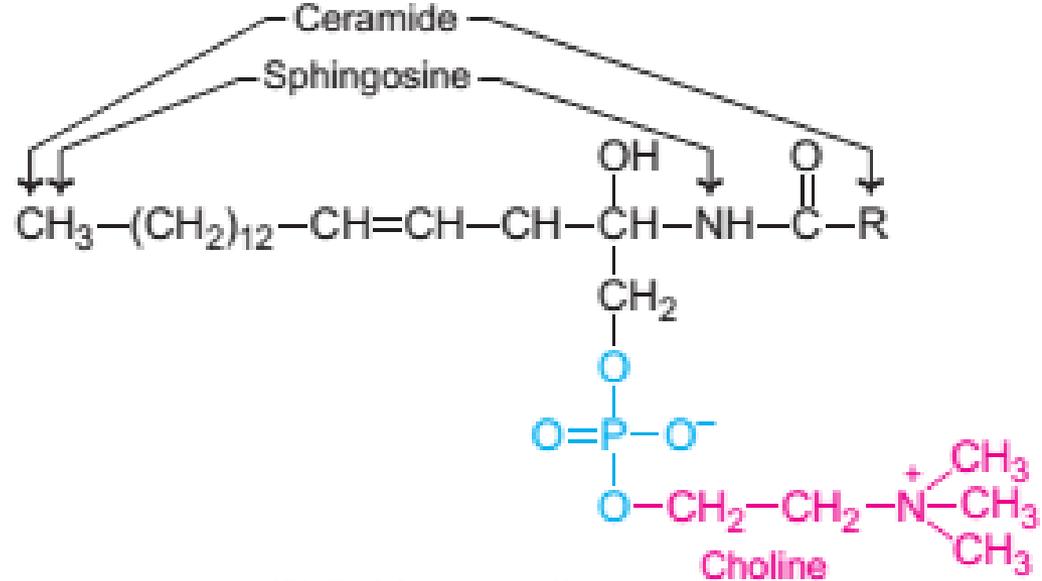
Cerebrosides: Cerebroside is a common name for a group of glycosphingolipids which are an important component of muscle and nerve cell membranes. They consists of a ceramide (sphingosine attached to a fatty acid) with a single sugar residue (glucose or galactose) at the 1-hydroxyl moiety.

Ganglioside: Brain, nerve tissue and other selected tissues, notably spleen; derivatives of cerebrosides and contain one or more molecules of N-acetylneuramic acid.

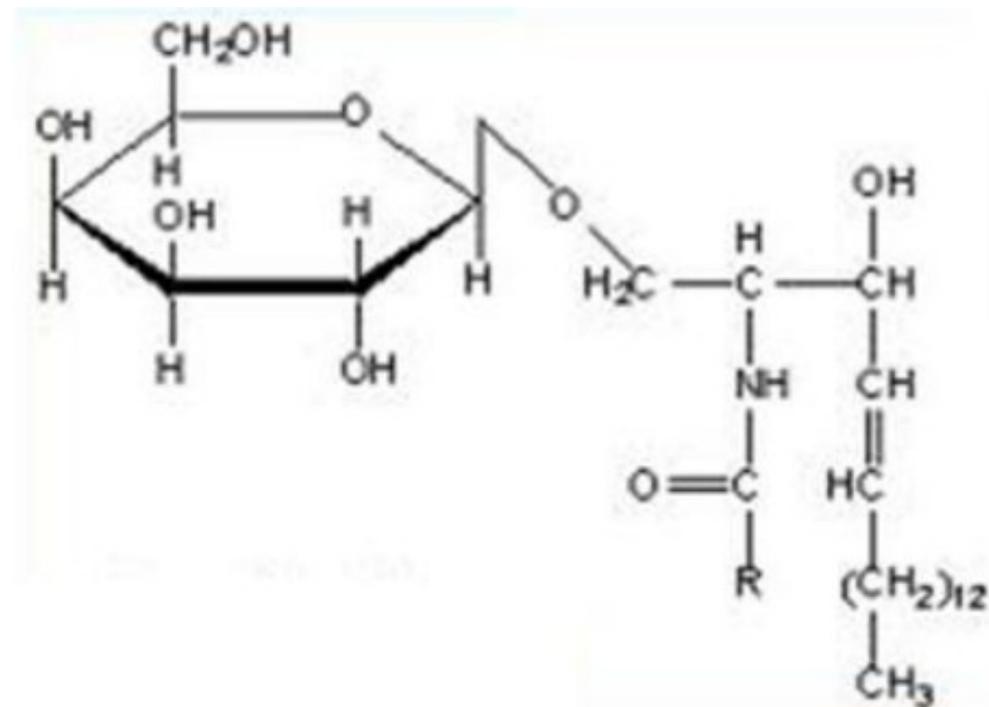
Sulfolipid: White matter of brain, liver, and testicle; also plant chloroplast. Sulfur containing glycolipid; sulfate present in ester linkage to galactose.

Proteolipids: Brain and nerve tissue. Complexes of protein and lipids having solubility properties of lipids.

c) Lipoproteins: Molecular complexes of lipid and protein. They are the transport vehicles for lipids in the circulation. 5 types are: Chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and free fatty acid-albumin complexes.



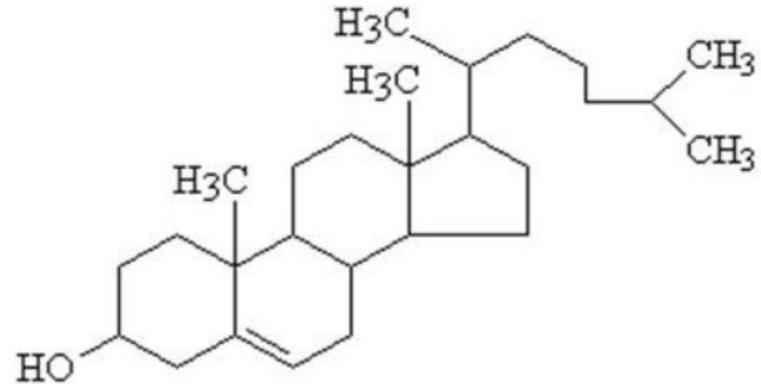
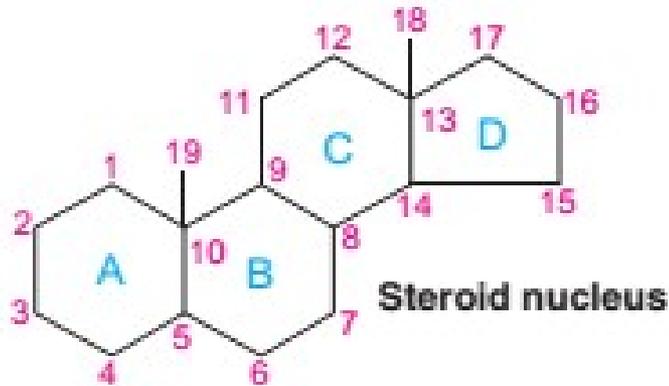
(8) Sphingomyelin



Structure of Cerebroside

Neutral Lipids: Uncharged lipids are neutral lipids.eg., cholesterol.

a) Cholesterol: Essential structural component of animal cell membrane. Found in egg yolk, dairy products, and animal tissues and one of abundant sterol. It is a precursor of bile acids and Vitamin D3. It has one hydroxyl group at C3 and a double bond between C5 and C6. An 8 carbon aliphatic side chain is attached to C17. Cholesterol contains 5 methyl groups.



Derived Lipids: These are the derivatives obtained on the hydrolysis of simple and compound lipids. Eg., soluble vitamins, fatty acids, hydrocarbons, glycerol and other alcohols, ketone bodies.

a) **Fatty acids:** They occur in plant and animal foods; also exhibit in complex forms with other substances. Fatty acids are carboxylic acids with hydrocarbon side chain. Classification of fatty acids is based on the length of the carbon chain (short, medium or long); the number of double bonds (saturated or unsaturated (mono or polyunsaturated); or essentiality in the diet (essential or non-essential). The most important omega fatty acids are:

✓ **Omega-6 fatty acids:** Linoleic acid (poultry, eggs, nuts, cereals, durum wheat, whole-grain breads, most vegetable oils, linseed oil, rapeseed oil)

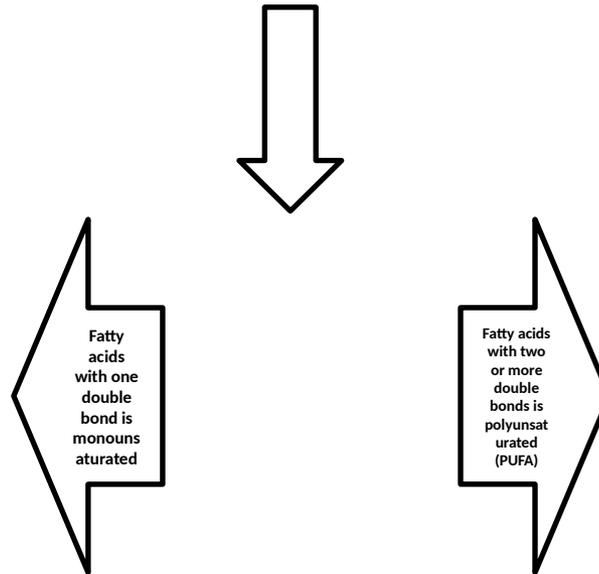
✓ **Omega-3 fatty acids:** Linolenic acid (ALA) found in plant oils and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) both commonly found in marine oils.

NOMENCLATURE OF FATTY ACIDS

- Depending upon their saturation and unsaturation, fatty acids are classified as saturated and unsaturated.

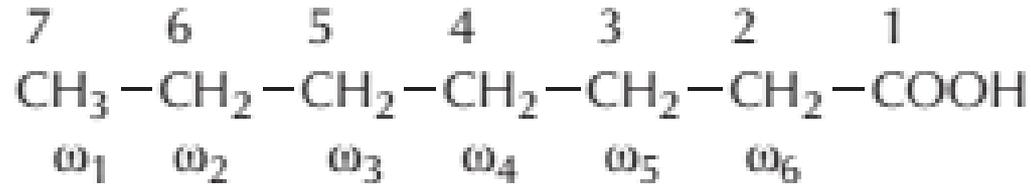
Saturated fatty acids do not contain double bonds

Unsaturated fatty acids contain one or more double bonds.



NOMENCLATURE OF FATTY ACIDS

The numbering of carbon atoms starts from the carboxyl carbon. The terminal carbon containing methyl group is called omega carbon.



The saturated fatty acids end with a suffix **-anoic** and unsaturated fatty acids end with **-enoic**

Depending upon the length of carbon chains, fatty acids are categorized into 3 groups: short chain with <6 carbons, medium chain with 8-14 carbons and long chain with 16-24 carbons.

<i>Common Name</i>	<i>Systematic name</i>	<i>Abbreviation*</i>	<i>Structure</i>
I. Saturated fatty acids			
Acetic acid	Ethanoic acid	2 : 0	CH ₃ COOH
Propionic acid	n-Propanoic acid	3 : 0	CH ₃ CH ₂ COOH
Butyric acid	n-Butanoic acid	4 : 0	CH ₃ (CH ₂) ₂ COOH
Valeric acid	n-Pentanoic acid	5 : 0	CH ₃ (CH ₂) ₃ COOH
Caproic acid	n-Hexanoic acid	6 : 0	CH ₃ (CH ₂) ₄ COOH
Caprylic acid	n-Octanoic acid	8 : 0	CH ₃ (CH ₂) ₆ COOH
Capric acid	n-Decanoic acid	10 : 0	CH ₃ (CH ₂) ₈ COOH
Lauric acid	n-Dodecanoic acid	12 : 0	CH ₃ (CH ₂) ₁₀ COOH
Myristic acid	n-Tetradecanoic acid	14 : 0	CH ₃ (CH ₂) ₁₂ COOH
Palmitic acid	n-Hexadecanoic acid	16 : 0	CH ₃ (CH ₂) ₁₄ COOH
Stearic acid	n-Octadecanoic acid	18 : 0	CH ₃ (CH ₂) ₁₆ COOH
Arachidic acid	n-Eicosanoic acid	20 : 0	CH ₃ (CH ₂) ₁₈ COOH
Behenic acid	n-Docosanoic acid	22 : 0	CH ₃ (CH ₂) ₂₀ COOH
Lignoceric acid	n-Tetracosanoic acid	24 : 0	CH ₃ (CH ₂) ₂₂ COOH
II. Unsaturated fatty acids			
Palmitoleic acid	<i>cis</i> -9-Hexadecenoic acid	16 : 1; 9	CH ₃ (CH ₂) ₅ CH = CH(CH ₂) ₇ COOH
Oleic acid	<i>cis</i> -9-Octadecenoic acid	18 : 1; 9	CH ₃ (CH ₂) ₇ CH = CH(CH ₂) ₇ COOH
Linoleic acid **	<i>cis, cis</i> -9,12-Octadeca- dienoic acid	18 : 2; 9, 12	CH ₃ (CH ₂) ₄ CH = CHCH ₂ CH = CH(CH ₂) ₇ COOH
Linolenic acid **	All <i>cis</i> -9,12,15-Octa- decatrienoic acid	18 : 3; 9, 12, 15	CH ₃ CH ₂ CH = CHCH ₂ CH = CHCH ₂ CH = CH(CH ₂) ₇ COOH
Arachidonic acid	All <i>cis</i> -5,8,11,14- Eicosatetraenoic acid	20 : 4; 5, 8, 11, 14	CH ₃ (CH ₂) ₄ CH = CHCH ₂ CH = CHCH ₂ CH = CHCH ₂ CH = CH(CH ₂) ₃ COOH

* Total number of carbon atoms, followed by the number of double bonds and the first carbon position of the double bond(s).

FACTORS AFFECTING FATTY ACIDS COMPOSITION

Feed and both lipid and non-lipid components affect milk fat composition. Milk produced by cow on low roughage diets may have only half of the fat content in comparison to the milk on high roughage diets.

The fatty acids (chain length of 16 or more carbon atoms) which are preformed from food fat (forage, fish supplements) is transferred to the mammary gland via the blood and lymph in the form of triglyceride and free fatty acids. Some of the fatty acids (short or medium chain length C4 to C14 and part of the C16) are being synthesized by the gland from the acetate and β -hydroxyl butyrate produced by rumen bacteria.

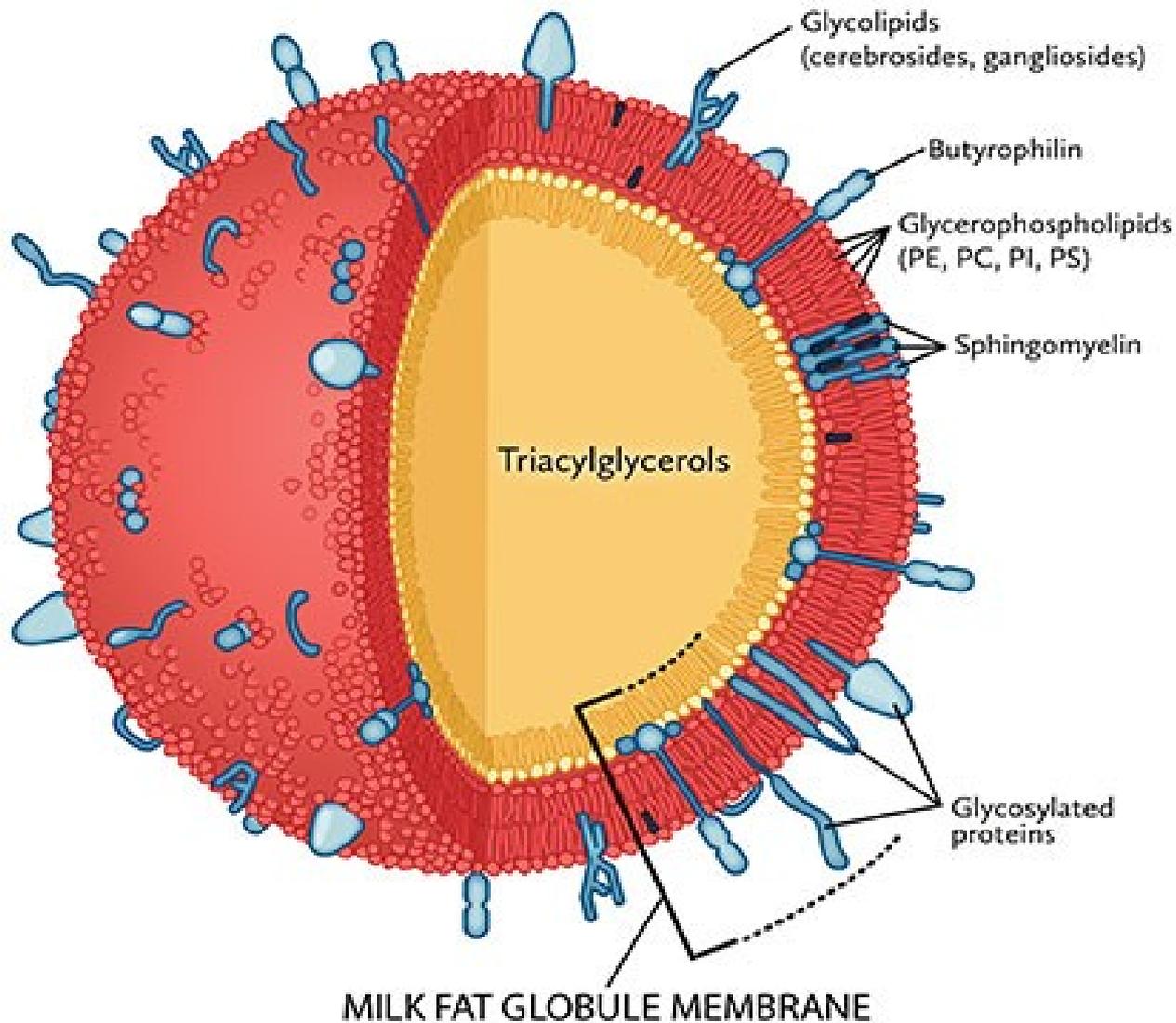
Feeding highly unsaturated oil such as safflower oil increases the C18:2 (linoleic acid) content of the milk fat.

Butyric acid (4:0) shows a maximum value during the first month of lactation, declining thereafter and becoming minimal at the end.

Hexanoic acid (6:0) to Myristic (14:0), the values increased during the first 4 to 8 weeks of lactation, remained relatively constant until the fifth or sixth month, and then decrease until the end of lactation.

Stearic (18:0) and oleic (18:1) acid contents were high in early lactation, then declining throughout the mid lactation and again increasing at the end of lactation.

STRUCTURE OF FAT GLOBULE

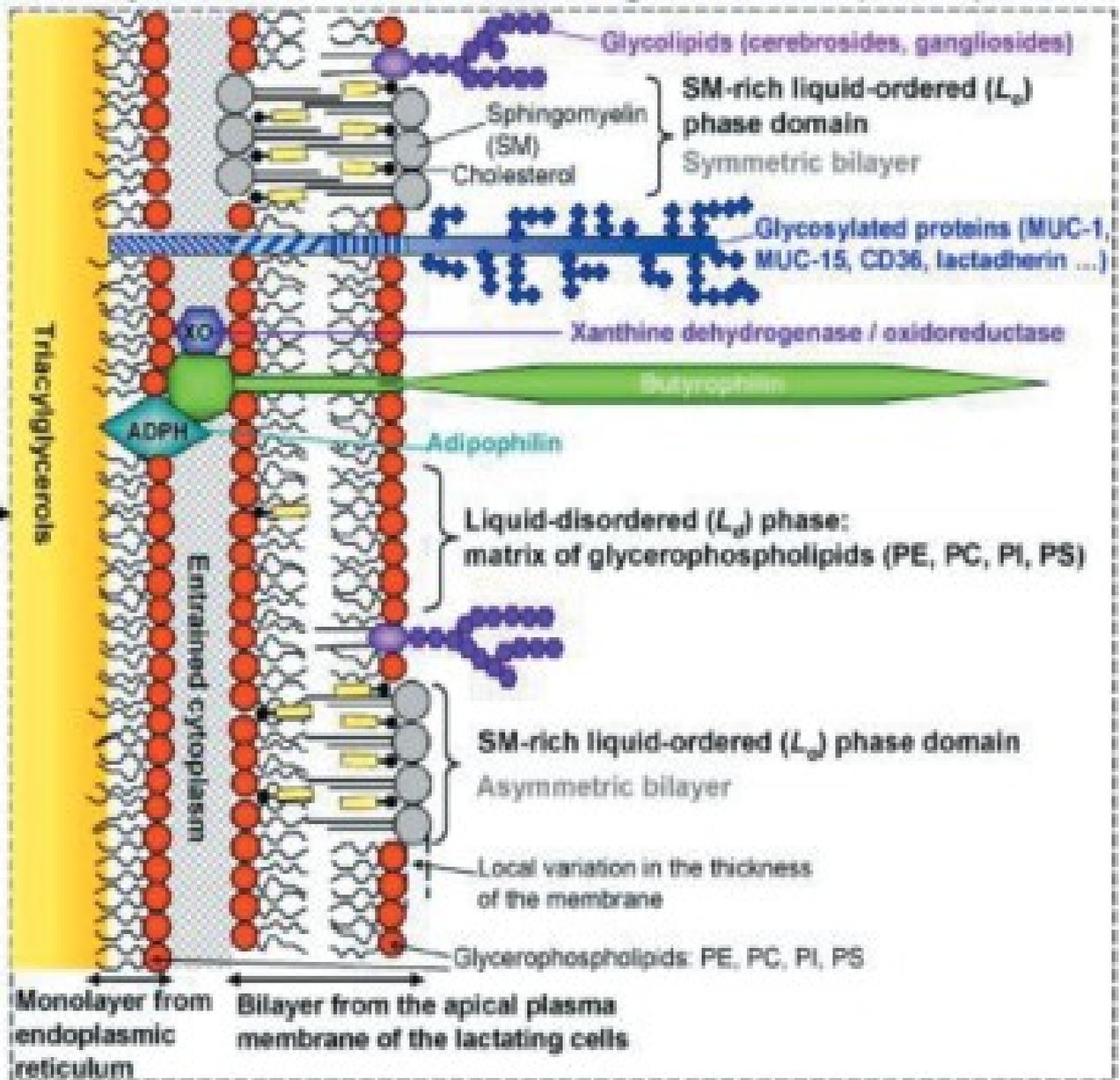
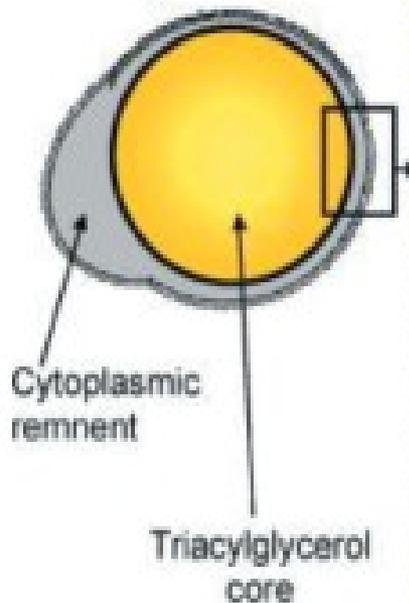


Human milk fat globules

Diameter: 4 to 5 μm

MFGM = 2 m^2 / g fat

$\zeta = -7 \text{ mV}$



STRUCTURE OF FAT GLOBULE

Milk fat is dispersed in milk as small, spherical globules (0.1-15 μm), stabilized in the form of emulsion by its surrounding membrane. This membrane (milk fat globule membrane (MFGM)) is created in the secretory cells of the mammary gland.

The interior of milk fat is made up of a triglyceride core surrounded by the fat globule membrane (10–20 nm in diameter) that protects the globules mainly against coalescence and lipolytic activity.

Structurally, the globule is composed of three layers:

- (1) a monolayer of polar lipids and proteins surrounding the interior lipid core**
- (2) a protein layer (protein coat)**
- (3) actual external lipid bilayer surrounding the entire fat globule, made of proteins, glycoproteins, enzymes, nonpolar and polar lipids and phospholipids.**

Protein constitutes nearly 25-60% of the total dry weight. MFGM is a rich source of enzymes including 5' Nucleotidase, alkaline phosphatase, acid phosphatase, aldolase, xanthine oxidase. Proteins of the MFGM could be in the form of polypeptides of varying molecular weight. Butyrophilin (BTN) is the main glycosylated protein of the fat globule membrane.

Lipids are the next major constituent of the MFGM and their content could vary from 0.5-1.2 mg per gram of protein. Phospholipids are the basic component of the membrane due to their amphiphilic structure and emulsifying properties. By interacting with membrane proteins and with sterols, they determine the structure, fluidity and stability of fat globule membrane. The major phospholipids present in the milk fat globule membrane are phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidylserine (PS).

Glycerides constitute a major portion of the neutral lipids of the membrane.

TESTS TO CHECK PURITY OF FATS AND OILS

IODINE NUMBER: It is defined as the grams (number) of iodine absorbed by 100 g of fat or oil. Iodine number is useful to know the relative unsaturation of fats and is directly proportional to the content of unsaturated fatty acids. Lower the iodine number, less is the degree of unsaturation. This unsaturation is in the form of double bonds which react with iodine compounds. The higher the iodine value, the more unsaturated fatty acid bonds are present in a fat.

SAPONIFICATION NUMBER: Number (mg) of KOH required to hydrolyze (saponify) one gram of fat or oil. It is the measure of the average molecular size of the fatty acids present. The value is higher for fats containing short chain fatty acids.

REICHERT-MEISSL (RM) NUMBER: Number of ml 0.1 N KOH required to completely neutralize the soluble volatile fatty acids distilled from 5 g of fat. RM number is useful for testing the purity of butter since it contains a good concentration of volatile fatty acids (butyric acid, caproic acid and caprylic acid). This is in contrast to other fats and oils which have a negligible amount of volatile fatty acids. Butter has a RM number in the range 25-30 while it is <1 for most other edible oils.

ACID NUMBER: Number of mg of KOH required to completely neutralize free fatty acids present in one gram of fat or oil. In normal circumstances, refined oil are free of fatty acids but on chemical or bacterial decomposition, it yields free fatty acids. Therefore, oil with increased acid number are unsafe for human consumption.

LIPOLYSIS AND RANCIDITY OF MILK FAT

Rancidity represents the deterioration of fats and oils resulting to unpleasant taste.

Fats containing unsaturated fatty acids are more susceptible to rancidity.

Rancid flavour is due to the accumulation of proper concentration and type of free fatty acids which are released due to the hydrolysis of milk fat due to the action of lipases which are normally present in milk.

Hydrolytic rancidity is caused by enzyme “milk lipoprotein lipase” which is attributed of leakage from blood through mammary tissues rather than true secretion.

Lipolysis takes place at an oil water interface.

The factors which influence enzyme activity are surface area availability , permeability of the emulsion, type of triglyceride employed, the physical state of the substrate (complete solid, complete liquid or liquid-solid) degree of agitation of the reaction, medium, pH, temperature, the presence of inhibitors and activators, concentration of the enzyme and substrate, light.

Lipolysis has been classified as spontaneous or induced. Spontaneous lipolysis has been defined as lipolysis caused without apparent mechanical agitation. Induced lipolysis is caused by some form of mechanical agitation of raw milk.

LIPID OXIDATION

Lipid oxidation in fluid milk and dairy products has been a concern of the dairy industry for the last several years.

To prevent or to retard this defect, low-temperature refrigeration of butter and butter oil, inert gas or vacuum packing of dry whole milk is preferred.

The rate of autoxidation is influenced by the complex composition of dairy products, the physical state of the product (liquid, solid, emulsion, etc.) and the presence of natural anti or pro-oxidants as well as processing, manufacturing and storage conditions.

Oxidation of unsaturated fats (RH) is a free radical process that involves initiation, propagation and termination steps.

Initiation

- The reaction involves removal of a hydrogen atom from a methylene group adjacent to a double bond in an unsaturated fat molecule.
- This generates the fatty acid radical (alkyl radical). The formation of R from RH is influenced by light exposure, metal catalysts, heat, active oxygen species and other factors.

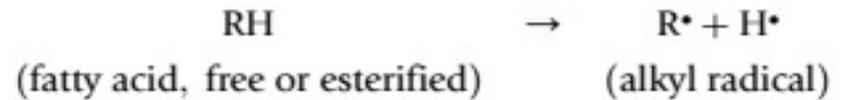
Propagation

- The alkyl radical is extremely reactive and can combine with molecular oxygen to produce a peroxy radical which can combine with another unsaturated fatty acid molecule and generate the corresponding hydroperoxide and a new alkyl radical.
- Hydroperoxides are unstable and degrade to produce volatile, non-radical end products leading to off-flavors.
- Alternatively, the hydroperoxides can degrade to produce an alkoxy radical and a hydroxy radical, both of which are extremely reactive and propagate the chain reaction by reacting with further RH molecules.

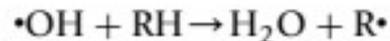
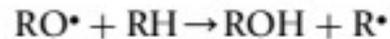
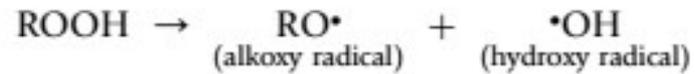
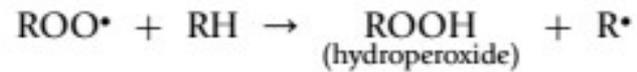
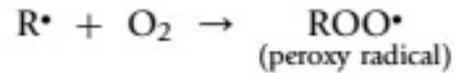
Termination

- The reaction ultimately terminates if the supply of RH molecules is exhausted, if oxygen becomes limiting, if radicals react with each other to generate non-radical end products

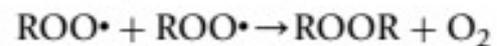
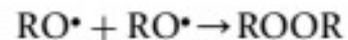
Initiation



Propagation



Termination



COMPOUND LIPIDS IN MILK

Lipids which on hydrolysis yield phosphates, carbohydrates and nitrogenous compounds (ethanolamine, serine, choline) in addition to the glycerol and fatty acids are known as compound lipids.

MFGM contains phospholipids, (glyco)proteins, glycolipids (cerebrosides and gangliosides), total and partial glycerides, free fatty acids and cholesterol. Phospholipids comprise approx. 1% of the total lipid in bovine milk.

PL belong to the class of polar lipids or defined “lipids containing phosphorus”). Polar lipids are fundamental in milk for the emulsification of fat in water, because together with proteins, they are the main constituents of the milk fat globule membrane (MFGM), which encircles the lipid droplets secreted by the mammary gland cells.

CHARACTERISTICS OF MILK PHOSPHOLIPIDS (PLs)

Glycerophospholipids and sphingolipids are the two main groups belonging to the class of PLs.

Glycerophospholipids are formed by glycerol, phosphoric acid, fatty acids and a hydroxy compound (e.g., choline, ethanolamine, serine, inositol).

The two fatty acids, mainly represented by unsaturated FAs, are esterified at the sn-1 and sn-2 positions of the glycerol backbone.

Sphingolipids are compounds containing a long chain base, (sphingosine), fatty acids and sugars or phosphoric acid. Sphingosine is the principal sphingoid base in mammalian sphingolipids, forming a ceramide when its amino group is linked (amide bond) with a saturated fatty acid.

In milk, cerebrosides are found 1.7 mg / 100ml in the globule membrane and 0.8 mg / 10 ml in skim milk.

However small amounts of plasmalogen which are the vinyl ether form of glycerophospholipid are also being reported

COMPOSITION OF MILK PHOSPHOLIPIDS

Phospholipids	Moles (%)
Phosphatidyl choline	34.5
Phosphatidyl ethanolamine	31.8
Phosphatidyl serine	3.1
Phosphatidyl inositol	4.7
Sphingomyelin	25.2
Plasmalogens	3
Diphosphatidylglycerol	Traces
Ceramides	Traces
Cerebrosides	Traces
Gangliosides	Traces

ROLE OF MILK PHOSPHOLIPIDS (PLs)

Milk and some by-products of dairy production are an interesting natural source of PLs.

Interest in these molecules is high due to both the possible positive effects on human health such as coronary heart disease, inflammation or cancer.

Technological properties in the food industry as emulsifiers or emulsion stabilizers.

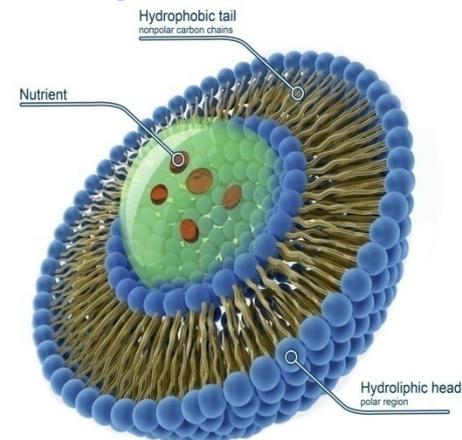
PROPERTIES OF MILK PHOSPHOLIPIDS (PLs)

Carriers of essential polyunsaturated FAs, very important molecules for the fluidity of the membrane. These lipids play an important role during storage and processing of milk due to their susceptibility to oxidation of the polyunsaturated fatty acids.

The emulsifying properties of PLs are due to the simultaneous presence in the molecule of a water-loving hydrophilic head and an oil-loving hydrophobic tail.

Milk PLs have been successfully tested for the preparation of liposomes, PL bilayer vesicles used both for the encapsulation and controlled release of bioactive compounds and for enhancing their stability and bioavailability.

Important role in the formation and secretion of the milk fat apart from forming a stable colloidal suspension or emulsions in aqueous sc



Structure of liposome

CHOLESTEROL-UNSAPONIFIABLE LIPID

Saponifiable lipid is one with an ester functional group, that can be hydrolyzed under basic conditions. These include triglycerides, phospholipids, glycolipids, sphingolipids, and the waxes. Non-saponifiable lipids cannot be hydrolyzed by sodium hydroxide because they have no ester bond.

The principal known compounds are cholesterol and related sterols, fat soluble vitamins A, D, E and K, unidentified waxes and series of n-alkylmethyl ketones containing odd number of carbons C3 to C15.

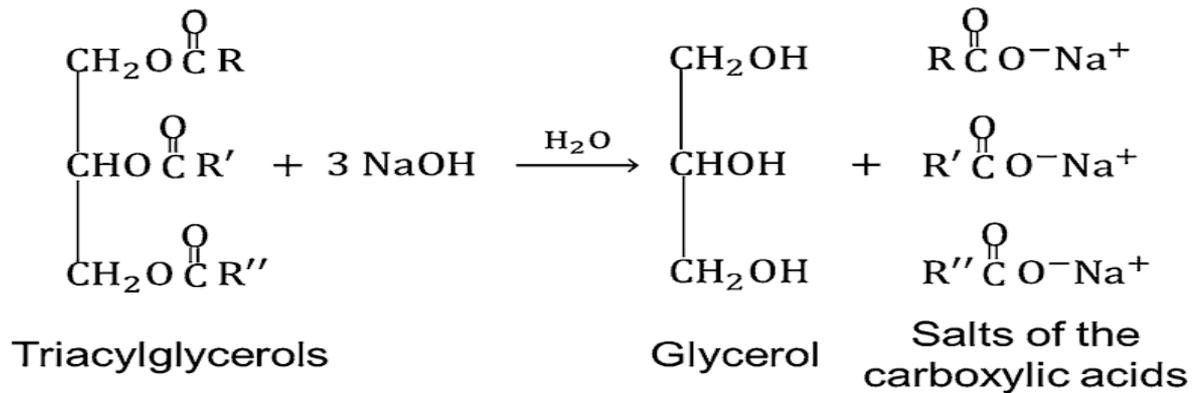
MAIN STEROLS IN UNSAPONIFIABLE MATTER OF MILK LIPIDS

Sr. No	Sterol	Per cent
1	Cholesterol	90.5
2	Campesterol	1.8
3	Stigmasterol	0.7
4	β sitosterol	0.2
5	Δ^5 Avenasterol	0.03

Triglycerides with approximately 0.5 N alcoholic base (heating) for 1 hr would decompose to salts of fatty acid, glycerol and small amount of other ester components. The resulting soap solution is diluted with water and extracted under specified conditions with ethyl ether. The ether extract after some washing to remove impurities is evaporated and dried to constant weight. This dry residue represents the unsaponifiable matter which for milk fat generally falls within the range of 0.30-0.45% by weight.

CHOLESTEROL

The principal sterol of milk is cholesterol ($C_{27}H_{45}OH$) that ranges from approx. 0.25-0.4% by weight of the fat. Since the total unsaponifiable matter ranges from 0.3 to 0.45% (by weight of the fat) giving an understanding that the unsaponifiable matter of milk fat is largely cholesterol.



There is a possibility that cholesterol values may include plant and animal hormone as well as other sterols all of which may be present in milk. The sterols are very apolar, but they easily associate with phospholipids, a small fraction of cholesterol is esterified, so this may be saponifiable.

PROPERTIES OF CHOLESTEROL

Cholesterol can be oxidized in various ways and cholesterol oxidized products (COP) are formed. These oxidized products of cholesterol are found in animal foods including the dairy products. A higher concentration of cholesterol oxidized products can be found only in processed dairy products exposed to harsh storage conditions where the impact of oxygen and light or oxygen and low water activity are associated.

Oxycholesterol or 5,6-epoxycholesterol is a form of oxidized cholesterol implicated in atherosclerosis. It is commonly formed from the reaction of fats and oxygen during high temperature cooking such as frying.

Oxysterols are oxidized derivatives of cholesterol which are important in many biological processes including cholesterol homeostasis, sphingolipid metabolism, platelet aggregation and apoptosis.