CC:7 Unit 10
MILK FAT (PART 1)
composition and characterization

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The milk of all mammals' contains lipids but the concentration varies widely between species. The principal function of the lipids is to serve as source of energy for the infants and young ones. It is also serves as a source of essential fatty acid i.e., fatty acids which cannot be synthesized by higher animals especially linolenic acid, and soluble vitamins such as A, D, E, and K. The lipids also help in contributing to the flavour and the rheological properties of the dairy products and foods in which they are used. The lipids in milk are unique for the species although they are termed as milk lipids or milk fat. This is because of the fact that the fatty acid composition and its biosynthesis is different in each species. The role of milk fat in nutrition is its capability to yield approximately ~37 kJ per g (9 Kcal/g) apart from carrying the fat soluble vitamins viz., A, D, E and K. The presence of significant amounts of essential fatty acids viz., linoleic and arachidonic acids also play an important role in the nutrition of the new born. In cow milk, the milk fat plays a vital role in body and texture and flavour of the milk products.

Milk and milk products occupy a significant position in the human diet which is primarily attributed to its nutritional properties and also the body, texture and flavour of these foods. The fatty acids composition of the lipid plays a significant role in the characteristics of the products. Milk fats especially ruminant fats, contain a wide range of fatty acids. However, the vast majority of these occur at only trace concentrations.

In the present chapter the properties of milk fat are covered under following sections:

1. Composition and structure
2. Fatty acid profile of milk fat
3. Refractive index
4. Fat constants:

Saponification value
Iodine value
RM value
Polensky value and
Peroxide value

Composition of Lipids in cow Milk

The milk fat is present in milk as oil-in-water emulsion in globular form. The bulk of cow milk lipids are triacylglycerols (TGs) which are 97-98 % of the total lipids found in pooled milk. Sterols mostly cholesterol and phospholipids are next in the quantity. Although phospholipids represent less than 1% of the total lipid, they play an important role, being present mainly in the milk fat globule membrane (MFGM) and other membranous martial in milk. In addition to stabilizing the emulsion of fat in the milk and preserving the individual identities of the fat globules, it protects the free fat from the lipase enzyme. Phospholipids represent a considerable proportion of the total lipid of buttermilk. The structure of the triglycerols of fatty acids is shown **in fig 1** and general composition of milk lipids in **Table 1.**

Structure of Fat Globule

Milk fat is predominantly present spherical droplets which range in diameter from less than 0.2 to 15 μm. The bulk of the fat is in globules 2-8 μm diameter. The size distribution found may depend greatly on the measurement method employed. Globule size can considerably altered by various treatments particularly homogenization. Lipids are insoluble in water and an interfacial tension therefore exists between the phases when lipids are dispersed in water. Owing to the interfacial tension, the oil and water phases would quickly coalesce and separate. However, coalescence can be prevented by use of emulsifiers which forms a film around each fat globule and reduce interfacial tension. In case of raw milk, the emulsifying film is much more complex than that in artificial emulsion, and is referred as milk fat globule membrane (MFGM). This layer is predominantly proteinaceous in nature. The composition of individual lipid sample lipids and total phospholipids in milks of some species are presented in Table 2.

Fatty Acid Profile of Milk fat

Cow milk is composed of triacylglycerol (97.0 to 98.5%). These are formed by the esterification of the hydroxyl groups of glycerol with the fatty acids. The nature of these fatty acids varies vastly and has a significant influence on the chemical, physical and organoleptic properties of the fat. Although several fatty acids have been identified in the milk fat only few of them are present in significant quantity and are of nutritional, physical and chemical importance. Further 80% of the total fatty acids are distributed among the five fatty acids namely the oleic, palmitic, butryic, stearic and myristic acids. These fatty acids are usually grouped on the basis of saturation (saturated, monounsaturated and polyunsaturated). Similarly they can also be grouped on the basis of geometric isomerism as straight chain, branched chain, on the basis of chain length as short, medium and long chain. The chain length of fatty acids in milk fat varies from $C_4$to C_{26}. C_4 indicates that the fatty acid chain has 4 carbon atoms and C_{26} indicates that the fatty acid chain has 26 carbon atoms. Normally the fatty acids percentage is not expressed on the weight basis but on molar percentage. The Principal fatty acids (wt % of total) in milk triacylglycerides in cow milk is presented in Table 3.

The major components of fats are the acids. In case of milk fat, the fatty acids account for about 85% and the glycerol for approximately 12.5% of the weight. Glycerol is a non-varying component of all fats, whereas the fatty acids represent the significant variable. Hence the chemistry of a particular fat depends primarily on its component acids.

Ruminant milk fat contain a high level of butyric acid ($C_{4:0}$) and other short chain fatty acids. The concentration of butyric acid in milk fat is the principle of the widely used criterion for the detection of and quantification of the adulteration of butter fat with other fats. (i.e RM value and Polensky numbers which are the measures of volatile water soluble and volatile insoluble fatty acids respectively).

Short chain fatty acids have strong and characteristic flavours which are released by the action of lipase enzyme in milk and milk products. They impart strong flavours which are undesirable in milk and butter while they contribute to the characteristic desirable flavour in cheeses. Hence the chemical reaction of milk fat i.e. hydrolysis and auto-oxidation plays an important role in spoilage of fat rich dairy products.

Unsaturated fatty acids may occur in *cis* or*trans* isomers. *Trans* isomers have higher melting points than the corresponding *cis* isomers which are considered to be nutritionally undesirable. Bovine milks contain low level of *trans* fatty acids in comparison with chemically hydrogenated vegetable oils.

Factors Affecting Fatty Acid Composition

The composition of milk fat is never constant and it very varies. The important cause for variation is feed and both lipid and non lipid components affect milk fat composition. As, fatty acids are preformed from food, the fat is transferred to the mammary gland via the blood and lymph in the form of triglyceride and free fatty acids. Most of these fatty acids are having a chain length of 16 or more carbon atoms. Milk produced by cow on low roughage diets may have only half of the fat content when compared to the milk from cows on high roughage diets and the proportion of the short and medium chain saturated acids which are synthesized from acetate is greatly diminished.

Melting point of milk fat

Milk fat exhibits a wide range of melting point range. Triacylglycerol molecule of milk fats are mixture of fatty acids forming. As a result of mixture of the glycerides which is a characteristic of the composition of any fats, they exhibit a range of melting point depending on the particular fat. There is no possibility to obtain a specific temperature to be regarded as melting point similar to pure chemical compounds. As such, melting point of milk fat is the end of melting range. There are several methods available for the determination of melting points. The average softening point for milk fat is attained at 30°C for soft butter and 38°C for hard butter. This has practical importance with reference to the granulation in ghee which determines the quality of ghee by the consumers.

The melting point of triglycerides is determined by the fatty acid profile and the position of the fatty acids in the triglyceride. The melting point of fatty acids increases with increase in the length of the fatty acid chain, position of double bond. The melting point decreases with decrease in the number of double bonds in the molecule. The traditional method of estimation of melting point of milk fat is by calorimetric method.

The melting point of milk fat has of great importance in granulation of ghee, the most popular indigenous fat rich product. Granulation is one of the important characteristic of good quality ghee. Since milk fat is a mixture of saturated and unsaturated fatty acids, the ghee forms good granules at room temperature of around 28 to 35^0C.

Milk fat constants: For the purpose of characterization, certain well-known physical and chemical constants have been derived for the more common fats. These constants serve as indications of the type of component fatty acids present in fats. They also enable the detection of fat adulteration qualitatively and in some cases quantitatively. The physical and chemical constants of fats are helpful in characterizing the fat. The type of the fatty acids present in the fat can also be identified with the help of these constants. The fat constants of some of the common fats and oils is summarized in the table 4

Refractive index

The basic principle in determining the refractive index is the fact that the degree of bending of light wave passing through a liquid or transparent solid is a characteristic for the particular liquid or solid. When a beam of light passes from one medium to another, the light rays bends. The bending or refraction of the light is expressed as its refractive index. The refractometer reading is a constant for any particular medium at a constant temperature. This physical constant for milk fat is determined by using the Abbey refractometer. The reading is usually obtained at 40℃. This instrument directly gives the refractive index. Butyrorefractometer is also useful for determining the refractive index of milk fat. The reading obtained with this instrument has to be converted in to refractive index or can be expressed as butyrorefractometer reading (B.R. Reading). The reading for the milk fat ranges between 1.4527and 1.4566.due to the large proportion of saturated glycerides and short chain acids in the bovine milk fat it is low in comparison to that of other fats and oils mainly because of the greater number of saturated glycerides and short chain acids in milk fat. The refractive index of a fat is influenced by the both the molecular weight and the degree of unsaturation of the component fatty acids. The refractive index reading is normally made at 40^0C due to the fat that at that temperature all the fat will be in the liquid form. This value id low in comparison to that for other fats and oils mainly because of the greater number of saturated glycerides and short chain acids in the milk fat. Refractive index increases with increasing saturation and with chain length of fatty acids.

Saponification Number

In fats a large portion is made up of glycerides esters which are saponifiable material. In addition to the triglycerides, the saponifiable matter also includes phospholipids and cholesterol. The saponification number is defined as the number equivalent to the number of milligrams of potassium hydroxide required to saponify one gram of fat. This value may range from 210to 233 for milk fat. This value of a lipid indicates the average molecular weight of fatty acids present in it. With exception of coconut and palm oil, this constant of milk fat is well above those for other oils and fats. Saponification value is more useful in detecting the mineral oils such as paraffin in ghee as they are not acted upon by alkali and such a sample does not form a homogenous solution upon saponification.

Iodine Number

Iodine number is the number grams of iodine absorbed by 100 g of fat under specified conditions. This value is a measure for the unsaturated linkages present in a fat. Absorption of either iodine bromide (IBr) or iodine chloride (ICl) is used for measuring this value. One molecule of halogen compound absorbed by each unsaturated linkage and the absorption is expressed as equivalent number of iodine absorbed by 100g fat. The iodine value for milk fat ranges between 26 - 35. The softness of fat is directly related to the iodine number. The iodine value for milk fat is generally low in comparison to most other oils and fats.

Reichert-Meissal Number

This value is the number of milliliters of 0.1N sodium hydroxide or aqueous alkali solution required to neutralize the steam volatile water soluble fatty acids distilled from 5g of fat under specified conditions. This value for milk fat is quite significant since it primarily measures the butyric acid and caproic acid content in the given fat. When compared to other fats this value for milk fat is high. This helps in the identification of milk fat from other fats. The R.M. value for milk fat ranges between 18 and 30.

Polensky number

It is the number of milliliters of 0.1N sodium hydroxide or aqueous alkali solution required to neutralize the steam volatile water insoluble fatty acids distilled from 5g of fat. The Caprylic and capric acid although steam volatile, are insoluble in water. Since most of the steam volatile fatty acids in milk fat are water soluble this value helps in identifying the presence of coconut oil content which contains higher proportion of these acids. The polensky value for milk fat ranges between 1.0 to 3.3.

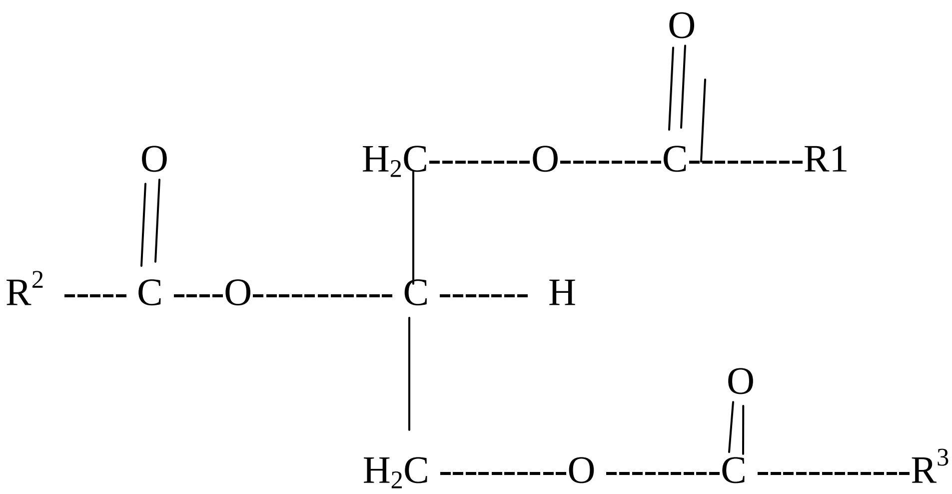
Peroxide Value: Peroxide value (PV) is one of the most widely used tests for oxidative rancidity. It is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Milliequivalents of peroxide per kg of fat are measured by titration with iodide ion. Peroxide values are not static and care must be taken in handling and testing samples high peroxide values are a definite indication of a rancid fat, but moderate values may be the result of depletion of peroxides after reaching high concentrations.

The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. Traditionally this was expressed in units of milliequivalents and more appropriately as millimoles per kilogram fat. The unit of milliequivalent has been commonly abbreviated as mequiv or even as meq. The determination of peroxide value is frequently used as a means of determining whether the quality of the oil or fat is spoiled due to oxidative rancidity. In general the oils and fats are spoiled by the chemical deterioration, and more particularly by the oxidation of fat. The determination of the PV is based on the reaction of potassium iodide in acid solution with the bound oxygen followed by titration of the liberated iodine with sodium thiosulphate.

Conclusion*:* The milk fat is present in milk as oil-in-water emulsion in globular form. The bulk of cow milk lipids are triacylglycerols (TGs) which are 97-98 % of the total lipids found in milk. The milk fat globule membrane helps in stabilizing the emulsion of fat in the milk and preserving the individual identities of the fat globules, it protects the free fat from the lipase enzyme. The fatty acids are usually grouped on the basis of saturation i.e., saturated, monounsaturated and polyunsaturated. Ruminant milk fat contain a high level of butyric acid ($C_{4:0}$) and other short chain fatty acids. These short chain fatty acids play a vital role in development of off flavours in fat rich dairy products. The physical and chemical constants have been derived for the more common fats. These constants serve as indications of the type of component fatty acids present in fats. They also enable the detection of fat adulteration qualitatively and in some cases quantitatively. The physical and chemical constants of fats are helpful in characterizing the fat. The type of the fatty acids present in the fat can also be identified with the help of these constants.

Total number of words excluding tables = 2827

Fig 1: Structure of triglycerides



Where R^1, R^2 and R^3 are alkyle chains of fatty acids. The three fatty acids are R^1COOH, R^2COOH and R^3COOH which may be same or different.

Table 1. Composition of milk fat

Constituents	Range of occurrence	Location in milk
Triglycerides	98 – 99%	Fat globules
Phospholipids (lecithin, cephalin, sphingomyelin)	0.2 – 1.0%	Globule membrane and serum
Sterols (cholesterol, lanosterol)	0.25 – 0.40%	Globule membrane and milk serum
Free fatty acids	Traces	Fat globules and milk serum
Waxes	Traces	Fat globules
Squalene	Traces	Fat globules
Fat-soluble vitamins		Fat globules
Vitamin A	7.0 – 8.5µg./g. fat	
Carotenoids	8.0 – 10.0µg./g. fat	
Vitamin E (tocopherols}	2 – 50µg./g. fat	
Vitamin D	Traces	
Vitamin K	Traces	

Table 2. Composition of individual lipid sample lipids and total phospholipids in milks of some species (weight % of the total lipids)

Lipid class	Cow	Buffalo	Human
Triacyleglycerols	97.5	98.6	98.2
Diacyleglycerols	0.36	T	0.70
Monoacyleglycerols	0.027	T	T
Cholesteryl esters	T	0.1	T
Cholesterol	0.31	0.30	0.25
Free fatty acids	0.027	0.5	0.4
Phospholipids	0.6	0.5	0.26

Source Fox and McSweeney, Dairy Chemistry and Biochemistry, 1998)

Table 3 Principal fatty acids (wt % of total) in milk triacylglycerides in cow milk

Sl. No	Fatty acid	Notation	(%)	
			cow	buffalo
1	Butyric acid	4:0	3.3	3.6
2	Caproic acid	6:0	1.6	1.6
3	Caprylic acid	8:0	1.3	1.1
4	Capric acid	10:0	3.0	1.9
5	Lauric acid	12:0	3.1	2.0
6	Myrestic acid	14:0	9.5	8.7
7	Palmetic acid	16:0	26.3	30.4
8	Stearic acid	18:0	14.6	10.1
11	Oleic acid	16:1	2.3	3.4

(Source: Fox and McSweeney, Dairy Chemistry and Biochemistry, 1998)

Table 4 Physical and chemical constants of some common fats

Fat	Melting point	Refractive Index at 40^0C	Iodine number	Saponification number	RM number	Polensky number
Beef tallow	42- 48	1.4566 – 1.4596	35 – 43	194 – 200	1	1
Cocoa butter	28 – 33	1.4537 – 1.4580	32 – 42	192 - 198	1	--------
Coconut oil	20 - 28	1.4477 – 1.4495	6 – 10	245 - 262	6 - 8	15 20
Cottonseed oil	--------	1.4696 – 1.4718	103 – 112	192 - 196	1	
Lard	36 - 45	1.4580 – 1.4620	50 - 80	193 - 200	1	1
Milk fat	30 - 41	1.4538 – 1.4578	26 – 35	210 -223	17 – 35	1 – 3
	23 – 30				4 8	7 - 12
Palm kernel oil	-------------	1.4492 – 1.4543	10 – 18	243 - 255	1	----------
Peanut oil		1.4620 – 1.4653	88 - 98	186 - 194		