# CHEMISTRY OF MILK

**K.S. Sarma**

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*Author*

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*Module 1. Milk definition, composition and variation*

**Lesson-1**

**Definition of Milk as Per Food Standards and Safety Authority of India, 2006 (formerly Prevention of Food adulteration Act, 1954) and Average Composition of Milk from Cow and Different Species**

## 1.1. INTRODUCTION

Milk is a complex fluid containing many components in different states of dispersion. Understanding its properties and the changes that occur in it requires knowledge of all the components and their interaction with other constituents. An over view of the milk composition and their physical states of dispersion are being dealt in this lesson.

## 1.2. MILK DEFINITION

Milk in legal terms (PFA1954) may be defined as whole, fresh,clean, lacteal secretions, obtained by complete milking of one or more healthy milch animals, excluding that obtained 15 days before or 5 days after or such periods as may be necessary to render the milk practically colostrum free and containing legally prescribed minimum percentage of fat and Solids-not-fat (SNF).

However in chemical terms milk may be defined as a complex chemical substance in which fat is present in the form of an emulsion, protein and some mineral matter in the colloidal state and lactose with some minerals and soluble proteins in the form of true solution.

All species of mammals secrete milk to provide nutrients required for the optimum growth of the new born,apart from protecting it from some of the common diseases. The development of the young one in all species of mammals is not uniform as such the composition of the milk secreted by these mammals will also vary depending up on the nutritional needs of the young one. It is a matter of academic interest to know the average composition of milk from some of the species along with the composition of milk from human. The scientific names of the mammals is given here

**Table-1.1: Scientific names for some species of mammals**

| Species of animals | Scientific name |
|---|---|
| Cows (Western) | *Bos taurus* |
| Cows (zebu) | *Bos indicus* |
| Water buffalo | *Bubalus bubalis* |
| Goat | *Capra hircus* |
| Sheep | *Ovis aries* |
| Humans | *Homo sapiens* |

**Table-1.2: Approximate Composition of Cow Milk (Western)**

| Component | Average Content (%, w/w) | Range (% (w/w) | Average (% On Dry matter basis) |
|---|---|---|---|
| Water | 87.3 | 85.5 -88.7 | |
| Solids not fat | 8.8 | 7.9-10.0 | 69 |
| Fat in dry matter | 31.0 | 21.0-38.0 | |
| Lactose | 4.6 | 3.8-5.3 | 36 |
| Fat | 3.9 | 2.4-5.5 | 31 |
| Protein | 3.25 | 2.3-4.4 | 26 |
| Casein | 2.6 | 1.7-3.5 | 20 |
| Mineral substances | 0.65 | 0.53-0.80 | 5.1 |
| Organic acids | 0.18 | -- | 1.4 |
| Miscellaneous | 0.14 | -- | 1.1 |

**(Source: Dairy Chemistry and Physics, Walstra and Jenness, 1984)**

## 1.3 DIFFERENCES IN THE COMPOSITION OF MILK FROM VARIOUS SPECIES

The Table 1.3 showing the composition of milk from various species is given for understanding the variation in the composition of the milk secreted by them. It could be observed that the buffalo milk is having the maximum fat while the fat content in sheep milk is also much close to that. Similarly the fat percent in goat milk is much similar to cow milk. Variation among the protein percent is also very less among these species. The lactose content in Human milk is characterized by having higher percent of lactose and fat while the protein and ash content is much less when compared with other species. The energy supplied through the milk is highest in buffalo and sheep while the difference is very much less between the milk from the remaining species

**Table- 1.3: Composition of milk from different animal species**

| Species of Animals | Composition (g/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Water | Fat | Casein | Whey proteins | Lactose | Ash | Energy (kcal/100g) |
| Human | 87.1 | 4.5 | 0.4 | 0.5 | 7.1 | 0.2 | 72 |
| Cow | 87.3 | 3.9 | 2.6 | 0.6 | 4.6 | 0.7 | 66 |
| Zebu | 86.5 | 4.7 | 2.6 | 0.6 | 4.7 | 0.7 | 74 |
| Water Buffalo | 82.8 | 7.4 | 3.2 | 0.6 | 4.8 | 0.8 | 101 |
| Goat | 86.7 | 4.5 | 2.6 | 0.6 | 4.3 | 0.8 | 70 |
| Sheep | 82.0 | 7.2 | 3.9 | 0.7 | 4.8 | 0.9 | 102 |
| Reindeer | 66.7 | 18.0 | 8.6 | 1.5 | 2.8 | 1.5 | 214 |
| Horse | 88.8 | 1.9 | 1.3 | 1.2 | 6.2 | 0.5 | 52 |
| Rabbit | 67.2 | 15.3 | 9.3 | 4.6 | 2.1 | 1.8 | 202 |
| Camel | 86.5 | 4.0 | 2.7 | 0.9 | 5.0 | 0.8 | 70 |

(Source: Jenness and Sloan,1970, Dairy science abstracts,32,599-612)

**1.4 LEGAL STANDARDS FOR VARIOUS CLASSES OF MILK**

According to the FSSAI (2006) the standards for different classes and designations of milk in India are presented in Table 1.4.

## Table: 1.4- Legal standards for various classes of milk

| Class of milk | Designation | Locality | Minimum Fat % | Minimum SNF % |
|---|---|---|---|---|
| Buffalo milk | Raw, Pasteurized, boiled, flavoured and sterilized | Andaman and Nicobar, Andhra Pradesh,Dadra and Nagar – Haveli, Goa,Daman and Diu, Kerala, Himachal Pradesh, Lakshadweep, Tamil Nadu, Madhya pradesh, Manipur, Karnataka, Nagaland, NEFA,Orissa, Pondicherry,Rajasthan, Tripura | 5.0 | 9.0 |
| | Raw, Pasteurized, boiled, flavoured and sterilized | Assam, Bihar, Chandigarh, Delhi, Gujarat, Maharashtra, Haryana, Punjab, Uttar Pradesh, West Bengal | 6.0 | 9.0 |

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| Cow milk | Raw, Pasteurized, boiled, flavoured and sterilized | Chandigarh, Haryana, Punjab | 4.0 | 8.5 |
|---|---|---|---|---|
| | Raw, Pasteurized, boiled, flavoured and sterilized | Assam, Andaman and Nicobar, Andhra Pradesh, Bihar, Chandigarh Delhi, Daman and Diu, Dadra and Nagar – Haveli, Gujarat, Haryana, Goa, Himachal Pradesh, Karnataka Kerala, Lakshadweep, Maharashtra, Madhya pradesh, Manipur, Nagaland, NEFA, Orissa, Pondicherry, Punjab, Rajasthan, Tripura, Tamil Nadu, Uttar Pradesh, West Bengal | 3.5 | 8.5 |
| Goat or sheep milk | Raw, Pasteurized, boiled, flavoured and sterilized | Assam, Andaman and Nicobar, Andhra Pradesh, Bihar, Delhi, Daman and Diu, Dadra and Nagar – Haveli, Gujarat, Goa, Himachal Pradesh, Karnataka Kerala, Lakshadweep, , Maharashtra, Madhya pradesh, Manipur, Nagaland, NEFA, Orissa, Pondicherry, Rajasthan, Tripura, Tamil Nadu, Uttar Pradesh, West Bengal | 3.0 | 9.0 |
| Mixture of milk from different species | Raw, Pasteurized, boiled, flavoured and sterilized | All India | 4.5 | 8.5 |

(FSSAI, 2006)

**Lesson-2**

**Nature of Variation in Milk Composition, Genetic, Physiological and Environmental and the Sources of Variation**

**2.1 INTRODUCTION**

It is an established fact that the composition of milk is not uniform in all the species. Depending upon the needs of the new born there will be variation in the proportion of the major constituents in milk. In addition to these differences several other factors also influence the proportion of various milk constituents.

**2.2 FACTORS AFFECTING MILK COMPOSITION**

**The composition of milk will show variation due to several factors. These factors are grouped into:**

- **Animal factor**
- **Environmental and**
- **Miscellaneous factors**

**2.2.1 Animal Factor**

**a. Genetic:** The milk composition being an important trait of milch animals and is basically a inherited character influenced by genes. This factor can be modified by proper planning of breeding practices It plays role in the quality and quantity of milk produced

**b. Species:** The composition of milk varies between species and is designed to provide different amount of nutrients to new born of particular species to ensure optimum growth, and is an inherited trait for that species. The faster the rate of growth the more concentrated are the milk components needed for this growth. For instance Cat milk contains about 7.0% protein since the new born has to double its birth weight in 9.5 days, similarly the lamb will double its birth weight in about 15 days as such the sheep milk has only 4.8% protein. Human infants take substantially longer time (180 days) to double their birth weight, since the human milk least protein content (~ 1.0%).

**c. Breed:** There are several breeds in domestic animals which are reared exclusively for milk production. Milk fat being the costliest component of milk, there is considerable selection among the breeds to ensure milk with a considerably higher fat content. To a considerable extent this being an inherited trait selection of suitable breed will be based on the fat percentage in the milk produced by that breed. However, not much variation could be

observed in the remaining constituents. Composition of the milk for some exotic and zebu cattle are presented for understanding this influence of breed on the milk composition **(Table 2.1)**

**Table-2.1: Average composition (% w/v) of milk from different breeds of exotic cow**

| Breed | Water | Fat | Protein | Lactose | Ash | SNF |
|---|---|---|---|---|---|---|
| Jersey | 85.27 | 5.14 | 3.8 | 5.04 | 0.75 | 9.59 |
| Guernsey | 85.47 | 4.96 | 3.84 | 4.98 | 0.75 | 9.57 |
| Ayrshire | 87.1 | 3.85 | 3.34 | 5.02 | 0.69 | 9.05 |
| Short Horn | 87.43 | 3.03 | 3.32 | 4.89 | 0.73 | 8.94 |
| Friesian | 88.01 | 3.45 | 3.15 | 4.65 | 0.68 | 8.48 |

(Source: Rai, Dairy Chemistry and Animal Nutrition , 1964)

Zebu cattle also show variation in milk composition between the different breeds. The data of the milk composition due to breed of the cow is given here under ( Table 2.2).

**Table-2.2: Average composition (% w/v) of milk from different breeds of indigenous cow**

| Breed | Water | Fat | Protein | Lactose | Ash |
|---|---|---|---|---|---|
| Sindhi | 86.07 | 4.9 | 3.42 | 4.91 | 0.7 |
| Gir | 86.44 | 4.73 | 3.32 | 4.85 | 0.66 |
| Tharparker | 86.58 | 4.55 | 3.36 | 4.83 | 0.68 |
| Sahiwal | 86.42 | 4.55 | 3.33 | 5.04 | 0.66 |

(Source: Rangappa and Achaya, Indian Dairy Products,1974)

The variation among the milk constituents contributing to the SNF is very marginal the influence of breed on the fat and SNF composition is presented in the Table 2.3

**Table-2.3: Average composition (% w/v) of milk from different breeds of buffalo**

| Breed | % Fat | % SNF |
|---|---|---|
| Murrah | 6.8 | 10.1 |
| Jaffarabadi | 7.3 | 10.1 |
| Surti | 8.4 | 10.3 |

(Source: Rangappa and Achaya, Indian Dairy Products,1974)

**d. Individual Cow**: Although individual animals maintain the breed characteristic of milk produced by them. However, there will be a variation with the individual animals. This also depends on the genetic characteristics of the individual animals. By following good breeding practices it is possible to get higher fat per cent in the individual or milch animal. A similar variation could be observed in the protein and lactose levels in individual animals within a given breed. Thus, selective breeding can be used to upgrade milk quality apart from the inherited characteristics the environment and various physiological factors influence the composition of milk produced by individual animal.

**e. Lactation Period:** Similar to the variations observed in the breed and individual animals, variation during the lactation period is also observed in the fat and SNF percentage during a lactation period. Fat content is higher after parturition, which decreases to a low during the first and second months of lactation and then gradually increases during the remaining period of the lactation. The composition of the fat also found to be changing during the lactation. A slight decrease in Lactose percentage could be observed during the last stage of lactation. An increase in the chloride content was observed with the advancement of lactation, Calcium concentration reduces to a minimum and further increase whereas the total phosphorous declines throughout the lactation period. Natural acidity was observed to be at higher level at the beginning of lactation and will decrease to normal value during the first three months and remains fairly steady during the remaining lactation period till the last month it declines sharply.

## 2.2 ENVIRONMENTAL

2.2.1. Interval between milking: The fat content of milk varies considerably between the morning and evening milking due to shorter interval of time between the morning and evening milking than between the evening and morning milking. If cows are milked at 12-hour intervals, the variation in fat content between milkings would be negligible, however this is not practicable on most farms. Normally, SNF content varies a little even if the intervals between milking vary considerably.

2.2.2. Age: Due to several physiological changes in the individual cow with advancing age, the composition of milk produced by these animals too shows variation in their composition. As cows grow older, the fat content of their milk decreases by about 0.02 percentage units per lactation. The fall in SNF content is much greater.

2.2.3 . Feeding Regime: All the precursors of milk constituents are derived from blood of milch animal. As such providing the suitable and well balanced feed to milking animal,plays an important role in maintaining requisite levels of constituents in the milk produced. Underfeeding a lactating animal will only reduces the milk yield but also level of fat and SNF. However changes in SNF content are more sensitive to the feeding level than the fat content. Fat content and its composition are influenced more by the roughage (fiber) intake.

2.2.4. Disease: Milk production, being a physiological function of a lactating animal, general health condition of individual cow plays a very important role in maintaining good milk composition. Specific diseases have great influence on the composition of milk,both fat and SNF contents reduce when animal suffers from any specific disease.Mastitis, being a disease specific to the udder, has substantial effect on milk composition; it reduces lactose content with a corresponding increase in salt content of milk.

2.2.5 . Completeness of Milking: During the interval between the two milking, milk will be held in glandcistern and teat cistern, consequently there is possibility that fat being the lightest component of milk would be moving to the surface, resulting in the variation of fat percentage in different portions of milk during the process of milking. It is advised to draw the milk completely from the udder during each milking. This will not only ensure that all the milk produced by the animal is drawn but also the fat is not left in the udder. The last part of the milking designated as stripping has a higher fat content as compared to the fore milk which is drawn first during the milking operation. As such completeness of milking ensures uniform quality of milk.

**2.2.6**. **Season:** There is wide variation in climate and also type of vegetation available in different seasons of the year. Due to seasonal variation in the availability of fodder, there are changes in the milk composition. In India,butter fat content in milk is maximum in the month of May and minimum in the month of November.Similarly the SNF is highest in October , and minimum in July and September . The variation in the milk production is dependent on several other factors *viz.*, temperature, hours of sun shine,length of the day, exercise, rain fall, drought, stall feeding or pasture feeding, etc. that are associated with the season.

**2.2.7. Weather:** In dry months the total yields of milk tend to decrease along with a decrease in SNF and increase in fat percentage. In wet months there may be a decrease in both SNF and fat percentage along with or without the increase in the yield of milk which depends on the level of nutrition.

**2.3: MISCELLANEOUS FACTORS**

**2.3.1. Exercise:** Subjecting the lactating animal to regular exercise will ensure good blood circulation through out the body and keeps the animal in good condition. In order to ensure sufficient reserve of energy such animals will eat more which would indirectly increase the milk yield. It was observed that the feed consumption of the animal will increase resulting in an increase in milk yield along with an increase in the fat percentage when compared with the period of rest.

**2.3.2. Excitement:** Hormones play an important role in the animal, especially during the milking time. Favourable conditions during milking of animals result in good milk yield . On the contrary, harassment and frightening conditions such as barking of dog, presence of strangers, bursting of crackers,etc. disturbs the animal and reduces milk yield. A change in the milk composition may also be expected by these conditions. Animal in oestrus will also reduce the milk yield. However , these factors cannot be generalized as they maybe specific to certain individual animal at a given geographic location.

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*Module 2. Structure of Milk*

**Lesson-3**

**Structural Elements of Milk: Fat Globules, Casein Micelles, Globular Proteins, Lipoprotein Particles and their Properties and Grading of Milk**

## 3.1 INTRODUCTION

The multi cellular organisms organize the cells into tissues and further into specific organs with specific functions. Consequently the secretions of these cells will have specific composition with the arrangement of these components in a specific manner giving unique physical structure to it. Depending up on the requirements , the structure of milk is either maintained or being disturbed in order to prepare certain products. As such a knowledge of these elements will give better understanding of the structure of the milk.

## 3.2 STRUCTURE OF MILK

Milk being a secretion of the epithelial cells of the mammary gland has specific interactive forces working between its components which help in maintaining the integrity of the whole system under different conditions and thereby maintaining its specific structure. The physical structure i.e., the arrangement of the components in space must also be known. The interactive forces between the components are important; they determine the integrity of the whole system under various conditions. The main structural elements are shown schematically in Figure 3.1. They are microscopicor sub-microscopic in scale. The structure of milk is very ~~has~~ simple and extensive studies are being conducted on this aspect. Since milk constituents exist in different physical state influencing milk properties in addition to the chemical nature of the constituents.
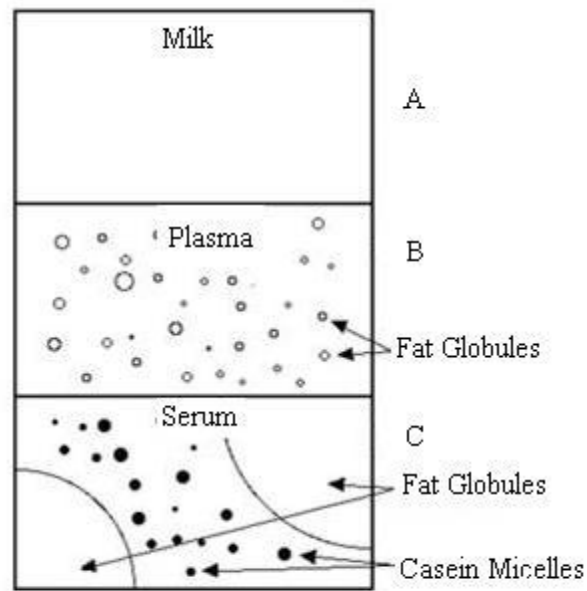
A-Magnified x 5     B-Magnified x 500     C-Mafnified x5000

**Fig.3.1Milk Viewed at Different Magnifications**

(Source: Mulder and Walstra, The milk fat globule, 1974)

**3.3 STRUCTURAL ELEMENTS**

The properties of various structural elements of milk are shown in the Table 3.1

**Table-3.1: Properties of the Main Structural Elements of Milk**

| Structural elements of milk | Fat globules | Plasma | | |
| --- | --- | --- | --- | --- |
| | | Casein micelles | Serum | |
| | | | Globular proteins | Lipo – protein particles |
| Main components | Lipids | Casein, Water, Salts | Serum proteins | Lipoprotein particles |
| Considered as | Emulsion | Fine dispersion | Colloidal solution | Colloidal dispersion |
| Content(% D.M) | 3.8 | 2.8 | 0.6 | ~ 0.01 |
| Volume fraction | 0.042 | ~0.06 | 0.006 | ~0.0001 |
| Particles Diameter | 0.1-10µm | 10-300nm | 3-6 nm | ~ 10nm |
| Number per ml | $10^{10}$ | $10^{14}$ | $10^{17}$ | $10^{14}$ |
| Surface area(cm$^2$/ml milk) | 700 | 40000 | 50000 | 100 |
| Density (20$^0$C) g/ml | 0.92 | 1.11 | 1.34 | 1.10 |
| Visible with | Microscope | Ultra microscope | Electron Microscope | Electron Microscope |
| Light scattering | Very turbid, white | Turbid bluish | Light haze | negligible |
| Separable with | Dairy separator | High – speed centrifuge | Gel filtration | Gel filtration |
| Filterable with | Fine sintered glass | Chamberland filter | Cellophane | Cellophane |
| Diffusion rate (mm in 1 hr) | - | 0.1-0.3 | 0.6 | 0.4 |
| Flocculation | By agglutinin in cold | By acid Or by rennet | By heat | - |
| Isoelectric pH | ~3.8 | ~4.6 | ~4.5-5.5 | ~ 4 ? |

(Source: Dairy Chemistry and Physics, Walstra and Jenness, 1984)

**3.3.1. Milk fat:** In milk, fat exists in the form of fat globules. The unique feature of these fat globules is that these fat globules are surrounded with a membrane which is derived from the apicalmembrane of the mammary secretory cell. Milk minus fat globules is called "Milk plasma".

**3.3.2. Proteins:** Casein is the major protein of bovine milk and exists mainly as micellar form, where as in human milk major proteins are whey proteins. The Casein micelle consists of water, casein, salts and some minor components including lipase and proteinase. Casein micelles are built of smaller particles called sub-units or sub-micelles. Milk plasma free from caseinmicelles is known as milk serum. The liquid that is obtained on clotting of milk either with rennet or by acidification to pH 4.6 (isoelectric point of casein) is known as whey. This whey differs in its composition from that of serumin having some of the polypeptides cleaved from casein by the action of rennet.The proteins present in whey are termed as whey proteins of milk. The whey proteins are mainly globular proteins. Lipoprotein particles sometimes called microsomes vary in their quantity, composition, and shape. They consists remnants of cell membranes, microvilli, etc.

**3.3.3 Somatic cells:** The somatic cells consist of mainly leukocytes of various types is considered as extraneous particles although they are always present. They are about 10 µ m in diameter; number is about 100,000 permilliliter and accounts for about 0.005% of the volume of milk. They contain all cytoplasmic components notable nucleic acids and enzymes. They are rich in catalase. In mastitic milk the cell counts are much higher

**3.3.4. Physical Structure of Milk:** If we try to see a drop of milk with only 5 times magnification it appears as uniform liquid, however, it cannot be homogeneous since it is turbid in nature.With an increase in the magnification by another 100 times one can observes pherical droplets of fat floating in plasma. A further increase of magnification by another 100 times the proteinaceous particles (casein micelles)become visible. Fat, casein micelles and other proteins form the major structural elements for milk, and these elements are microscopic or submicroscopic in their size. Although fat is considered to be present in the form of an emulsion but it is not just a simple emulsion, because of the fact that the fat globules are being covered with the fat globule membrane which is derived during its biosynthesis. In addition to the fat globules some of the fat in the milk is found outside the globules while the membrane material includes several enzymes and proteins and polar lipids. As such fat and fat globules are not identical.

**3.3.5. Milk Plasma:** Milk gets its structure with the structural elements. The main structural element being the fat, all that portion of milk which is free from the fat is considered as milk plasma. It is often a practice to use cream separators for getting the skim milk, but all the fat present in milk will not be separated as such we cannot consider milk plasma to be synonymous to skim milk. Here we consider fat free -milk to be plasma but not skim milk. Although the words serum and plasma are used for fractionation of blood here in milk these words are denoting the physical fractions of milk.

**3.3.6. Milk Serum :** All that portion of milk excluding the casein micelles is referred as milk serum. By using either rennet or dilute acids the casein micelles could be separated from the plasma to obtain milk serum. The globular proteins consists mainly the whey proteins. They also bind some cations and a little water. Lipoprotein particles present in milk are also referred as the milk microsomes.

**3.3.7. Casein micelles:** Water,casein and salts along with some minor components such as lipases and proteinases constitute this structural element of milk.Casein micelles do es not completely account for the casein content since some of the casein is also present in a solution form. As casein binds cations especially the Ca$^{--}$ and Mg$^{++}$ ions at the normal pH of milk i.e ~6.6, it is referred as caseinate. Amorphous calcium phosphate and small amounts of citrates are the constituents associated with the casein micelle. Calcium caseinate phosphatecomplex is the term applicable to describe the casein micelles. Phosphatebeing a part of the colloidal particles, the term colloidal phosphate is also used.

**3.3.8. Whey proteins:** The non micellar proteins constitute the whey proteins, but it is often the globular proteins present as such are considered to be the whey proteins

**3.4 TESTS FOR GRADING OF MILK**

The milk procured at the dairy plants is not only sold as fluid milk but also used for the preparation of various dairy products. As such it is necessary to grade the milk procured at

the dairy plants by conducting various tests. The tests that are commonly conducted for grading of raw milk are:

A)Sensory Tests

Organoleptic tests

B) Physical tests

- Sediment test
- Clot on boiling test
- pH

C) Chemical Test

- Alcohol test
- Alizarin-alcohol test

**3.4.1. Organoleptic tests:** The first test that is expected to be conducted in all the dairies as soon as milk is received at the milk procurement section is organoleptic test. This test is primarily based on smell (odour) and appearance. An experienced person with an ability to detect the various abnormalities in the milk flavours should conduct this test. This will help in grading the good and bad quality milk. During this examination it is possible to detect the abnormalodours such as garlic, onion, cabbage etc., and sourness of the milk. Similarly,the abnormalities in the colour could be detected in these tests, apart from presence of various extraneous objects in the milk, such as hair, fibres, dung cakes etc.

**3.4.2 . Sediment test:** This test reveals the extent to which visible insoluble matter has gained entry into milk. The sediment test presents a simple rapid and a quantitative measure of indicating the cleanliness of milk with respect to visible dirt. However it is necessary to give the interpretation judiciously since absence of visible dirt never ensures good quality milk.

**3.4.3 Clot-On-Boiling (COB) Test:** This is a quick test to determine developed acidity and the suitability of milk for heat processing. Milk giving a positive test has acidity generally above0.17% lactic acid and is not suitable for distribution as liquid milk or for heat processing.

**3.4.4 pH:** The hydrogen ion concentration or the pH value is a measure of true acidity of milk. The pH of normal cow milk ranges from 6.6 to 6.8. Due to the development of acidity by the conversion of lactose into lactic acid this value would be reduced. Milk obtained form the animals suffering from mastitis will have pH above 7.0. The pH test is mainly used for detecting of abnormal mastitis milk.

**3.4.5Alcohol Test:** The alcohol test is used for rapid assessment of stability of milk to processing particularly for condensing and sterilization. The alcohol test is used as an indicator of disturbed salt balance in milk but not as an index for developed acidity. This test helps in detecting abnormal milk such as colostrums, late lactation milk, and mastitis milk.

**3.4.6Alizarin-Alcohol Test:** This test is similar to the alcohol test and the addition of alizarin helps in detecting the acidity by judging of colour as well . The interpretation of the

results is based on the size of the clot and the colour developed which in turn helps in grading of the milk.

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*Module 3. Milk Proteins*

**Lesson-4**

**Introduction, Definition and Nomenclature of Milk Proteins**

**4.1. INTRODUCTION**

About two thirds of all cells consist of proteins and they have functional and structural roles in human body such as catalyst (enzymes), regulator (hormones), protection (Immunoglobulins), carrier proteins (haemoglobulin, lactoferrin, etc.) and as structural proteins (collagens). Milk Protein is widely consumed human food proteins for people of all ages, particularly infants and children. Casein is unique due to their characteristic physical properties which are different from globular proteins. These proteins influence the behaviour and properties of other milk constituents. The protein content in a given milk products influences the structure of that product.

**4. 2. DEFINITION**

Proteins are defined as high molecular weight polymers of α-amino acids that are formed by living organisms. All these amino acids have L-configuration except glycine. The primary structure of proteins consists of a polypeptide chain of amino acids residues joined through peptide bonds.



(Source: http://www.web-books.com/MoBio/Free/Ch2B.htm)
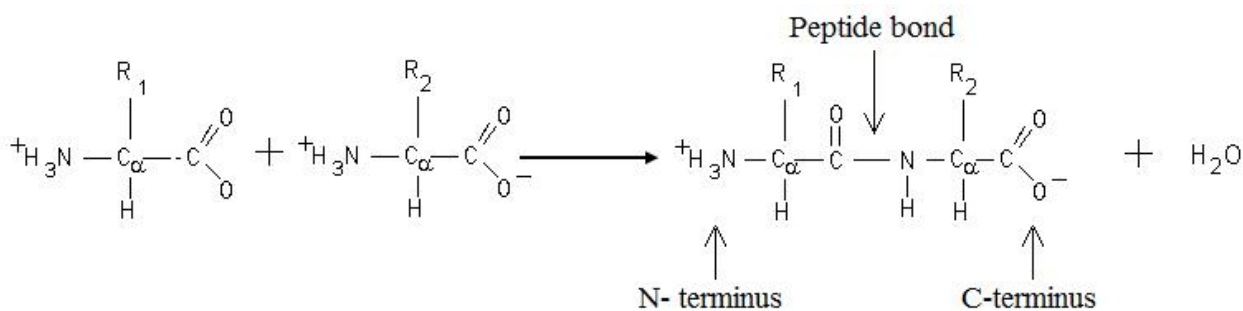
Proteins differ from each other not only in their length of polypeptide chain but also in the proportion and sequence of the amino acids residues in them. Proteins of various species are said to be homologous if they are considered to have originated from a common ancestor. The most definitive criterion of homology is similarity of the amino acid sequence. The homologous proteins usually exhibit a common biological function but coincidence of function also can arise by convergence from different ancestral lines

## 4.3. NOMENCLATURE OF MILK PROTEINS

The Committee on Milk Protein Nomenclature,Classification, and Methodology of the Manufacturing Section of American Dairy Science Association (ADSA) for has made efforts to suggest suitable nomenclature to the milk proteins. Several research workers made attempts to suggest nomenclature basing on the resolution of this protein on the free boundary electrophoresis. The proteins which are having similar electrophoretic characteristics at pH 8.6 were actually heterogeneous mixtures of proteins.Waugh and Von Hipp *et al.* (1956) have shown that α-casein could be separated into calcium sensitive α $_s$-casein and calcium insensitive crude k-casein based upon its dissociation and differential solubility in the presence of calcium ions. A new terminology was suggested by Waugh *et al* (1956) for the action of rennin on α-casein fraction k casein for the primary reaction product of renninon k-casein and $α_s$-casein for the clots which are formed. But the role of other α-casein components and of βand or γ-casein in this transformation was not explained. The committee felt that in view of the advancements in the knowledge of the casein systems the committee preferred not recommend any nomenclature for the α-casein complex.

The American Dairy Science Association's committee on milk protein nomenclature, classification and methodology has given a classification system for the known proteins in milk (Eigel*et al.*, 1984; Farrell *et al.*, 2004). According to this system of classification a Greek letter with or without a numerical precedes the class name when necessary to identify the family of proteins. The genetic variant of the protein is indicated by an upper case Arabic letter with or without a numerical super script immediately following the class name. Post-translational modifications are added in sequence: βcasein B 5P (f1 to 105) indicates that

a. Class of the protein is caseins

b. The family is β

c. The genetic variant is B

d. There are 5 post translational phosphorylations

e. The amino acid sequence from N-terminal amino acid through residue 1 through residue 105.

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**Lesson-5**

**Milk Proteins: Classification, Distribution, Genetic Polymorphism and**

**its Importance**

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## 5.1. INTRODUCTION

In the earlier years proteins were classified largely on the basis of rather empirical fractionation procedures. In most cases the proteins were classified partly on the basis of their solubility and partly on the basis their composition. With the availability of better an alytical techniques it is established that the proteins which were earlier considered to be individual proteins are actually a mixtures of several other fractions.Presently the proteins in milk are classified on the basis of their fractionations and their behaviour during electrophoresis, difference in their solubility in various solutions, difference in their sedimentation rate etc.

## 5.2. CLASSIFICATION AND DISTRIBUTION OF MILK PROTEINS

A common method for the classification and distribution of milk proteins is represented as follows

**Genus *Bos* (30-35 g/L )**

**5.2.1. Caseins** (24-28 g/L)

**5.2.1. 1. $\alpha_{s1}$ -Caseins** (12-15 g/L)

1. $\alpha_{s1}$-Casein X-8P ( X = Genetic variants-A, B, C, D-9P, and E)

2. $\alpha_{s1}$-Casein X-9P (X = Genetic variants-A, B,C, D-10P, and E)

3. $\alpha_{s1}$-Casein fragments'

**5.2.1.2. $\alpha_{s2}$ caseins** (3-4 g/L)

1. $\alpha_{s2}$ -Casein X-l0P ( X =Genetic variants-A, B, C-9P, and D-7P)

2. $\alpha_{s2}$ -Casein X-l1P (X =Genetic variants-A,B, C-10P, and D-8P)

3**.** $\alpha_{s2}$-Casein X-12P (X =Genetic variants-A, B, C-11P, and D-9P)

4. $\alpha_{s2}$ Casein X-13P (X =Genetic variants-A,B, C-12P, and D-10P)

**5.2.1.3. β - Casein** (9– 11 g/L)

Ø βCasein X-5P (X =Genetic variants-$A^1$, $A^2$, $A^3$,B, C-4P, D-4P, and E)

Ø β-Casein X-lP (f 29-209) (X=Genetic variants- $A^1$, $A^2$, $A^3$,and B)

Ø β-Casein X-(f 106-209)(X =Genetic variants- $A^2$, $A^3$, and B)

Ø β-Casein X-(f 108-209) (X =Genetic variants-(Fragments from $A^1$, $A^2$, $A^3$ ) and B)

Ø β - Casein X-4P (f 1-28) *

Ø β -Casein X-5P (f 1-105) *

Ø β -Casein X-5P (f 1-107) *

Ø β -Casein X-lP ( f 29-105) *

Ø β Casein X-lP (f 29-107) *

**5.2.1.4. κ -Caseins (2-4 g/L)**

1.κ -Casein X-lP (**X** = Genetic variants-A andB)

2. Minor κ -caseins X -l, -2, -3, etc. ( x = Genetic variants-Aand B)

**5.2.2. Whey proteins (5-7 g/L)**

**5.2.2.1. β Lactoglobulins** (2-4 **g/L**)

1. β -Lactoglobulins X ( X = genetic variants-A,B, C, D, Dr, E, F, and G)

**5.2.2.2. α-Lactalbumins** (0.6-1.7**g/L**)

1. α-Lactalbumin X ( X = Genetic variants-Aand B)

2. Minor α-Lactalbumins

**5.2.2.3. Bovine serum albumin** (0.2-0.4 **g/L**)

**5.2.2.4. Immunoglobulins** (0.5-1.8 **g/L**)

**a. IgGimmunoglobulins**

Ø $IgG_1$, Immunoglobulins

Ø $IgG_2$, Immunoglobulins

Ø IgG fragments

**b. IgMimmunoglobulins**

**c. IgA immunoglobulins**

Ø IgA Immunoglobulins

Ø Secretory IgA Immunoglobulins

**d. IgE Immunoglobulins**

**e. J-chaincomponent**

**f . Free secretory component**

**5.2.3. Milk Fat Globule Membrane (MFGM) Proteins**

**MFGM – A$_{15}$ – 127, C, S**

Where MFGM indicates the protein satisfied the operational definition for a MFGM protein, A$_{15}$ designates the zone in a 15% Laemmliacrylamide gel, 127 is the "apparent molecular" weight in k-daltons, and C, S designates that the protein is both Coomassie brilliant blue and periodic acid /Schiff positive (PAS).

1. Six major proteins readily stained with Coomassie brilliant blue are XDH/XD; CD36, BTN,ADPH, PAS 6/7 And FABP.

2. Two stained by Periodic Acid/ Schiff (PAS) or silver stain are Muc-1 and PAS III.

(Source: Nomenclature of proteins of cows' milk-5th and 6th revision, *J. Dairy Sci.*, year: 1984 and 2004)

**5.2.4. Minor proteins**

Ø Serum transferrin

[*] Genetic variants of these fragments have not been specifically identified

(Source: Fundamentals of Dairy chemistry, Wong *etal.*, 1988)

**5.3 POLYMORPHISM IN MILK PROTEINS**

In 1956 As chaffenburg and Drewry discovered that the βlactoglobulin exists in two forms A and B which differs from each other by only a few amino acids. The milk of any few individual animal may contain β lg A orβ lg B or both and the milk is indicated as AA, BB or AB with respect to β lg.This phenomenon is referred as genetic polymorphism and has since been shown to occur in all the milk proteins. A total of about 30 variants have been demonstrated by Polyacrylamide Gel Electrophoresis (PAGE). Since PAGE differentiates on the basis of charge and molecular weight, only polymorphs which differ in charge alone are detected i.e a charged residue is replaced by a non charged or vice versa. Therefore it is likely

that more than 30 polymorphs exist. The genetic variant present is indicated by a lain letter eg: $\alpha_{s1}$ -CN A-8P, $\alpha_{s1}$- CN B – 8P and $\alpha_{s1}$ CN B – 9P etc.

The frequency with which certain genetic variant occur is breed specific and hence genetic polymorphism has been useful in phylogenic classification of cattle and other species various technologically important properties of the milk proteins are related to the genes that occur in the primary polypeptide chain of milk proteins, which will result in variation of the behaviour of that protein either on electrophoretic behaviour or its gel filtration pattern. This is usually due to the changes occurring in genetic code for that protein. This deviation or change is referred as polymorphism. There are several such variations observed in the milk proteins.

The casein genotype significantly influences the milk yield, fat yield and protein yield with highest yields obtained for the genotype BB. Cheese yield on a fixed amount of milk and fat yield were significantly related to κ polymorphism observed to be β lg genotype with highest estimates obtained for BB. Protein percentage was influenced by $\alpha_{s1}$- casein and κ casein with the genotype BC and BB respectively having the highest percentages

## 5.4 DISTRIBUTION OF MILK PROTEINS

The distribution of the proteins in milk is presented in a Figure 5.1. It could be observed that the proteins in milk are chiefly the caseins, whey proteins and some minor proteins. Since enzymes are protein in nature they are also grouped along with other proteins. Caseins being the major fraction of the entire milk proteins are present in micellar form while the whey proteins being considered as soluble proteins and which exists in colloidal form. Enzymes play a significant role in the raw milk storage and processing of the milk and also found to have                                              industrial                                              importance.
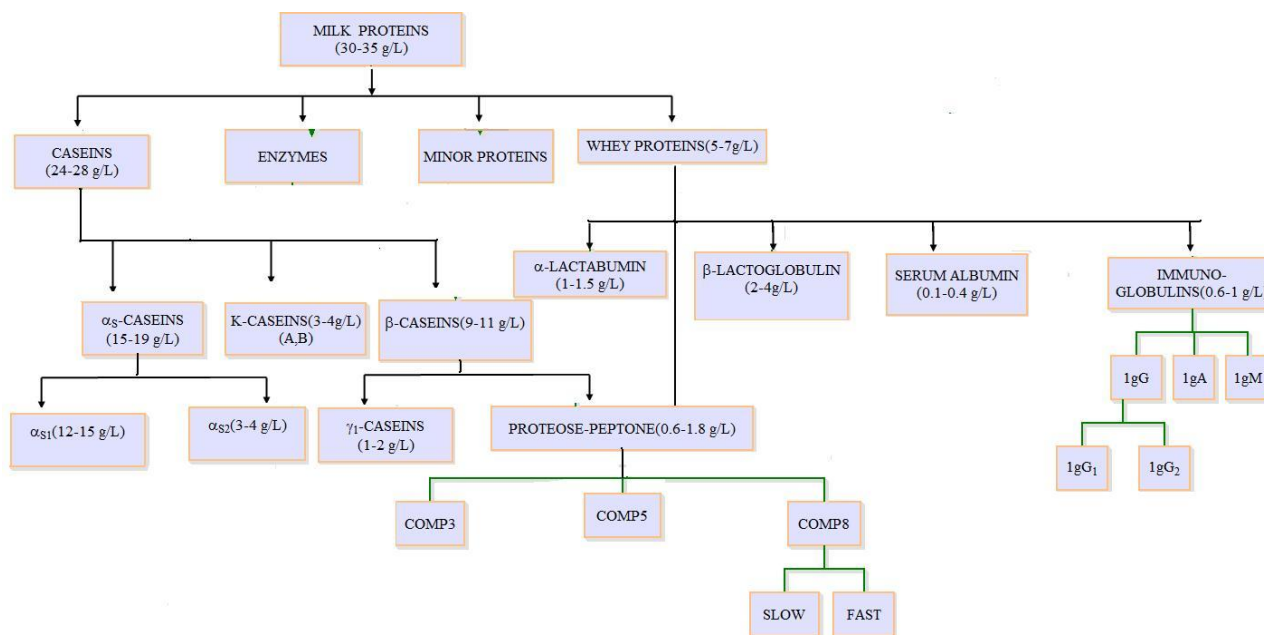
**Fig.5.1 Distribution of Protein Fractions in Bovine Milk**

(Source: Developments in Dairy chemistry – 1, Proteins, Fox, 1982)

Minor proteins include vitamin binding protein, lactoferrin, metallo-protein, MFGM protein,Ceruloplasmin, etc.

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**Lesson-6**

**Caseins: α s$_1$ -casein, α s$_2$- casein, β-casein and κ-casein**

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## 6.1. INTRODUCTION

It is difficult to define caseins in a way that includes all proteins belonging to this class and excludes all others. Their common property of low solubility at pH 4.6 serves as the basis for a rather convenient operational definition (at least for bovine milk). At this pH, all of the caseins except some of the proteolytic derivatives precipitate. As the solubility of caseins is much less and the whey proteins are having better solubility than casein the separation of the casein has become possible by lowering the pH value to 4.6.

As mentioned earlier obtaining molecular components individually from a mixture of casein is difficult. The ion exchange chromatography using DEAE-cellulose or hydroxyapatite columns would give satisfactory fractions. However, it is necessary to control the interaction of casein molecules. The unique feature of the caseins is their ester-bound phosphate. All of the casein polypeptide chains have at least one such group per molecule; whereas, none of the whey proteins have any ester bound phosphate. The $\alpha_{s1}$ and β-caseins contain no cysteine residues,while $\alpha_{s2}$ and κ–caseins each have two cysteine residues. Proline contents of caseins are rather high ( $\alpha_{s1}^-$, $\alpha_{s2}^-$ , β $^-$ ,and κ$^-$ caseins contain 17,5, 17 and 12 mol %, respectively). There is no organized secondary structure for the various fractions of casein. From the various analytical studies carried out on this protein revealed that they have short lengths of α -helix or β -sheet structure in them. Their ionizable groups of the amino acids are accessible to titration and are also involved in several other side chains to reaction. Denaturing agents and heating seems to have no effect on the secondary structure of these proteins. Thus their conformation appears to be much like that of denatured globular proteins. As the protein is having large proportion of proline residues, closely packed orderly secondary conformation is being prevented in this protein molecule. The four caseins differ greatly from each other in charge distribution and the tendency to aggregate in the absence and presence of Ca$^{2+}$ ions.

## 6.2. αs$_1$-CASEINS

The polypeptide chain of αs$_1$-casein consists of two predominantly hydrophobicregions (residues 1-44, and 90-199) and a highly charged polar zone (residues 45-89).All but one of the phosphate groups is in the 45-89 residues segment, and the prolines are distributed at intervals in the hydrophobic segments. Thus, this protein can be visualized as a rather loose flexible polypeptide chain.Self-association of αs$_1$-casein depends markedly on its concentration and on the pH, ionic strength, and kind of ion in the medium, but it is relatively independent of temperature. Physical measurements such as lights cattering, sedimentation, and viscosity indicate that αs$_1$- casein is completely dissociated to a flexible random chain monomer of MW = 24,000 at pH 12, in 3 M guanidinium chloride and at neutral pH and 0.01 ionic strength, it associates at neutral pH and higher ionic strength, the

extent of association depending on protein concentration, it binds about 8 moles $Ca^{2+}$ per mole near pH 7, probably to the ester phosphate groups. It aggregates and precipitates at very low concentrations of $Ca^{2+}$ (7 mM $Ca^{2+}$,28 mM NaCl). A small amount of peptides, sometimes called A-casein, is present in milk; these appear to originate from proteolysis of $\alpha s_1$-casein.

### 6.3. $\alpha s_2$-CASEIN

$\alpha s_2$-casein has a remarkable dipolar structure with a concentration of negative charges near the N-terminus and positive charges near the C-terminus. Its properties have not been investigated as thoroughly as those of the other caseins, but certainly it binds Ca strongly and is even more sensitive to precipitation by $Ca^{2+}$ than $\alpha s_1$-casein. It self-associates at neutral pH in the absence by $Ca^{2+}$, and the association depends markedly on ionic strength and is at a maximum at an ionic strength of about 0.2.

### 6.4. β-CASEIN

β-casein has a strong negatively charged N-terminal portion. The net charge of the 21-residue N-terminal sequence is 12 at pH 6.6, and the rest of the chain has virtually nonet charge. The large number of Pro residues effectively precludes extended helix formation. Thus, the β casein molecule is somewhat like that of an anionic detergent with a negatively charged head and an uncharged essentially hydrophobic tail. The outstanding characteristics of the association ofβ-casein in both the absence and the presence of $Ca^{2+}$ are its strong dependence on temperature. In the absence of $Ca^{2+}$, only monomer is present at 4°C, but large polymers (20-24 monomers) are formed at room temperature. Removal of the 20-residue C-terminal sequence destroys the ability of β-casein to associate, suggesting that specific hydrophobic interactions maybe involved. β -casein tightly binds about 5 $Ca^{2+}$ per mole, consistent with its ester phosphate content.

### 6.5. γ-CASEINS

Group of caseins designated as γ-caseins have been known for some time to correspond to C-terminal portions of the β -casein sequence. These are formed by cleavage of β-casein at positions 28/29, 105/106, and107/108 by the enzyme plasmin. The fragments 29-209, 106-209, and 108-209constitute the y-caseins. The smaller fragments resulting from the cleavage appear in the whey when casein is precipitated by acid and constitute part of what has long been designated as the proteose-peptone fraction of the whey. Thus, fragments 1-105 and 1-107 were called as whey component 5, fragment 1-28was whey component 8-fast, and fragments 29-105 and 29-107 were named as whey component8-slow. However the revised nomenclature for the fragments of β-caseins in milk is given here under (Table 6.1).

Table-6.1: Revised nomenclature for the fractions of β-casein

| Fragment | Recommended Nomenclature | Former Nomenclature |
|---|---|---|
| 29-209 | β-casein X# (f29-209) | $\gamma_1$-casein |
| 106-209 | β-casein X(f106-209) | $\gamma_2$-casein |
| 108-209 | β-casein X(f108-209) | $\gamma_3$-casein |
| 1-105 | β-casein X(f1-105) | PP* Component 5 |
| 1-107 | β-casein X(f1-107) | PP Component 5 |
| 29-105 | β-casein X(f29-105) | PP Component 8-slow |
| 29-107 | β-casein X(f29-107) | PP Component 8-slow |
| 1-28 | β-casein X(f1-28) | PP Component 8-fast |

(Source Walstra, et. al., Dairy chemistry and physics 1984)

X# = genetic variants of the casein fractions

PP* = Proteose-peptone

## 6.6. κ – CASEINS

About one-third of the κ casein molecules are carbohydrate-free and contain only one phosphate group (SerP-149). At least six other components differing in charge also occur. They have varying numbers of N-acetyl neuraminic acid (NANA) residues and one, at least, appears to have a second phosphate (SerP-127). Three different glycosyl oligomers linked to Thr-133 have been identified. The N-terminal residue of κ-casein is glutamic acid. In the isolated protein it is present as the cyclic derivative pyroglutamic acid. κ-casein as isolated from milk consists of a mixture of polymers probably held together by intermolecular disulfide bonds; these polymers range in molecular weight from about 60,000 (trimers) to more than 150,000. κ-casein is rapidly hydrolyzed at the Phe (l05)-Met (l06) bond by the enzyme chymosin (EC 3.4.23.4), and by other proteases, yielding N-terminal fragment called para- κ casein, which contains the two cysteine residues, a C-terminal fragment of 64 residues called the macropeptide, containing all of the carbohydrate and phosphate groups as well as the genetic substitutions. κ-casein binds about 2 moles $Ca^{2+}$ per mole of protein at neutral pH but differs markedly from the other caseins in its solubility over a wide range of $Ca_2^{++}$ concentration.

The naturally occurring mixture of bovine κ-casein variants polymerizes, as previously mentioned, through -S-S- linkages to subunits containing three to eight monomers. These further polymerize by no covalent association to particles of about 6,50,000 daltons. This polymerization is insensitive to concentration of $Ca^{2+}$ and to temperature.

Lesson-7

**Caseins: Fractionation, Structure and Conformation, and Physico Chemical Properties**

**7.1 DEFINITION**

The behaviour of individual milk proteins and their concentration will be known only when they are fractionated from milk. This can be done by chemical or by several other means. A classical scheme for fractionation of milk proteins by chemical method was developed by Rowland (1938) which was further modified by Aschaffenburg and Drewry (1959).

**7.2 ROWLAND METHOD OF FRACTIONATION**

In this method of fractionation the total protein in milk will be fractioned initially into protein nitrogen and non protein nitrogen using the insoluble property of NPN compounds in trichloro acetic acid (TCA). The casein components are precipitated by the utilizing solubility of non-casein proteins in 10 % acetic acid. The NPN components are further fractioned by utilizing the insolubility of the components with variation.
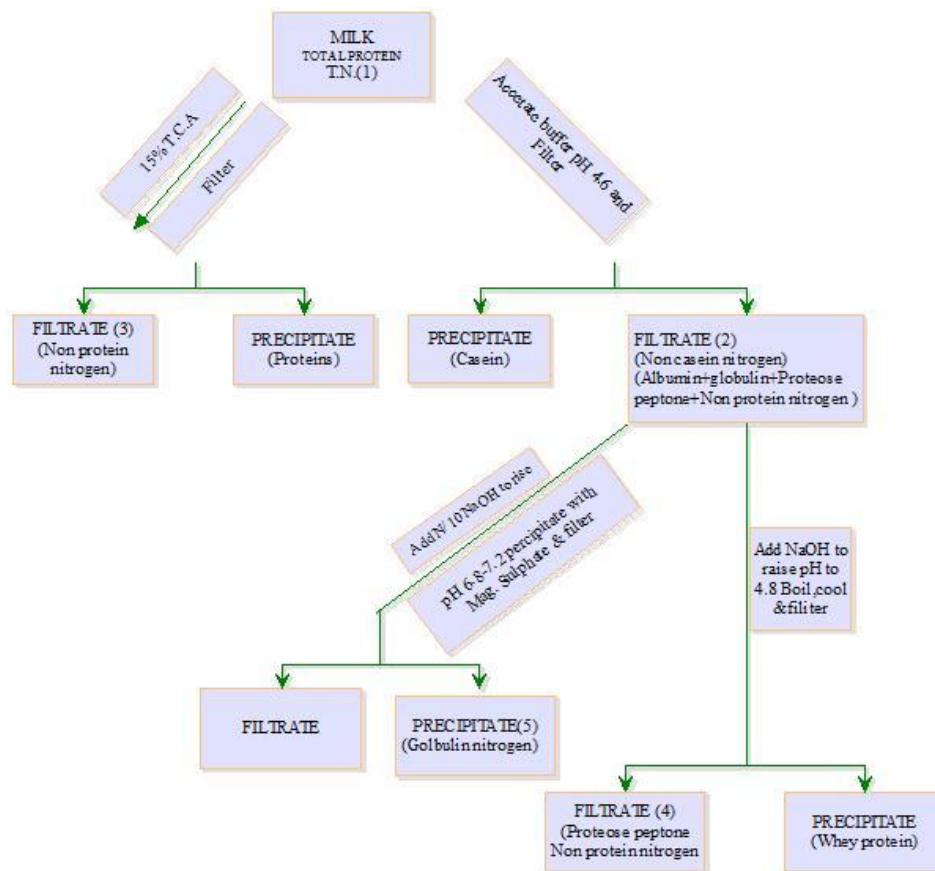


**Fig 7.1 Rowland Fractionation** (Source: Rowland,J.Dairy Res,1938)

## 7.3. ASCHAFFENBURG AND DREWRY METHOD

In the modified method suggested by Aschaffenburg and Drewry (1959) the solubility of some fractions of the proteins in sodium sulphate solutions is utilized where the total albumin along with NPN compounds will be soluble. While in saturated sodium sulphate the albumins get precipitated (Figure 7.2).



**Fig.7.2 Protein fractionation by Aschaffenburg and Drewry (1959)**
(Source: Aschaffenburg and Drewry,Int.Dairy congr,1959)

## 7.4 DIFFERENTIAL SOLUBILITY METHODS

Several methods have been developed to obtain one or more of the various caseins from whole casein or directly from skim milk based on their differential solubility. Hipp et al (1952) [*] developed two procedures which have been used extensively or partly incorporated into oth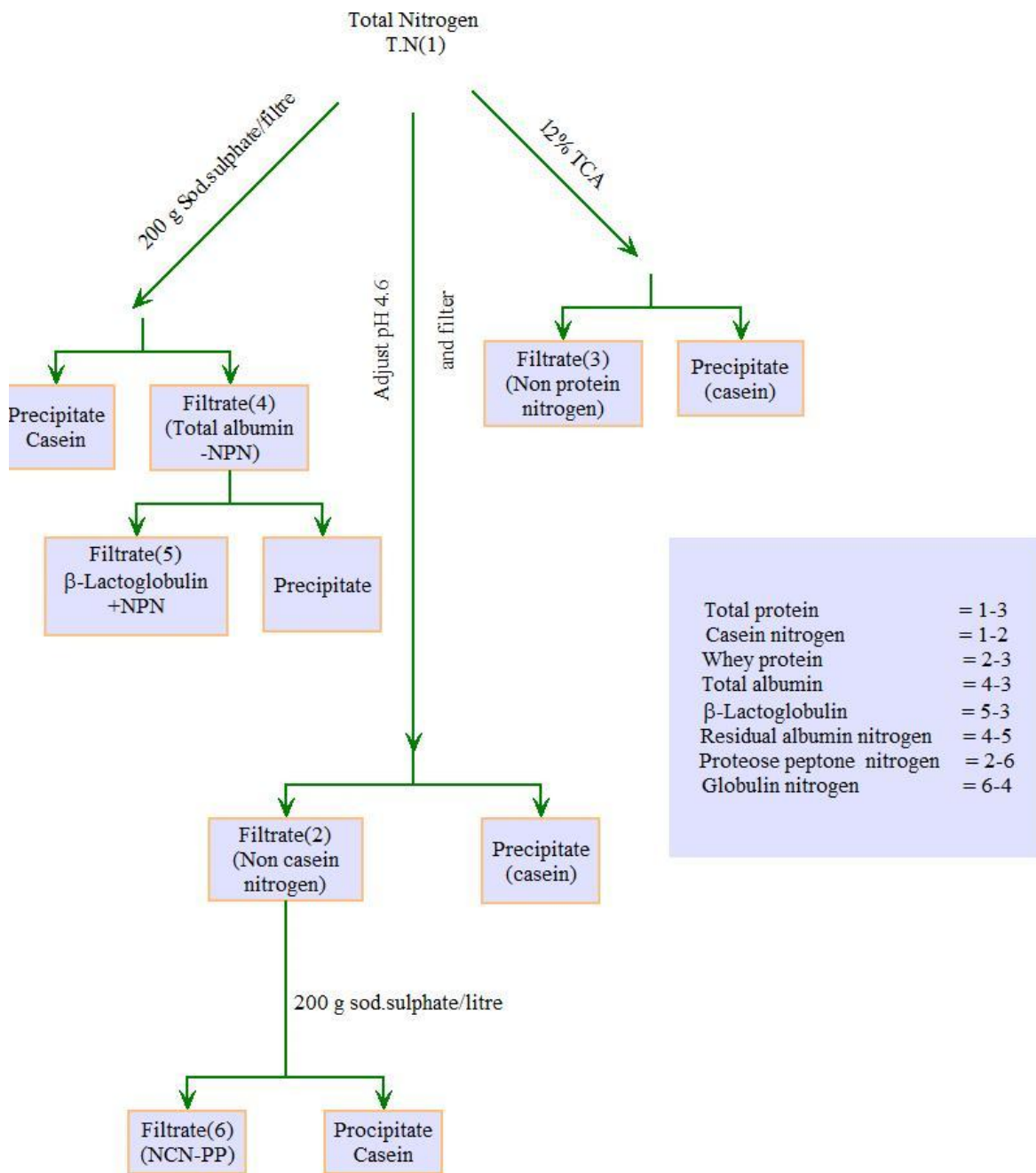er methods. The first one is based on the differential solubility of casein in 50% alcohol in the presence of ammonium acetate by carrying the pH temperature and ionic strength. The second procedure involves the dispersion of whole casein in 6.6M urea and separation of casein fractions by dilution, pH adjustment and finally addition of $(NH_4)_2 SO_4$. The order of precipitation of the casein in both methods is α-casein , β-casein and then γ- casein. Warner's casein fractions are now identified as follows, α- casein is a mixture of $α_s$ and κ - caseins.

The $α_s$ caseins and κ - caseins have been prepared largely from either whole casein or the α-casein fraction of Hipp and co workers. Crude $α_s$ -casein is prepared from casein obtained by $CaCl_2$ precipitation at $37^0C$ by removing calcium with oxalate to solubilize the casein and reprecipitation with 0.25M $CaCl_2$ at $37^0C$ and pH 7.0. The κ-caseins are removed from the supernatant by precipitation with $Na_2SO_4$, followed by re-precipitation from 50% ethanol with ammonium acetate or by using calcium oxalate as a precipitate to enhance the removal of the other caseins. Adjustment of the pH of whole casein dispersions in urea also has been used to precipitate the $α_s$ -casein either by adjusting 6.6 M urea dispersion to pH 1.3 to 1.5 with $H_2SO_4$ or by adjusting a 3.3M urea to pH 4.5. The crude k caseins can be obtained from supernatant of the $H_2SO_4$ method, by precipitating with $(NH_4)_2 SO_4$ and purified by reprecipitation from aqueous ethanol. More than 90% of $α_{s1}$ -caseins have been recovered from acid casein by precipitation with 75mM $CaCl_2$ at $5^0C$.
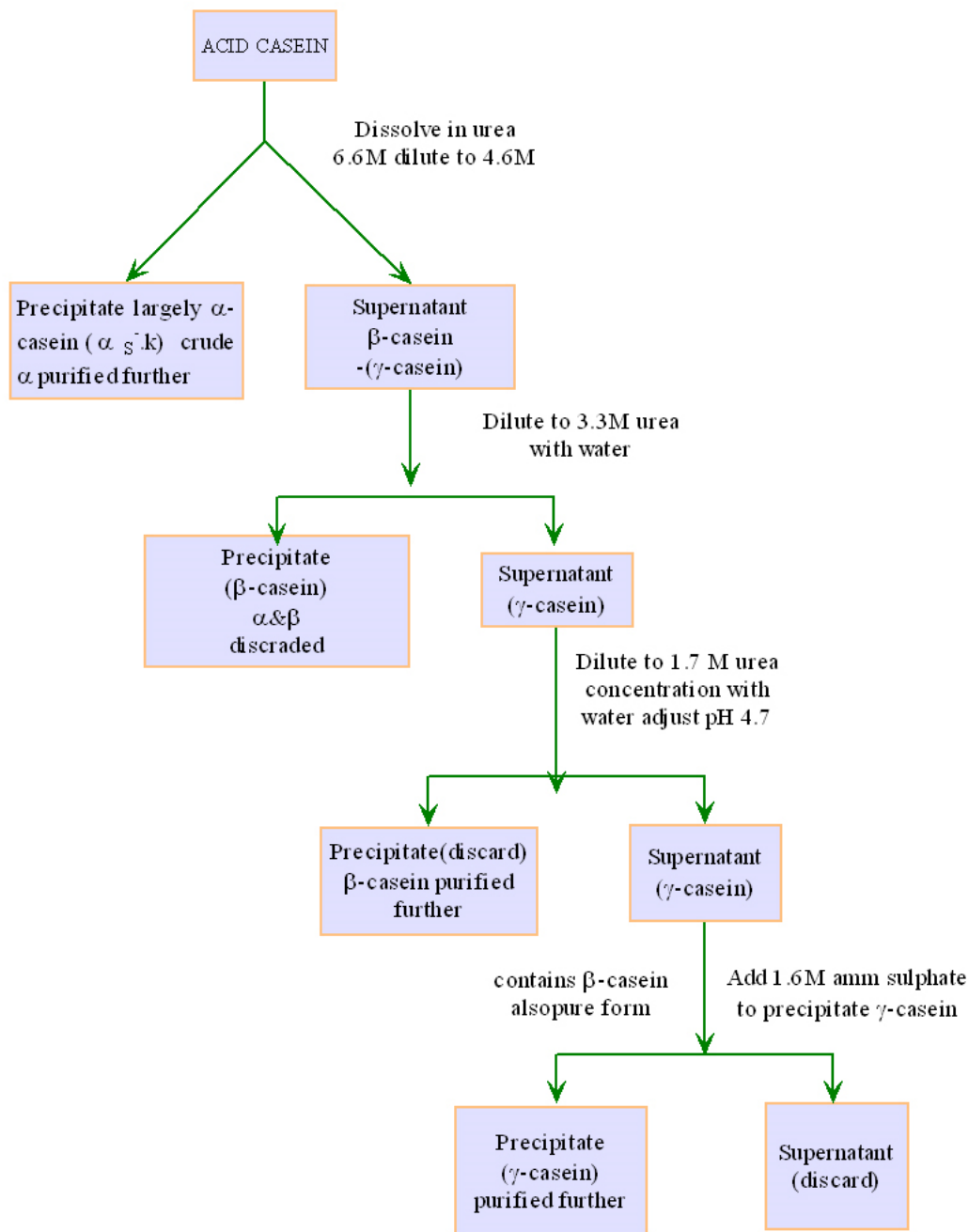
**Fig.7.3 Fractionation of casein by Hipp et al (1952) base on urea solubility**

(Source:Hipp *et.al*., J.Dairy Sci,1952)

Starting with α -casein fraction of Hipp and co workers the $\alpha_s$ -caseins can be precipitated by Ca $Cl_2$ treatment and the κ-caseins can be removed from the supernatant by pH adjustment to 4.7. Some research workers have added 12% tricholoro acetic acid (TCA) to a 6.6 M urea dispersion of the same fraction at $3^0C$ and precipitated $\alpha_s$-fraction . After removal of the urea and TCA from the supernatant they adjusted the pH to 7.0 added $CaCl_2$ to 0.25 M and removed the precipitate. κ-casein was finally obtained from the supernatant at pH 4.4. Wake (1959 ) prepared κ-casein from the supernatant remaining from the β- casein precipitation by the first procedure of Hipp and his co-workers by adjusting the pH to 5.7. A κ-casein concentrate has been prepared from commercial casein based on the differential solubility's of the caseins in $CaCl_2$ solutions.

The major β-casein component can be prepared by a simplification of the urea method of Hipp and his co workers. Whole casein is dispersed in 3.3M urea at pH 7.5 and adjusted to pH 4.6 which precipitates the bulk of $\alpha_{s1}$ and κ-caseins . The supernatant is adjusted to pH 4.9 diluted to 1.0M urea and warmed to 30 °C precipitating the major β- casein.

## 7.5. PHYSICO CHEMICAL CHARACTERISTICS OF CASEINS

The physico-chemical parameters for the casein fractions are shown in Table 7.1. Number of these parameters is derived from the composition based on determination of the primary structure.

Table-7.1: Physico-chemical characteristics of Caseins

| Protein | Charge at pH 6.6 | Isotonic pH | Partial specific volume | Absorptivity (cm²/g) 280nm | Molecular weight |
|---|---|---|---|---|---|
| $\alpha_{s1}$-casein | | | | | |
| A | -20.0 | 4.97 | 0.722 | 1.13 | 22066 |
| B | -20.9 | 4.96 | 0.725 | 1.05 | 23612 |
| C | -20.0 | 5.00 | 0.725 | 1.06 | 23540 |
| D | -22.6 | 4.91 | 0.723 | 1.05 | 23722 |
| $\alpha_{s2}$ - casein A | | | | | |
| $P_{10}$ ($\alpha_{s6}$) | -13.2 | 5.39 | 0.722 | 1.11 | 25148 |
| $P_{11}$ ($\alpha_{s4}$) | -14.8 | 5.32 | 0.721 | 1.10 | 25228 |
| $P_{12}$ ($\alpha_{s3}$) | -16.4 | 5.25 | 0.720 | 1.10 | 25308 |
| $P_{13}$ ($\alpha_{s2}$) | -18.0 | 5.19 | 0.718 | 1.10 | 25388 |
| β-casein | | | | | |
| A³ | -12.8 | 5.11 | 0.742 | 0.46 | 23.971 |
| A² | -12.3 | 5.19 | 0.742 | 0.46 | 23980 |
| A¹ | -11.8 | 5.27 | 0.742 | 0.46 | 24020 |
| B | -10.8 | 5.35 | 0.742 | 0.46 | 24089 |
| C | -8.2 | 5.53 | 0.742 | 0.46 | 24939 |
| κ-casein | | | | | |
| A | -3.9 | 5.43 | 0.734 | 0.95 | 19037 |
| B | -3.0 | 5.64 | 0.734 | 0.95 | 19005 |

(Source: Developments in Dairy Chemistry, Fox,1982)

Lesson-8

## Casein Micelle: Structure, Properties and its Importance

### 8.1. INTRODUCTION

In order to provide proteins along with a considerable portion of its calcium and phosphate to it's offspring, the casein in cow milk forms intricate particles which are recognized as casein micelles. Highly insoluble material (calcium phosphate) has to be carried without disturbing either stability or increase in its size. Apart from this , it must be able to form a clot once it enters the stomach of the calf. Considering these limitation nature s has formulated these casein micelles with several intricacies in it.

### 8.2. STRUCTURE

The whole casein will form aggregate when it is in solution at a concentration, pH, and ionic strength as in milk and low $Ca^{2+}$ activity. The micelles contain about ten to one hundred casein molecules. The aggregates like globular proteins will have a dense hydrophobic core in which most hydrophobic parts of the casein molecules are buried and a more loosely packed, hydrophilic outer layer containing most of the acidic (carboxylic and phosphoric) and some of the basic groups. Each of these small aggregates of the whole casein,usually called submicelles contains different casein molecules. The relative proportion of $\alpha_{s1}$: $\alpha_{s2}$ : $\beta$ and $\kappa$ in casein micelle is 3: 0.8: 3:1 respectively.More over $\kappa$-casein probably exists in milk as an oligomer containing several molecules and held together by covalent bonds (S-S linkages). Consequently there may be essentially two types of submicelles with and without $\kappa$-casein.

Earlier casein micelle models described were

1) Core-coat model

2) Internal structure model

3) Sub-unit model

However, Encyclopaedia of Dairy Sciences (2004) states what those is no universally accepted model for the structure if the casein micelle. The major contenders are the sub –unit model, the Holt model and the dual-binding model introduced to overcome criticism levelled at the first two.

**Dual Binding model for micelle assembly and structure** : According to this model micellar assembly and growth take place by a polymerization process involving two distinct forms of bonding:cross linking through hydrophobic regions of the casein or bridging across calcium phosphate nanoclusters. Central to the model is the concept that micellar integrity and

hence stability is maintained by a localised excess of hyrophobic attractions over electro static repulsion. Each casein micelle effectively functions as a block co polymer, with each bloc possessing different different and possibly multiple functionality for the cross linking paths. κ-casein is the most important of the caseins in this model of micellar assembly and structure. It can link in to the growing chain through its hydrophobic N-terminal block but its C-terminal block has no phophoserine cluster and therefore cannot extend the polymer chain through an anocluster link. Therefore, chain and net work growth are determined here leaving the casein micelle net work with a surface layer of κ-casein molecules, a prime reason for                any                structural                model.
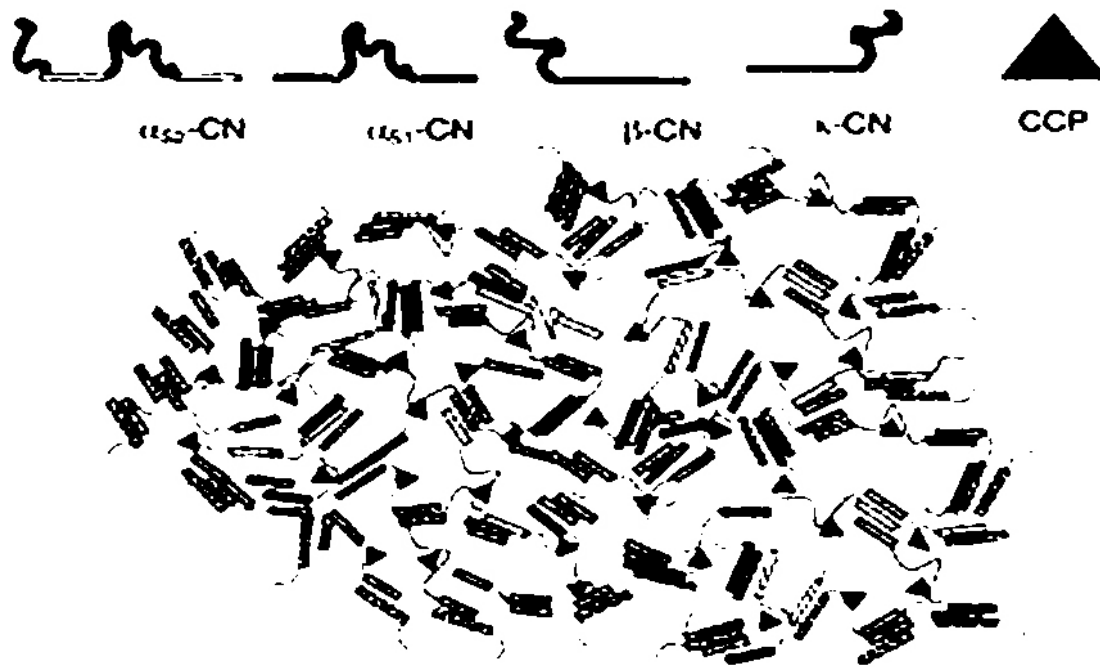


**Fig-8.1: Dual binding model of casein micelle**

**(Source: Horne,Opinion Colloidal Interface,2006)**

There will be considerable increase in the association of the casein micelle with increase in the $Ca^{2+}$ activity. Under conditions as in milk which means in particular that besides calcium phosphate is present submicelles aggregate in to micelles. The calcium phosphate acts as cementing agent. The aggregation will go on until a gel or a precipitate was formed if there were noκ -casein, which thus acts as a "protective colloid". The C-terminal part of κ -casein is very hydrophilic particularly those molecules that contain carbohydrate and it also has a considerable negative charge. This part of the molecule sticks partly out in to the surrounding medium as a flexible "hair" more or less behaving as a random coil polymer chain. There will be some areas which having steric repulsion causing hinderance for aggregation of the sub micelles. In this way aggregation of sub micelles would go on until the surface of the micelle was more or less covered with κ-casein. The κ- casein will be inters paced with other caseins, instead of covering the entire surface of the micelles.

Most of the k- casein is at the outside and the protruding chains of its C- terminal end give the micelles a hairy surface which are flexible and show perpetual Brownian motion. The effective thickness of the hairy layer is at least 5nm. A small part of κ- casein is in the interior. The casein micelle is fairly voluminous; electron micrographs indicate that the

voluminosity 'V' is about 2 ml per gram of casein. About half of the voluminosity of casein micelle is due to the sub micelles while remaining volume is serum between sub micelles.The serum inside the micelles however is depleted of part of the large solutemolecules. The pore width in the micelles is probably a few nanometers, Permitting easy access by many solute molecules but restricting the access of globular proteins. Taking the hairy outer layer in to consideration micelles voluminosity is higher on average about 4 ml per gram of casein. The hydrodynamic voluminosity increases with the decreasing micelles size. The casein micelles are not static and have three dynamic equilibrium with its surroundings. The equilibrium is between the free casein molecules and sub micelles(a tiny part of the casein is in true solution) the equilibrium strongly depends on temperature, between the dissolved and colloidal Ca and phosphate,between free sub-micelles and micelles. The casein micelles are rather variable particularly in milk of individual cows. Casein composition varies though not very much. The proportion of κ-casein varies by about 9-15% which will be reflected in the average casein micelle size. The voluminosity varies by a factor of 2 variations in the size and voluminosity usually from 6-9g per 100gdry casein. There will also be a variation in the composition of inorganic matter.

The quantity of calcium phosphate in the micelles increases on heating and very much decreasing when lowering the pH. The casein micelles will be dispersed into smaller units by any treatment that dissolves a considerable proportion of the calcium phosphate at constant pH such as adding Na Cl.Micelles disperse in to submicelles when milk is subjected to high pressure(100MPa for 1 hr) this disintegration is irreversibly and it may be caused by the formation of hydroxyl apatite from the calcium phosphate. Casein micelles also dissolve when submicelles dissociate. In the sub micelles the molecules are held together mostly by hydrophobic interaction and by 'H' bonds in the hydrophobic interior of the submicelles. Consequently addition of large quantities of urea or guanidinium chloride and sodium dodocyl sulfate in small quantities will dissolves the micelles. Electro static interactions (i.e internal salt bridges) also participate in keeping the submicelles together, but they cannot be broken without dissolving the calcium phosphate. Reagents that break S-S linkage do not disintegrate the micelles fully. Lowering the temperature (eg: to 5 °C)considerably effects the casein micelles. Hydrophobic interactions become much weaker and part of the casein particularly of the beta casein dissociate from the micelles. The voluminosity of the micelles increases, probably in part from increased "hairiness" as β-casein chains may protrude from the micelle surface.A small part of the calcium phosphate dissolves. These changes may be the cause of the slight disintegration of the micelles despite the increase in voluminosity their average size decreases to some extent ( b y 15%).

## 8.3. PROPERTIES

Almost all casein in un-cooled milk is present in roughly spherical particles mostly 0.02 to 0.30 μ m in diameter, comprised of some 20 to 150000casein molecules. These micelles also contain inorganic matter mainly calcium phosphate,about 8 g per 100g casein. The casein micelles are voluminous and hold more water than pure casein. They also contain small quantities of other proteins such as the milk enzymes lipase and plasmin and part of proteose-peptone

## 8.4. IMPORTANCE

The existence and properties of casein micelles have several consequences in the use of milk. The stability of milk products during heating, concentrating, and holding is largely determined by these micelles. The rheological properties of sour milk and concentrated milk are largely determined by the changes occurring in these micelles. The interaction of these

micelles with air- water and oil- water interfaces is important in homogenization. The casein micelles also influence some of the properties of the homogenized products. As such a study of the properties and structure of casein micelles will help in proper understanding of various phenomena occurring in milk and milk products.

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Lesson-9

## Whey Proteins: α-Lactalbumin and β-Lactoglobulin, their Fractionation and Physico-Chemical Properties

### 9.1. INTRODUCTION

Several procedures have been suggested for isolating the whey proteins from other whey components. The study conducted by Hansen and co- workers (1971) has revealed that the whey proteins could be separated by complexing the whey proteins with carboxymethyl cellulose. Similarly ferripolyphosphate, hexameta phosphate and polyacrylic acid were also used to isolate whey proteins by forming a complex. Gelfiltrationwith Sephadex G-25, ultrafiltration using cellulose acetate membranes, reverse osmosis and electro dialysis are some of the methods available for isolation of whey proteins from whey. As such it is necessary to know about the separation of whey proteins and also to study the properties of these proteins is also necessary for proper understanding of chemistry of milk and applying them to study in detail their behaviour in milk. This information could be used to develop suitable procedure in the manufacture of several dairy products and also to prevent some important and undesirable changes which are likely to interfere in developing some new products.

### 9.2. FRACTIONATION OF WHEY PROTEINS

Separation of individual components of whey protein mixture was originally accomplished by fractionations based on differences in solubility and by crystallization. At present the preferred methods of fractionation for both analytical and preparative purposes make use of gel filtration (which fractionates on the basis of molecular size) and ion exchange(which separates on the basis of net charge on protein). Gel filtration technique is particularly useful for fractionation of bovine whey proteins because of their great variation in their molecular size. The fact that β-lactoglobulin (MW = 18,277) forms a noncovalent dimer makes it readily separable from α-lactalbumin (MW = 14,175), as the latter does not aggregate.

### 9.3. α-LACTALBUMIN

This family of proteins consists of a major component and several minor components. Three genetic variants of α-lactalbumin have been identified.

Two genetic variants, A and B, of this protein exist. They differ by a single substitution,A having Gln and B having Arg at position 10. In the milk of European breeds and yaks only B variant is observed while both A and B variants occur in the milk of Indian cattle. Some minor forms of bovine α-lactalbumin are revealed by electrophoresis. Some of these contain covalently bound carbohydrate groups;the major component of bovine α-lactalbumin is

devoid of carbohydrate. Other minor components seem to have fewer amide groups than the major ones, and one minor α-lactalbumin containing three instead of four disulfides has been reported. In total, the major components do not account for more than 10% of the α-lactalbumin.

The complete primary structure of the major α-lactalbumin has been determined . The B variant consists of123 amino acid residues with a calculated molecular weight of 14,178 and the A variants differ from it only in having Gln instead of Arg at position 10.

The amino acid sequence of α-lactalbumin is similar to that of lysozyme. Indeed, bovine α-lactalbumin B and chicken egg white lysozyme have identical amino acid residues at 49 positions, and the four disulfide groups are located identically ( positions between 6and 120; 28 and 111; 61 and 77; and 73 and 91, respectively) in α-lactalbumin. The two proteins have different biological activities without mutual interference.The biological activity of α-lactalbumin is its interaction with galactosyltransferase to promote the transfer of galactose from uridine diphosphate galactose (UDP - galactose) to glucoseto form lactose. It has been shown that α-lactalbumin binds two atoms of Ca $^{2+}$ very tenaciously. In fact it is probable that all preparations of this protein have carried this Ca undetected. Removal of the bound Ca with ethylenediamine tetraacetate renders α-lactalbumin more susceptible to denaturation by heat or by addition of guanidine.

## 9.4 β-LACTOGLOBULIN

As chaffenburg and Drewry (1957) demonstrated that there are two components of β-lactoglobulin in the electrophoretic pattern of this protein in the western cattle. However, two more variants have also been identified by other workers. These genetic variants differ in their electrophoretic mobilities in starch or poly acrylamidegel in the ascending order as A > B > C > D. Bovine β-lactoglobulin'B' consists of 162 amino acid residues. Their calculated molecular weight for monomer is 18,227 and dimer is 36000 respectively **.** The dimer contains five cysteine residues per mole, of which four are involved in disulfide linkages. Location of one disulphide bond always occurs between Cys residues at 66 to 160 positions and the other link is between 106 and119 or 121. The single free thiol appears to be equally distributed between Cys119 and Cys 121. The existence of this thiol group is of great importance for changes occurring in milk during heating, as it is involved in reactions with other proteins, notably κ-casein and α-lactalbumin. It is likely that considerable portions of the sequence of β-lactoglobulin exist in the α-helix and β-sheet structures. Regions that are most likely helical are residues21-37, 51-63, 127-143, and 154-159 respectively. β-sheet structures are likely in 2-19, 39-43,76-88, 91-99, and 101-107, while the structure of the sequence 114-125 is not clearly established. β-Lactoglobulin exists naturally as a dimer of two monomeric subunits which is covalently linked. When more than one genetic variant is present, hybrid dimers are formed. Dissociation to the monomer occurs below pH 3.4. β-Lactoglobulin Aassociates to form an octamer at pH 4.5 and low temperature. The B variant (predominant in Western cattle) octamerizes to a smaller extent.

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**Lesson-10**

**Bovine Serum Albumin, Immunoglobulin, Proteose-Peptone, Other Whey Proteins and Non-Protein-Nitrogenous Compounds**

## 10.1 INTRODUCTION

Whey being an important byproduct from cheese industry has a large number of important nutrients which must be utilized effectively and efficiently. It is also necessary to have some knowledge about these components for their incorporation in some of the dairy products or to be used independently.

## 10.2 BOVINE SERUM ALBUMIN

This protein, a major component of blood serum, is synthesized in the liver and gains entrance to milk through the secretary cells, but it comprises only about 1.2% of the total milk protein. The protein as isolated from bovine milk could not be differentiated from that isolated from bovine blood by methods available in 1950. Since that time little work has been done on the serum albumin isolated from milk; it has been assumed to be identical to that in blood. The protein isolated from blood consists of a single polypeptide chain of 582 amino acid residues. Its tertiary structure reveals three equal-sized globular domains. It has one free thiol and 17 disulfide linkages, which neatly hold the protein in a multi loop structure. There is no specific role of this protein in the function of mammary gland. The behaviour of this protein in milk and milk products and its possible influence on their properties is not known.

## 10.3 IMMUNOGLOBULINS

Immunoglobulins are antibodies synthesized in response to stimulation by macromolecular antigens foreign to the animal. They are polymers with two kinds of polypeptide chains, light chain (L) of MW 22,400, and heavy chain (H). The latter are of several types, including $\gamma$ (MW 52,000), $\alpha$ (MW 52,000-56,000) and $\mu$ (MW 69,000).Each of the L and H chains consists of a relatively constant and highly variable sequence and appears to be coded for by two genes. The nomenclature of the classes of chains and of antibodies was developed for human antibodies and was extended to other species, including cattle, on the basis of homology of the heavy chains as revealed by immunological cross reaction with those of humans. IgG1 and IgG2 are each polymers of two light chains and two heavy chains of the $\gamma$ type ($\gamma_1$and $\gamma_2$). The chains are joined by disulfide linkages to form two antibody sites, each consisting of the variable portion of an H and L chain. IgGI and IgG2 have about 2.9% bound carbohydrate and MW of about 1,50,000. They differ slightly in electrophoretic mobility.

IgA and IgM immunoglobulin likewise have the basic structure of two H and two L chains joined by disulfide bridges. In IgA, the H chains are of α type, and in IgM they are of μ type. IgA, is secreted as a dimer of two of the basic four-chain units joined by a polypeptide of MW about 25,000, called J-component, and associated with another called secretory component, SC. This complex is called secretory IgA (SIgA) and has a MW of about 3,85,000. The secretory component is a protein of MW about 75,000, consisting of a single polypeptide chain with number of internal disulphide salt bridges. The carbohydrate content is high consists of N-acetyl galactose amine, D-glucose, D-mannose, L-fucose and N-acetyl neuraminic acid. These sugars are bound to the SIgA. IgM consists of pentamer of the basic four chain units joined by J component and has molecular weight of 9,00 ,000 and carbohydrate content of 11-12%.
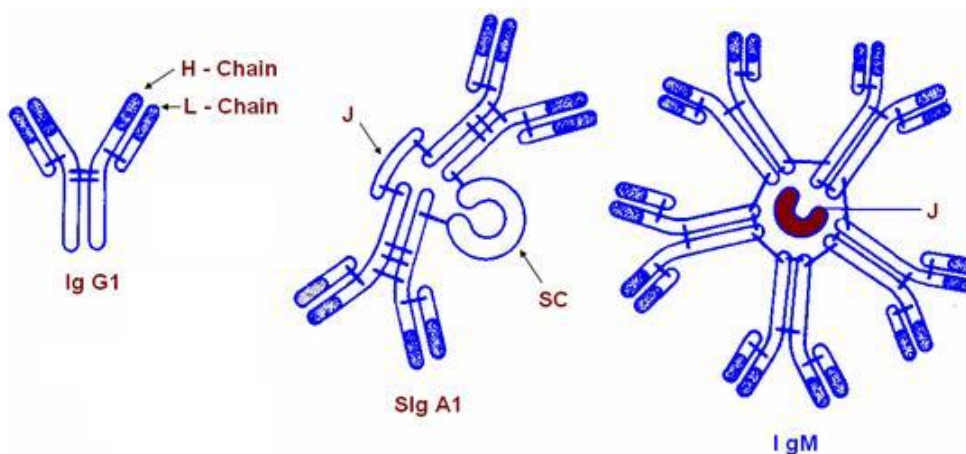


Fig 10.1 : Schematic Conformation of immunoglobulin I gG, SIgA, and IgM.⸺ = di sulfide linkage. Hatched = Variable portion

**(Source: Dairy chemistry and physics,Walstra and Jenness, 1984)**

## 10.4 PROTEOSE PEPTONE

Rowland has defined the conditions and procedures for obtaining the various fractions of protein in milk and to quantify them. It was observed by him that if milk is heated, about 80% of the whey proteins consisting mainly of α lactalbumin and β-lactoglobulins which precipitate with the casein when it is precipitated by aciditification to pH 4.6. The remaining 20% is a separate protein to which he applied the name proteose-peptone. Proteose and peptones are the polymers of amino acids which are of lower molecular weight than proteins. They often are formed by the partial hydrolytic degradation of proteins. They are usually not heat denaturable and hence it was easy for Rowland to reason by analogy that the proteins of milk which remain soluble in acid after heating are proteoses and peptones. The nature of proteins in this fraction has not been clearly established. The issue is complicated by the fact that the fraction may consist in part of native proteins and in part of breakdown products resulting from heat treatment.

## 10.5 NON-PROTEIN-NITROGENOUS COMPOUNDS IN MILK

Addition of 12% TCA to milk would result in the precipitation of all the caseins, α lactalbumin and β lactoglobulins leaving the non protein nitrogenous compounds in the filtrate. The major compounds identified from this are uric acid, creatine, creatinine, orotic

acid, α-aminonitrogen, hippuric acid, indicans,phosphoglyceroethanolamine, o-phosphoethanolamine and phenylacetylglutamine. The compounds present in the urine of dairy animals have a remarkable similarity between those present in non protein nitrogenfractions of milk. As a matter of fact the compounds or substances present in the urine of dairy animals are the resultant waste metabolites of dairy animal's body. It is apparent that the bulk of these waste metabolites in the urine of dairy animals originate from the blood and hence this entry or appearance and levels in milk or urine are due to the protein metabolism of the animals. Intake of feed by the animals is directly proportional to the presence of these compounds or substances n milk. NPN in milk varies from season to season and has no biological value as protein. It cannot be utilized by the body as a substitute of protein nor can it increase the cheese yield. Pasteurization by itself has no effect. However pasteurization with homogenization causes an increase in the non protein and amino nitrogen content. Increase in NPN was observed when concentrated milk is stored.

## 10.6 OTHER WHEY PROTEINS

**10.6.1. Lactoferrin and Transferrin:** These are the two iron-binding proteins are found in milk. One of them, transferring (Tf), is a common blood plasmaprotein; the other, lactoferrin (Lf), is secreted not only by mammary glands but also by lacrymal, bronchial, and salivary glands and by kidney and endometrial mucosa. It also is found in specific granules in heterophilicleukocytes. Both Tf and Lf appear to be large single chain polypeptides of 600-700 amino acid residues. Reported molecular weights differ somewhat; recent work favours 75,000 to 77, 000 for Tf, but values for Lf are not so consistent, 77, 000 or 93,000 being reported. In both proteins about 4 mol % of the residues are Cys, and both have covalently linked carbohydrate consisting of N-acetylglucosamine, mannose, galactose, and N-acetylneuraminic acid. All transferrins and lactoferrins appear to bind 2 mol of $Fe^{3+}$ per mole. Tf and Lf differ markedly from each other in amino acid composition and in electrophoretic mobility. They can be detected readily in electrophoretic patterns by auto radiography with [59]Fe. Electrophoretic patterns of milk and blood preparations from individual animals reveal the occurrence of genetic variants of both proteins. No immunological cross-reaction between Tf and Lf has been demonstrated even when both are from a single species. Amino acid analyses and partial sequences of human Lf and Tf indicate some degree of homology between the two and some internal homology of peptide segments with in each; sequencing is far from complete, however.

Both Tf and Lf can be determined quantitatively in a biological fluid by immuno diffusion method using a specific antiserum. The concentrations and ratios of Tf and Lf in milk vary greatly among species and with stage of lactation. The concentration of Lf in colostrums is about 1250 mg. $kg^{-I}$ in mid-lactation, the concentration falls to less than 100 mg $kg^{-I}$. Concentrations of Tf in milk have not been determined accurately but may be similar to those of Lf. Lactoferr in is an inhibitor of bacteria because it deprives them of iron. The concentration of Tf in milk have not been determined accurately but may be similar to those of Lf. Lactoferrin is an inhibitor of bacteria because it deprives them of iron. The concentration of Lf in bovine milk is so low,however, that it does not exert any significant antibacterial effect.

## 10.6. 2. Fat Globules Membrane Proteins

The fat globulemembrane contains approximately 50% protein and accounts for about 1% of the total protein of the milk. Some of the protein constituents of the membrane are enzyme, but it is not possible at present to estimate the ratio of enzymatic and non-enzymatic components. The fat globule membrane proteins are difficult to resolve analytically and to

separate preparatively because they interact strongly with one another and with lipids, and most of them are insoluble. Part is released from the globules by cooling the milk. Some success has been achieved in solubilizing the membrane proteins with detergents such as sodiumdodecyl sulfate coupled with reduction of disulfides.

The current nomenclature for milk fat globule membrane (MFGM) proteins recommended by the Milk Protein Nomenclature Committee of the American Dairy Science Associaion, the major proteins of MFGM in order of increasing mobility upon SDS-PAGE separation,are the mucin MUC-1, the redox enzyme, xanthine dehydrogenase/oxidase (XDH/XO),glycoprotein (PASIII), cluster of differentiation 36 (CD36), butyrophilin (BTN),adipose differentiation related protein (ADRP), and glycosylated variants of some polypeptide backbone designated PAS6/7 and fatty acid binding protein (FABP).

### 10.6.3. Acid Glycoproteins

Formerly called orosomucoid. It has been isolated from human serum, colostrum, and milk, and from bovine serum and colostrum, but it has not been detected in bovine milk.It consists of a polypeptide chain of 181 residues to which five heteropolysaccharide groups are linked to asparagines residues. The carbohydrate constitutes about 45% of the total molecule. The function of this protein is not known. In any event, $\alpha_1$-acid glycoprotein comprises only a small portion of the acid glycoproteins obtainable from fractionation of colostrum or milk on DEAE-cellulose.Five other fractions have been obtained in varying states of homogeneity. All contain carbohydrate and phosphate and promote the growth of Bifidobacteriumbifidum var. pennsylvanicus (formerly Lactobacillus bifidus). The possibility that some of these glycoproteins represent partial degradation products of caseins or membrane materials has not been elucidated.

### 10.6.4. Folate Binding Protein

A specific protein that binds folate (FBP) has been isolated from milk. Affinity chromatography on Sepharose to which folate has been attached is especially effective in isolating this protein. Its concentration in normal milk is about8 mg/ 100 ml.

### 10.6.5. $\beta_2$-Microglobulin

This protein consists of a single polypeptide chain of about 100 amino acid residues and MW of 11,800 daltons. It is present in several body fluids and in membranes of various types of cells. Its amino acid sequence indicates homology with the constant regions of immunoglobulin light and heavy chains. A protein that had long previously been crystallized from bovine milk and designated lactollin was shown in the year 1977 to be bovine $\beta_2$-microglobulin. It is a polypeptide of 98 amino acid residues,two of which are Cys. Direct quantification of microglobulin concentration have not been made in bovine milk; the amounts of lactollin that have been isolated from colostrum and milk are about 6 and 2mg per liter, respectively.

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Lesson-11

## Protein Denaturation and Hydrolysis

### 11.1. INTRODUCTION

Proteins in milk play an important role in the human nutrition. The processing and handling of the milk will change the physico chemical environment which would result in the denaturation of the protein molecule. As such knowledge of these changes is necessary for having a comprehensive idea about the behaviour of the proteins in the changed environment.

### 11.2. DEFINITION

Denaturation is a phenomenon that involves transformation of a well-defined, folded structure of a protein, formed under physiological conditions, to an unfolded state under non-physiological conditions.

### 11.3. AGENTS CAUSING PROTEIN DENATURATION

Change in the structure of proteins can be caused by a variety of factors. Some of these are encountered frequently while others are more of theoretical interests. A change in the structure of protein by heat, acid, alkali, or other agents such as sound waves, surface forces, pressure, UV radiation and ionizing radiations, results in loss of solubility and coagulation which is otherwise known as denaturation. Treatment with organic solvents such as alcohol, acetone, and solutes like urea, guanidine and ionic detergents would also result in protein denaturation. It is normally irreversible. Denatured proteins lose their biological activity (e.g. as enzymes), but nott heir nutritional value. Indeed, their digestibility is improved compared with the native structures, which are relatively resistant to enzymatic hydrolysis.

### 11.4. THERMAL DENATURATION

When proteins are exposed to increasing temperature, losses of solubility or enzymatic activity occurs over a fairly narrow range. Depending upon the protein studied and the severity of the heating, these changes may or may not be reversible. As the temperature is increased, a number of bonds in the protein molecule are weakened. The first affected are the long range interactions that are necessary for the presence of tertiary structure. As these bonds are first weakened and are broken, the protein obtains a more flexible structure and the groups are exposed to solvent. If heating ceases at this stage the protein should be able to readily refold to the native structure. As heating continues, some of the cooperative hydrogen bonds that stabilize helical structure will begin to break. As these bonds are broken, water can interact with and form new hydrogen bonds with the amide nitrogen and carbonyl oxygen of the peptide bonds. The presence of water further weakens nearby

hydrogen bonds by causing an increase in the effective dielectric constant near them. As the helical structure is broken, hydrophobic groups are exposed to the solvent.

The effect of exposure of new hydrogen bonding groups and of hydrophobic groups is to increase the amount of water bound by the protein molecules. The unfolding that occurs increase the hydrodynamic radius of the molecule causing the viscosity of the solution to increase. The net result will be an attempt by the protein to minimize its free energy by burying as many hydrophobic groups while exposing as many polar groups as possible to the solvent. While this is analogous to what occurred when the protein folded originally, it is happening at a much higher temperature. This greatly weakens the short range interaction that initially direct protein folding and the structures that occur will often be vastly different from the native protein. Upon cooling, the structures obtained by the aggregated proteins may not be those of lowest possible free energy, but kinetic barriers will prevent them from returning to the native format. Any attempt to obtain the native structure would first require that the hydrophobic bonds that caused the aggregation be broken. This would be energetically unfavorable and highly unlikely. Only when all the intermolecular hydrophobic bonds were broken, could the protein begin to refold as directed by the energy of short range interactions.

The exposure of this large number of hydrophobic groups to the solvent, however, presents a large energy barrier that make such a refolding kinetically unlikely. Exposure of most proteins to high temperatures results in irreversible denaturation. Some proteins, like caseins, however, contain little if any secondary structure and have managed to remove their hydrophobic groups from contact with the solvent without the need for extensive structure. This lack of secondary structure causes these proteins to be extremely resistant to thermal denaturation.

The increased water binding noted in the early stages of denaturation may be retained following hydrophobic aggregations. The loss of solubility that occurs will greatly reduce the viscosity to a level below that of the native proteins.

## 11.5 EFFECT OF pH ON PROTEIN DENATURATION

Most proteins at physiological pH are above their isoelectric points and have a net negative charge. When the pH is adjusted to the isoelectric point of the protein, its net charge will be zero. Charge repulsions of similar molecules will be at minimum and many proteins will precipitate. Even for proteins that remain in solution at their isoelectric points, this is usually the pH of minimum solubility. If the pH is lowered far below the isoelectric point, the protein will lose its negative and contain only positive charges. The like charges will repel each other and prevent the protein from readily aggregating. In areas of large charge density, the intramolecular repulsion may be great enough to cause unfolding of the protein. This will have an effect similar to that of mild heat treatment on the protein structure. In some cases the unfolding may be extensive enough to expose hydrophobic groups and cause irreversible aggregation. Until this occurs such unfolding will be largely reversible.

Some proteins contain acid labile groups and even relatively mild acid treatment may cause irreversible loss of function. This generally results from the breaking of specific covalent bonds and thus should be considered separately from denaturation. Exposure to strong enough acid at elevated temperatures will first release amide nitrogen from glutamine and asparagine groups and eventually lead to hydrolysis of peptide bonds. The effects of high pH are analogous to those of low pH. The proteins obtain a large negative charge which can cause unfolding and even aggregation. The use of high pH to solubilize and alter protein

structure is very important to the formation of fibers from proteins of plant origin A number of reactions can cause chemical modification of proteins at alkaline pH's that are commonly encountered in protein processing. Many of these involve cysteine residues. Perhaps the most important are the base catalyzed beta eliminations of sulfur to yield dehydroalanine which can react with lysine to form lysinoalanine. This result in a loss of nutritive value of the protein and the products of there action may be toxic. Exposure of protein molecules to high pH should be minimized as much as is possible. Exposure to very high pH at elevated temperatures results in alkaline hydrolysis of peptide bonds.

## 11.6. CHANGES IN DIELECTRIC CONSTANT

The addition of a solvent that is miscible with water, but that is less polar will lower the dielectric constant of the system. This will tend to increase the strength of all electrostatic interactions between molecules that were in contact with water. Many of the protein hydrogen bonds are effectively removed from the solvent and will not be affected. The presence of the less polar solvent will also have the effect of weakening the hydrophobic bonds of the proteins. These bonds depend upon an increase in the order of water when they are broken for their existence. As there is less water in the system, this becomes less important and at some level of replacement, these groups are at a lower energy level when in contact with the solvent. The structure of the protein will be changed and hence, it will be denatured. The reversibility of the process depends to a large extent on the nature of the non-polar solvent, the extent of unfolding the temperature of the system and the rate of solvent removal. When large amounts of the solvent are present, the protein will be largely unfolded with extensive exposure of the hydrophobic groups. If the protein could be instantaneously transferred to pure water at room temperature, the protein would most likely aggregate and precipitate. The sudden exposure of the hydrophobic groups to water would cause them to try to remove themselves from the aqueous phase as soon as possible. Even before the short range interactions could redirect the folding of the protein aggregation would occur. If the solvent exchange were slow, there would be a better chance that the hydrophobic groups would be able to return to the interior of the molecule and prevent aggregation. If the exchange occurred at low temperatures, the chances of regaining the native structure would be even better. At low temperatures, the hydrophobic groups may in part be stable in the aqueous phase or at least not as unstable. In this case, the removal of the solvent has little affect. When the temperature is subsequently increased, the normal course of protein refolding can occur. Solvent precipitation is often utilized as a means of purifying and concentrating enzymes. It is extremely important that both the solvent and the protein solution be cold when they are mixed and that the subsequent removal of the solvent be performed at reduced temperature. This helps to insure the recovery of enzyme activity.

## 11.7. DENATURATION AT INTERFACES

When proteins are exposed to either liquid-air or liquid-liquid interfaces, denaturation can occur. As a liquid-liquid interface, the protein comes into contact with a hydrophobic environment. If allowed to remain at this interface for a period of time, proteins will tend to unfold and place as many of their hydrophobic groups as possible in the non-aqueous layer while maintaining as much charge as possible in the water layer. To understand why protein unfolds at hydrophobic interfaces, it must be realized that the tertiary structure of a protein is not rigid. There are continued fluctuations about an average configuration. Any change in conformation that yields a higher energy state will spontaneously go back to the state of lowest energy. As a part of this process, hydrophobic groups will occasionally be positioned so that they have increased contact with the aqueous phase. When this occurs, these groups will assume the configuration of lowest free energy and will be removed from the water. If a

hydrophobic group is exposed while a protein is in contact with a polar solvent, these groups will find a state of lower energy exists if they enter into the solvent phase. This will continue to occur until random fluctuations in protein structure can no longer yield a configuration of lower free energy.

The amount of unfolding that occurs at such an interface will depend on how rigid the three-dimensional protein structure is on the number and location of hydrophobic groups in the molecule. A flexible, non-cross linked protein will be able to unfold easier than will a highly structured and cross linked one. If energy is applied to cause shear, the process will be accelerated. The shear can cause the protein to unfold, thus exposing its hydrophobic groups to the non - aqueous phase. It can also increase the interfacial area between the two phases and allow more proteins to come into contact with the non-aqueous phase. This unfolding is essentially non-reversible because of the large energy barriers. Even if the phases should separate and the protein is forced into the aqueous phase the protein will not regain its original structure. Rather an association of hydrophobic groups will cause the protein to aggregate.

The same forces are in operation when a protein migrates to a liquid-air interface. Hydrophobic groups tend to associate in the air and the protein unfolds. The presence of shear helps to unfold the protein and to introduce more air into the solution. Both of these effects can be minimized by keeping the temperature low (to weaken hydrophobic bonds) and by minimizing the interfacial area. If the interface is limited, then only a small amount of protein will be able to denature. The presence of this denatured protein will serve as a barrier to further denaturation. Proteins are often utilized in food products to stabilize emulsions or to incorporate air.These cases will be examined in more detail when emulsions and foams are discussed.

## 11.8. IONIC STRENGTH

Proteins are usually more soluble in dilute salt solutions than in pure water. The salts are thought to associate with oppositely charge groups in the protein. This combination of charged groups binds more water than do the charged groups alone and protein hydration is increased. With most proteins there is little change in solubility as more salt is added until some very high salt content is reached. At very high levels of salt there is a competition between the ions and the proteins for water of hydration. When the salt concentration is high enough, the proteins will be sufficiently dehydrated to lose solubility. Removal of the salt or dilution to a low enough concentration will usually result in the recovery of native structure.

## 11.9 THE EFFECT OF PROTEIN CROSS LINKERS

The presence of groups that cross link protein molecules will tend to lower the extent of protein denaturation. There are two main reasons for this type of behavior. First, when proteins are cross linked it is more difficult for them to unfold. As energy is added to the system and secondary bonds are weakened, the presence of cross linkers will tend to maintain structure. This is especially true if the cross links are covalent as in the case of disulfide bonds. The more compact the molecule is and the greater the number of disulfide linkages present, the greater the stability of the protein. While secondary forces may be weakened and some bonds can be broken, the cross linkers will tend to keep these groups in fairly close proximity. They also tend to prevent the exposure of large numbers of hydrophobic groups to the solvent. When conditions are returned to the native state, there is now a much greater chance for the proper secondary interaction to occur and for the protein to assume the native configuration.

A second effect has to do with the differences in entropy between the native and unfolded states. If a protein can be caused to assume a completely random coil conformation, there will be a large increase in entropy compared to the native structure. This entropy must be overcome if the protein is to refold into a native conformation. When cross linking groups are present, a completely random coil conformation can not be assumed. These groups introduce order into the structure and there is a considerable loss in the amount of disorder that can be achieved in the most denatured state. Because of this, the entropy change between the native and denatured state is not nearly as great and there will be less of a driving force for denaturation. If the cross linking groups are broken before denaturation and thus allowed to randomly form after denaturation, no stability will be added to the protein by the pressure of these groups.

No reactions involving primary covalent bonds (such as peptide linkages) occur during denaturation the unfolding of the molecules often exposes groups which may undergo chemical reactions (oxidation of sulfhydryl groups by the atmospheric oxygen).

### 11.10 HYDROLYSIS OF PROTEIN

Proteins are polymers of amino acids which are held by the polypeptide bonds between them. When these bonds are broken the protein splits in to smaller peptide and may even proceed to that extent of release for amino acids or smaller peptides. For these reactions, the presence of a suitable agent, or enzymes. The solution containing these smaller peptides and even amino acids is called hydrolysate solution. This process will help the digestion of the proteins and also study of the various amino acids present in the protein.

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**Lesson-12**

**Fat Globule Membrane Proteins their Properties and Role**

## 12.1 INTRODUCTION

Proteomics which involves in a large scale study of proteins especially with reference to their structure and function it has become possible to know the presence of some minor milk protein which are having a significant role in the physiological and abnormal functioning of the human system. Milk-fat globules originate near the basal region of the secretory cells as small droplets of fat. They migrate through the cytoplasm, gradually increasing in size, as the synthesis of triacylglycerol proceeds. The milk fat globules are secreted from the apical surface of the cell, surrounded by a membrane thin bilayer, the Milk Fat Globule Membrane (MFGM). MFGM are formed by a unique and quantitatively small subcategory of milk proteins (approximately 2–4% of total protein in human milk), the content of which is still largely unknown.

## 12.2 MILK FAT GLOBULE MEMBRANE PROTEINS

The MFGM proteins are having the nutraceutical and biological importance. Realizing the importance of these proteins there is an increase in the studies on MFGM. The MFGM is a rich source of membrane proteins, and applied proteomic analysis of these membrane proteins, has highlighted some of the possible signaling and secretory pathways used by the mammary gland .MFGM glycoproteins seem to contribute to the prevention of pathogenic organisms infections, being able to act as specific bacterial and viral ligands in the stomach of newborns, to prevent the attack of the intestinal mucosa The diversity of the glycans found in MFGM is thought to enable the glycoproteins to perform this function in the acidic environment of the stomach. It has been noted that some forms of gastric diseases such as peptic ulcer, chronic type B gastritis and gastric cancer can be attributed to the colonization of gastric mucosa by *Helicobacter pylori* Nondefatted and defatted MFGM preparations, given orally, caused equal healing effect on *H. pylori infection* of gastric mucosa in BALB/CA mice, leading to the conclusion that the major role in inhibition of *H. pylori* infection is played by the protein

## 12.3 COMPOSITION OF FAT GLOBULE MEMBRANE PROTEINS

The composition of the fat globule membrane is not consistent and varies considerably depending on different factors. The membrane is predominantly protein aciousin nature. The fat globule membrane contains approximately 50% protein and accounts for about 1% of the total protein of the milk. There is also wide variation in the nature of these proteins several enzymes have been reported to be associated with this membrane. AT least 25 different enzymes have been reported to be isolated from the fat globule membrane which includes the 5'nucleotidase, alkaline phosphatase,acid phosphatase , aldolase, xanthine oxidase. Although a major portion of the membrane material contains higher proportion of enzymes, presently it is not possible to estimate the ratio of enzymatic and non-enzymatic components. A part from the enzymes the proteins of the fat globule membrane protein exists as

polypeptides of varying molecular weight. These micro lipid droplets are encircled by a special membrane composed of lipid bilayer and proteins. This membrane has been designated the milk fat/lipid globule membrane The MFGM originates from the plasma membrane of the mammary gland secretory cells by extrusion of lipid droplet from the cell. Because each lipid droplet in milk has its own membrane envelope,i.e., the MFGM, the lipid droplets are present in milk in a dispersed form and, therefore, do not aggregate with each other.Only after destruction of the structure of the MFGM through, for example, a mechanical force like churning, do the lipid droplets aggregate and subsequently form large fat clumps known as butter. Milk fat globule membrane is composed of proteins and lipids in a 1:1 weight ratio. Many of these proteins are present in the MFGM as glycoproteins.

The main protein of the MFGM is the glycoprotein butyrophilin (about 40% of the total proteins of the MFGM), and the second representative protein of the MFGM is xanthine oxidase, which comprises 12 to 13% of the total proteins. Other proteins are present in MFGM each at 5% or less. The physiological role of the MFGM proteins is not completely clear despite numerous research studies. However, there are some reasonable suggestions about the physiological function of these proteins in the cell because these proteins are an integral part of the plasma membrane of secretory mammary gland cell.

## 12.4. ROLE OF FAT GLOBULE MEMBRANE PROTEINS

For the last 15 years, a great deal of knowledge hasbeen accumulated on health beneficial factors, protein andnon-protein, of bovine milk fat globule membrane (MFGM). Among thehealth beneficial components of the MFGM are cholesterolemia loweringfactor, inhibitors of cancer cell growth, vitamin binders, inhibitor of *Helicobacter pylori*, inhibitor of beta-glucuronidase of the intestinal *Escherichia coli*, xanthine oxidase as a bactericidal agent, butyrophilin as a possible suppressor of multiple sclerosis, andphospholipids as agents against colon cancer, gastrointestinal pathogens,Alzheimer's disease, depression, and stress. All of the above compelus to consider bovine MFGM as a potential neutraceutical.

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**Lesson-13**

**Quantification of Proteins in Milk**

**13.1. INTRODUCTION**

The proteins in milk are estimated by using either their component amino acids or by their chemical reactions or by their physical forms. Accordingly the methods available for estimation of proteins in milk are discussed in this lesson. The determination of the protein content of materials in which proteins occur in mixtures with other biological materials is not simple.

**13.2. PHYSICAL METHODS**

**13.2.1. Direct weighing:** Since the ultimate objective of protein analyses is to determine the weight of protein in a given quantity of material, it might seem that separation of the protein and direct weighing would be the simplest method to use. Since removing lipids, salts, other solutes, and water completely is difficult it is not generally employed for routine analyses. Obviously,however, direct weighing is the ultimate standard for all other methods. For example, the factor for converting nitrogen to protein was arrived at originally by preparing a sample of pure protein and determining the ratio between nitrogen and dry weight.

**13.2.2. Volume measurements:** Methods based on precipitating protein, centrifuging in a calibrated tube, and measuring its volume, have often been suggested. The Hart method for casein is of this type.Fat is extracted with chloroform and the casein precipitated with acetic acid,centrifuged, and measured. Methods based on volume measurements of proteins are not used to any extent in the dairy industry at present except for assessing the amount of in-soluble material in milk powders (solubility index).

**13.2.3. Turbidimetric methods:** It is sometimes convenient to form a suspension of insoluble protein and to estimate the protein content by optical methods. Either light transmittance or light scattering can be measured. Methods employing the former principle are called turbidimetric, those using the latter, nephelometric. In recent years a turbidimetric method devised by Harland and Ashworth has been used to a considerable extent for determining the concentration of undenatured serum proteins in heated and dry skim milks. In this method the casein and denatured serum proteins are precipitated by saturation with sodium chloride. The resulting filtrate is then diluted and acidified and its light transmittance determined. The protein content is determined from a calibration curve in which per cent transmittance is plotted against protein nitrogen content of the filtrate (determined by Kjeldahl). Methods of this kind are rapid, require little sample, and are conveniently adaptable to photometers and colorimeters found in most laboratories. Nevertheless, turbidimetric methods are not of the highest accuracy and precision because light absorption in turbid systems depends not only on the amount of dispersed material present but also on its degree of dispersion. The degree of dispersion depends on time of standing after development of turbidity, pH, salt concentration, method and rate of

precipitation, and concentration of material. Only by the most rigid standardization of all of the details of such methods can they be employed at all successfully.

**13.2.4. Refractive index measurements:** As previously pointed out most proteins have a refractive index increment of about 0.0018, meaning that one g. of protein dissolved in 100 ml. of solution increases the refractive index by that amount. With an accurate refractometer such as the Zeiss dipping refractometer, which has a sensitivity of ±0.00003, it is possible to determine protein concentrations satisfactorily. Of course, the refractive index of the solvent must be determined as well as that of the protein solution. Temperature must be carefully controlled since most proteins exhibit a considerable temperature coefficient for refractive index increment. The casein is isolated by acid precipitation, washed and dissolved in alkali,and the refractive index is determined.

**13.2.5. Absorption of ultraviolet radiation:** The absorption of ultraviolet radiation at wavelengths in the neighborhood of 280 nm, due to tyrosine, tryptophan, and phenylalanine residues in the protein, can be used as a method for determining protein content. This method is particularly valuable for a known pure protein whose extinction coefficient ($E_1^{1\%}{}_{cm}$) is known.It may also be useful as an approximate measure of the total protein content of a mixture if an average extinction coefficient can be assumed to apply. Obviously the extinction coefficients of proteins vary with their contents oftyrosine, tryptophan, and phenylalanine. The presence of materials other than proteins that absorb at this wavelength would seriously limit the method, but the common salts and other solutes do not absorb radiation at 280 nm. Methods based on this principle have not been used to any extent for routine determinations of milk proteins.

## 13.3 CHEMICAL METHODS

**13.3.1. Determination of nitrogen:** By all odds the most widely used method for determining protein content is the Kjeldahl procedure for nitrogen. It is natural that this should be so since nitrogen is a characteristic element in proteins.

This method involves the oxidation of the sample with sulfuric acid and a catalyst. Carbon and hydrogen are oxidized to $CO_2$ and $H_2O$, and reduced forms of nitrogen (such as $-NH_2$ and $>NH$) are retained in the digest as ammonium ions. The digest may be made alkaline and the ammonia distilled, and titrated, or it may be determined colorimetrically directly in the digest by means of Nessler's reagent.

Although it is widely accepted and used, the Kjeldahl method suffers from some rather serious difficulties. In the first place, there is the problem of separating protein from other nitrogenous materials. In milk about 5% of the total nitrogen is in the form of low molecular weight non-protein nitrogenous materials. The protein can be separated from these by precipitation with trichloroacetic acid. This precipitation, however, does not separate protein from nitrogen-containing lipids .Since the amount of lipid nitrogen is small, comprising only about 0.270 of the total milk nitrogen, it is usually neglected (i.e., included as protein nitrogen).

A second difficulty with nitrogen methods for determining protein arises from variation in the nitrogen content of various proteins. Proteins vary in nitrogen content from 14 to 19% and thus a single universal conversion factor cannot be used. Fortunately the principal milk proteins exhibit much less variation ranging from 15.3 to 16.0%. An average factor of 6.38 (corresponding to 15.65% nitrogen) is commonly used for milk proteins to convert nitrogen to

protein. In precise work on an individual milk protein the correct factor for that protein should be used.

A third group of difficulties concerned with the Kjeldahl method is the problem of proper digestion and no loss of nitrogen. A great many different modifications of the original Kjeldahl procedure have been suggested to accelerate the digestion while still attaining complete digestion. Copper, mercury, or selenium are used as catalysts and frequently $Na_2SO_4$ or $K_2SO_4$ is added to elevate the boiling point during digestion..

The use of boric acid to receive the ammonia as it is distilled off is an advantage in that only one reagent, the standard acid for titrating, need to be standardized and measured accurately.

**13.3.2. Formol Titration:** Considerable use has been made of titration with formaldehyde as a means of determining protein content in milk and milk fractions. Such titrations have been of particular interest as rapid methods of determining the casein content of milk for cheese making. As per Bureau of Indian Standards, for the estimation of total milk protein a factor of 1.7 is used and while for casein the factor 1.38 is used.

All such methods involve titration of a sample of milk to the end point of an indicator such as phenolphthalein, or adding a solution of formaldehyde, and titrating the acid liberated to the same end point. The amount of alkali used in the second titration is a measure of the amino groups that were originally present and combined with the formaldehyde.

## 13.4. INSTRUMENTAL METHODS

Several instrumental methods have been developed for the estimation of protein content in milk and milk products. A major advantages of these instrumental methods over the other techniques mentioned earlier are that these are nondestructive, require little or no sample preparation, and measurements are rapid and precise. For quality control purposes, it is often more useful to have rapid and simple measurements of protein content and therefore IR techniques are most suitable. For fundamental studies in the laboratory, where pure proteins are often analyzed, UV-visible spectroscopic techniques are often preferred because they give rapid and reliable measurements, and are sensitive to low concentrations of protein.

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**Lesson-14**

**Introduction and significance of Enzymes in Milk**

## 14.1 INTRODUCTION

Milk being a biological secretion of epithelial cells of the mammary gland has to undergo several biochemical reactions during its secretion. Consequently some of the enzymes will be entering the milk which have not been utilized during the biosynthesis of the milk. Similarly some enzymes get incorporated directly as a measure to protect the constituents after the production of milk. As such the enzymes naturally found in milk play a very significant role not only during the processing but also during their storage.

## 14.2 ENZYMES IN MILK

Approximately 50 enzymatic activities have been detected in bovine milk. Milk chemists are interested in quantitation of these enzymes, in deleterious or beneficial reactions that they catalyze in milk and dairy products, and in their inactivation. Catalysis of the enzyme is not similar for all enzyme some enzymes have a very fast activity Ex.Conversion up to 1,000,000 mols of reactant in one minute where as some enzymes catalyse only few hundred molecules of reactantper min in to the product. The rate of catalysis per molecule of enzyme is expressed as $k_{cat}$ sometimes called the turnover number for the enzyme. The $k_{cat}$ is a function of the efficiency of the enzyme and the chemistry of the reaction. Enzyme activity is frequently expressed in units. The Enzyme Commission has defined a unit of enzyme as the quantity that will catalyze the transformation of one micromole of substrate to product(s) per minute under standard conditions. For comparative purposes and quantization, initial velocities are preferred. In order to employ the relation between reaction velocity and enzyme concentration to quantitate the latter, three other parameters *viz.* substrate concentration, temperature, and pH-must be recognized and controlled. In general a plot of initial velocity against substrate concentration is a section of a rectangular hyperbola. Such a relation is rationalized and explained on the basis of the well-known Michaelis-Menten equation

$v_i = V_{max}[S]/(K_m+[S])$

where vi =initial velocity of reaction, $V_{max}$ = maximum velocity (or activity),$K_m$ = Michael is constant, and [S] = substrate concentration.

In general, enzymes are active only over a limited range of pH, and usually a distinct pH optimum is observed. This may result from an effect of pH on the V max, on affinity of enzyme for substrate,or on the stability of the enzyme. The activity of many enzymes depends on particular ionized groups in the active site. Obviously, these will be influenced by even small variations in pH and Ionic strength. More extreme high or low pH may denature and hence inactivate the enzyme.

The overall effect of temperature on enzyme-catalyzed reactions is a resultant of the accelerating influence of temperature on the reaction itself and the thermal denaturation and inactivation of the enzyme. Thus, the rate of the catalyzed reaction passes through a maximum as it is examined at a series of increasing temperatures. The optimal temperature therefore, is generally higher when the time during which the reaction is measured is shorter. The optimum differs greatly among individual enzymes because they differ in susceptibility to thermal denaturation. It should be apparent from this brief Introduction: that measurement of enzyme activity must be performed under rigidly controlled conditions in which observed activity can be translated into activity with 100% of the enzyme in the ES form. Therefore, enzyme activity is a value equivalent to V max for the concentration of enzyme available, that is, [E] $k_{cat}$ (The values given in represent such values.)

Enzymatic activities detected in bovine milk are listed in Table given below. They are named and classified according to the recommendations of the Enzyme Commission of the International Union of Biochemistry. All classes of enzymes except ligases have been detected in milk.

Some of the milk enzymes (e.g., catalase) are constituents of leukocytes, and some (e.g.,plasmin) may gain entrance to milk from blood. Most, however, are constituents or products of the mammary cells that enter milk as rather benign accidental constituents during the secretory processes. Some (e.g. the galactosyl transferase component of lactose synthase) are constituents of Golgimembranes, others (e.g., alkaline phosphatase) of the cell membrane. Enzymes of microbial origin are not considered in this chapter. Individual milk enzymes are associated with casein micelles, fat globules, or leukocytes, or are dispersed in the serum. Locations are specified in the appendix, for cases in which they are known.

## 14.3 ACTIVITIES OF SOME ENZYMES IN BOVINE MILK

The enzymes in milk play a significant role by having an activity specific to that enzyme. The important enzymes along with their EC No, pH of optimum activity and temperature and the ratio of their activity in relation to the activity in bovine and human are presented in Table No. 14.1

Table.14.1 Activities of Some Enzymes in Bovine Milk

| Enzyme | EC Number | Optimum | | Activity in Bovine Milk ($\mu$mole.min$^{-1}$.liter$^{-1}$) | Ratio of Activities Human/Bovine |
|---|---|---|---|---|---|
| | | pH | Temp ($^0$C) | | |
| Lactate dehydrogenase | 1.1.1.27 | | | 40. | 3.5 |
| Malate dehydrogenase | 1.1.1.37 | | | 40. | 1.8 |
| Xanthine oxidase | 1.2.3.2 | 6.-9 | 37 | 175. | - |
| Catalase | 1.11.1.6 | 7.0 | | 300. | 10. |
| Lactoperoxidase | 1.11.1.7 | 6.-7 | 20 | 22,000. | 0.03 |
| Galactosyl transferase | 2.4.1.38 | 7.2 | 37 | 50. | - |
| Lipoprotein lipase | 3.1.1.34 | | | 600. | - |
| Alkaline phosphatase | 3.1.3.1 | 9.8 | 37 | 500. | 0.02 |
| Phosphoprotein Phosphatase | 3.1.3.16 | 4.0-5.5 | 14-50 | 13. | 1.2 |
| Ribonuclease | 3.1.27.5 | 7.5 | 37 | 11.0-25.0 mg. liter$^{-1}$ | 0.3 |
| Lysozyme | 3.2.1.17 | 7.9 | 37 | 130.$\mu$g liter$^{-1}$ | 3000. |
| Glucose-6- phosphate isomerase | 5.3.19 | | | 155. | 2. |

(Source:Dixon and webb, Enzymes,1979)

Concentrations of milk enzymes vary greatly among species, and within a species large variations occur among individual animals and during the course of lactation. Data on such variations for most of the milk enzymes are not very complete or definitive.

A few of the enzymes found in milk (e.g., lactosesynthetase) have known functions in the biosynthetic processes of the mammarycells. Some milk enzymes act on substrates present as normal constituents ofmilk and may under suitable conditions play either beneficial or deleteriousroles in dairy processes and products. Hydrolases, such as lipase andproteinase, may facilitate resorption of milk constituents if and when milkingis stopped. There is no well-documented case of an enzyme in bovine milk beingof direct benefit to the primary consumer-the calf. It has been suggested,however, that a lipase in human milk facilitates digestion of fat by the humaninfant.

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**Lesson-15**

**Milk Enzymes its Source an d Significance-Part I**

## 15.1 INTRODUCTION

Activities of 14important enzymes in bovine milk are listed in by way of emphasizing the differences among species, ratios of activities in bovine and human milks are included. Xanthine oxidase, lactoperoxidase, and ribonuclease are especially prominent in bovine milk; lysozyme predominates quantitatively in human milk.

## 15.2 HYDROLASES

The enzymes which are responsible for the hydrolysis of various milk constituents are included in this group. This enzymes play a significant role in dairy industry especially the lipases which are responsible for the development of off flavours in milk and milk products. Similarly some of these hydrolases are useful in the detection of efficiency of milk processing. Enzymes which have the bacteriostatic action are also included in this group of enzymes.

### 15.2.1 Lipase (EC 3.1.1.34)

The principal lipolytic enzyme of bovine milk is a lipoprotein in the normal function of such enzymes is to liberate fatty acids from lipoproteins and chylomicrons of the blood; the fatty acids are then resorbed by the secretory cells of the mammary gland. A low molecular weight apoprotein cofactor is necessary for the enzyme to attack its acylglycerol substrate when the latter is present as or covered with lipoproteins. Blood serum contains such cofactors, and the lipoprotein lipase is said to be serum-stimulated. In bovine milk the lipoprotein lipase (about 2 mg per liter)is bound largely to casein micelles. It does not attack the glycerides of the fat globules unless the membranes of the latter are damaged (e.g.homogenization) or if lipoproteins containing the apoprotein cofactor are added. The latter effect may arise from leakage of blood serum into milk if the tight junctions between secretory cells are impaired. This enzyme is inactivated at pH 4.6 for one hour. Lipoprotein lipase has been isolated from bovine skim milk using affinity chromatography on Sepharose to which heparin has been linked covalently. Its molecular weight is about 50,000, and it exists as a non- covalently linked dimer under physiological conditions; it contains about 8% carbohydrate. Preparations with activities capable of releasing 300-700 µmol of fatty acids (from a triglyceride emulsion at pH 8.5) per min per mg protein have been achieved.

Cow's colostrum contains little of the lipoprotein lipase but has a different lipase that is not bound to casein, does not bind to heparin-Sepharose, is not activated by blood serum, and is stable at pH 4.6 for 1 h. It disappears after the first few milkings following calving. The two lipases do not exhibit immunological cross-reaction. The colostral lipase probably falls under the classification of a triacylglycerol lipase (EC 3.1.1.3) but does not appear to be homologous to the bile-salt stimulated lipase of human milk. Lipolytic activities of skim milk and colostrum are respectively about 600 and 200 µmol·min$^{-1}$·liter$^{-1}$(measured with tributyrin emulsion at pH 8.75, 37°C).

### 15.2.2 Milk Alkaline Phosphatase (EC3.1.3.1)

This is a phosphomonoesterase. The scientific name for this enzyme is orthophosphomonoester phosphohydrolase Two major isozymes have been identified, α and βphosphatase, mainly located in the milk plasma and fat globule membrane,respectively. The latter, more abundant, isozyme has been highly purified from bovine milk and found to be a dimer of two identical or very similar subunits each of MW ~ 85,000. It contains about five atoms of zinc per dimeric molecule. Its optimal pH for hydrolysis varies from one phosphate ester to another and with the composition of the medium. Monoesters, such as phosphoserine and β-glycerophosphate are hydrolyzed maximally near pH 9.0

Milk alkaline phosphatase is used as the method of preference for determining whether the milk has been pasteurized adequately. Alkaline phosphatase by heat bur reversibly reactivated when milk is chilled. Inactivation of alkalinephosphatase by pasteurization is an index of destruction of *Mycobacterium tuberculosis.* It is possible to determine the inactivation of phosphatase enzyme by easy chemical methods.

### 15.2.3 Acid Phosphomonoesterase (EC 3.1.3.2)

A second phosphatase present in milk has a pHoptimum at about 4.0. It has been assumed to be more similar to the phosphoprotein phosphatase (EC 3.1.3.16) of bovine spleen. It is primarily in the milk plasma. Its concentration is quite low (compared to alkalinephosphatase), though higher in colostrum.

Both the alkaline and the acid phosphatase can release inorganic phosphatase from caseins and from soluble esters, and this may occur in milk or fractions thereof under appropriate conditions. The acid phosphatase is the more active of the two at the pH of milk. These two enzymes differ greatly in susceptibility to inactivation by heat.

### 15.2.4: Ribonuclease (RNASE, EC 3.1.27.5)

Its content of mixed milk was found in one study to be 11 mg. liter$^{-I}$ and 25 mg.liter$^{-I}$ in another. RNase has been isolated from bovine milk by various methods; it appears to be identical to bovine pancreatic RNase in amino acid composition and immunological cross-reaction. Bovine pancreatic RNase has been well characterized. It consists of a single polypeptide chain of 124 residues, with MW 13,690. In spite of the low content of RNase in human milk (3mg·liter$^{-1}$), it has been isolated from that source by adsorption on a cation exchanger. Both RNase and lysozyme are so adsorbed; they can be differentially eluted.

### 15.2.5: Lysozyme (EC 3.2.1.24)

It is quantitatively an important fraction of the proteins of human milk (400 mg.liter$^{-1}$). It is a powerful bactericide as it attacks polysaccharides of the bacterial cell wall, causinglysis of the bacteria. Although the lysozyme content in bovine milk contains only about 0.1 mg. liter$^{-1}$ it could be isolated from it. The lysozyme of human milk appears to be identical to that found in other human secretions; it is a polypeptide of 129 residues, with MW 14,602.α-

lactalbumin and lysozyme are considered to be descendants of a common ancestor. Human lysozyme and α-lactalbumin, both obtained from milk, have different residues at 81 of 129 positions, Both have four disulfide bridges identically placed.

### 15.2.6. Plasmin (EC 3.4.21.7)

The principal milk proteinase belongs to the alkaline serine proteinase class; it is probably identical to the plasmin of blood Blood plasminogen (human) is a polypeptide of790 residues; activation involves proteolytic cleavage of the C-terminal 230residues and sometimes the N-terminal 76 residues as well. Apparently, this enzyme enters milk from blood mostly in the form of its zymogen, plasminogen. In fresh milk only a small proportion is in the active form; milk may contain a factor that slowly activates the plasminogen. The enzyme is associated with the casein micelles. It attacks peptide bonds at the C-terminal side of Arg and Lys residues and thus is trypsin-like. Cleavage of Lys-Xis faster than that of Arg-X. Optimal activity occurs at slightly alkaline pH and 37°C. The milk proteins most susceptible to plasmin are β- and $\alpha s_2$-caseins.$\alpha s_1$-casein also is attacked while κ-casein is relatively resistant, and the whey proteinsα-lactalbumin and β-lactoglobulin are not affected. Plasmin action on β-caseinis responsible for production of γ-caseins and the proteose-peptone fragments.Plasmin fully survives pasteurization and partially resists UHT treatments.Increased activity has even been observed after heating milk at $72^0$Cfor 15 s.

This has been attributed to conversion of plasminogen to plasmin, to inactivation of inhibitors, or to enhancing the accessibility of susceptible linkages in the substrate. The action of plasmin may produce serious defects in UHT milk products, such as development of a bitter flavor (caused by hydrophobicpeptides of low molecular weight) and changes in viscosity and appearance.

A second proteinase, with maximal activity at pH 4.0,also occurs in milk. Its molecular weight is 36,000, it is heat-labile(inactivated at 70°C for 10 min), and it is partly inhibited by SH-blockingagents. It cleaves $\alpha s_1$-casein faster than β- or γ-caseins (at pH 5,37°C). Its action on these proteins produces peptides that in electrophoretic behavior resemble those produced by chymosin. It is not yet clear how this enzyme should be classified; it may well be an aspartate proteinase.

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**Lesson-16**

**Milk Enzymes its Source and Significance-Part II**

## 16.1. INTRODUCTION

Among the various milk enzymes oxidases play a very important role especially the reactions involving oxidation and reduction. These oxidases influence these reactions influence the OR potential of the milk. Knowledge about these enzymes will help in proper utilization of the milk and its processing.

## 16.2. OXIDASES

Oxidases are a group of enzymes which catalyzes an oxidation-reduction reaction involving molecular oxygen ($O_2$) as the electron acceptor. In these reactions, oxygen is reduced to water ($H_2O$) or hydrogen peroxide ($H_2O_2$). Some of the enzymes which are included in this group are helpful in increasing the shelf life of the raw milk utilizing the activity of an oxidase for the oxidation of thiocynate. Presence of some of the oxidases like catalase will help in the detection of diseased condition of the udder.

**16.2.1 Xanthine Oxidase(XO, EC 1.2.3.2)** : This enzyme is very prominent in bovine milk. Most of it is associated with the fat globule membrane. Because of its relatively high XO content, milk is a source of choice for isolating this enzyme for investigation. Xanthineoxidase is much less prominent in milks of most other species; goat's milk has only about one-tenth the activity of cow's milk. The activity of XO in cow's milk depends to some extent on the Molybdenum content of the feed consumed.

The molecular weight of xanthine oxidase is about 2,83,000; it is a dimer of two identical subunits, each carrying one flavin-adenine dinucleotide,one Mo, four Fe, and four acid-labile Satoms. The specific activity of the pure enzyme is 5 $\mu mol.min^{-1} \cdot mg^{-1}$.It can oxidize 12 moles of xanthine per mole per second (assuming two active centers per dimer). Although hypoxanthine and xanthine are its normal substrates in the pathway of purine metabolism, it catalyzes oxidation of many other substrates, including various aldehydes, Under some conditions XO catalyzes a two-electron reduction of $O_2$ to $H_2O_2$;but at high pH, high $O_{22}^-$ is favoured. concentration, and low xanthine concentration, the one-electron reduction to superoxide ion, O

As isolated from milk, XO utilizes molecular $O_2$ as an electron acceptor, but if some of its disulfides are reduced with dithiothreitol it becomes a $NAD^+$ -dependent dehydrogenase. Reoxidizing the thiols to disulfides with the milk enzyme sulfhydryl oxidaserestores the specificity of the enzyme for $O_2$ as electron acceptor.

The activity of XO in fresh milk is increased about fourfold by storing at 4°C, by heating at 70°C for 5 min, by homogenization, or by incubation with commercial proteinase or lipase preparations. Such treatments also transfer much of the enzyme from fat globules to plasma.

**16.2.2. Sulfhydryl Oxidase:** (EC number not assigned): This enzyme is present in bovine milk , catalyzesoxidation of thiols to disulfides using molecular $O_2$ as the electron acceptor. It is an aerobic oxidase, reducing the $O_2$ to $H_2O_2$ratherthan to $H_2O$.

$$2 \text{ RSH} + O_2 \rightarrow \text{RSSR} + H_2O_2$$
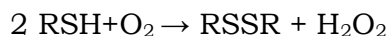
(It is completely different from thiol oxidase (EC 1.8.3.2) is not known to occur in milk, which forms $H_2O$ in the oxidation.)Sulfhydryl oxidase catalyzes oxidation of thiols in both small compounds and proteins. It is a large aggregate of subunits of MW 89,000. It contains about1.1 % carbohydrate residues by weight and 0.5 atom Fe per subunit. At optimal pH (7.0) and temperature (35°C) its $K_m$ for glutathione as substrateis 90 μM. Preparations with specific activities of more than 50 μmol· $min^{-1}mg^{-1}$ have been obtained. Milk may contain about 3 mg of this enzyme per liter. Sulfhydryl oxidase is closely associated with γ-glutamyl transferase (EC 2.3.2.2) in skim milk, and it has been suggested that the two activities reside in the same molecule. Such suggestions have been proved wrong, however, because the two activities can be separated. Sulfhydryl oxidaseim mobilized on glass beads has been used to oxidize thiols in UHT milk in an attempt to reduce cooked flavour.

**16.2.3. Catalase (EC1.11.1.6)** : This enzyme catalyzes the decomposition of $H_2O_2$ to $H_2O$ and $O_2$. It is found in many tissues and is particularly prominent in liver, erythrocytes, and kidneys of animals. In milk its activity parallels leukocyte count and is higher in mastitic milk and colostrum than in normal milk. It increases with the multiplication of bacteria. Cells associate with the fat globules:therefore, catalase accumulates in the cream layer. Crystalline catalases have been prepared from several sources, but because little is present in milk it has not been fully purified therefrom. Milk catalase has a molecular weight of about 210,000, its isoelectric pH ≈ 5.5, and it contains heme iron. Catalase activities on the order of 300 μmol per min per liter have been reported for milk.

**16.2.4 Lactoperoxidase(LP, EC 1.11.1.7)** : This enzyme catalyzes oxidation by $H_2O_2$ of a long list of electron-donor compounds,including aromatic amines, phenols, aromatic acids, leuko dyes, tyrosine and tryptophan, ascorbate, iodide, nitrite, and thiocyanate. LP may amount to as much as 1% of the total serum proteins of milk (i.e., 60 mg·$kg^{-1}$). Its activity in milk increases with advancing lactation to a maximum about 40days postpartum and thereafter declines somewhat. It is a glycoprotein (MW 77,500)containing 20-26 hexosamine residues (nosialic acid) and one heme per mole. Whether it consists of one or two polypeptide chains has not been settled. LP catalyzes oxidation of thiocyanate($SCN^-$) to a product hypothiocyanite ($OSCN^-$) that inhibits certain bacteria. Thiocyanate is a natural constituent of milk and $H_2O_2$ is produced by some bacteria themselves. Thus, in milk such bacteria exhibit self inhibition.

**16.2.5 Superoxide Dismutase (SOD, EC 1.15.1.1)** : This enzyme catalyzesthe dismutation of superoxide ion $O_2$ to $H_2O_2$and O2. The enzyme consists of two identical subunits of MW 16,000, each containing one Cu and one Zn per mole. The primary structure of SOD from bovine erythrocytes is known completely. It has one free thiol and one disulfide bond.It functions as a non covalently linked dimer, and its specific activity is3300 μmol/ min/ mg. Bovine milk serum contains a superoxide dismutase that is similar if indeed notidentical to that of the erythrocytes. Estimated content in milk range from0.15-2.4 mg/ kg. It is a rather heat-stable enzyme; the activity is not decreasedby heating milk at 63°C for 30 min. It may well be an important antioxidant protecting milk constituents from oxidation by superoxide ion generated by oxidations catalyzed by xanthine oxidase and lactoperoxidase as well as by riboflavin-sensitized photore actions.

### 16.3. TRANSFERASES

The only transferase discussed here is the galactosyl transferase component of lactose synthase (EC 2.4.1.22). In the absence of α-lactalbumin this enzyme transfers a galactosyl residue from UDP-galactose to an N-acetylglucosamine residue either free or in a protein-bound oligomer. In performing this function the enzyme is sometimes designated glycoprotein β-D-galactosyl-transferase (EC 2.4.1.38). α-lactalbumin modifies the transferase so that the $K_m$ for transfer of a galactosyl group to D-glucose is much reduced, and a β-1,4-glycosidic linkage is formed. The transferase is found in membranes of Golgi vesicles and there performs the synthesis of lactose as α-lactalbumin transiently combines with it. It enters milk with golgi membrane fragments. Its concentration in bovine milk is approximately 3-4mg/liter. The specific activity of the purified enzyme is 14 μmol/min/mg proteins at 37°C and neutral pH. As isolated from milk, the transferase has a molecular weight of about 42,000. The transferase contains 12-13% carbohydrate consisting of about 8% neutral sugars, 1% glucosamine,1% galactosamine, and 2% sialic acid.

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**Lesson-17**

**Lactose: Nomenclature and Structure**

## 17.1. INTRODUCTION

The characteristic carbohydrate of milk is lactose (4-O-β-D-galactopyranosyl-D-glucopyranose). It is commonly referred as milk sugar. Milk of mammals is the sole source of lactose. Of all the mammalian milk human milk contains highest lactose content (7.0%)while the average lactose content of normal bovine milk is 4.8% which accounts for about 50 to 52% of the total solids in skim milk. Besides lactose small amount of other carbohydrates are found in milk partly in a free form and partly bound to proteins, lipid or phosphate. Cow's milk contains monosaccharide glucose and galactose in concentrations of about 10mg/10ml. the amount of oligosaccharides is 100 mg/litre. Lactose is a disaccharide which upon hydrolysis will yield one molecule of glucose and one molecule of galactose. This disaccharide would act as a controlling factor in fermented and ripened dairy products. It contributes to the nutritive value of milk and milk products and plays essential role in the body and texture and solubility of certain stored products. It has an essential role in the color and flavour of heated products. Essential role in the color and flavour of the high heated dairy products.

## 17.2 NOMENCLATURE

Lactose is a disaccharide that yields D-glucose and D-galactose on hydrolysis.It is designated as 4-0-β-galactopyranosyl-D-glucopyranose and occurs in both alpha and beta forms. The predominant carbohydrates encountered in the body are structurally related to the aldotriose **glyceraldehyde** and to the ketotriose **dihydroxyacetone** . All carbohydrates contain at least one asymmetrical (chiral) carbon and are, therefore, optically active. In addition,carbohydrates can exist in either of the two conformations, as determined by the orientation of the hydroxyl group about the asymmetric carbon atom. farthest from the carbonyl. With a few exceptions, those carbohydrates that are of physiological significance exist in the D-conformation. The mirror-image conformations, called **enantiomers** , are in the L-conformation.

## 17.3. STRUCTURE OF LACTOSE

The two monosaccharides of lactose are linked through the aldehyde group of D-galactose. Thus the aldehyde portion of lactose is on glucose residue. The configuration of the D-galactoseresidue is of the beta form, which was shown by using the enzyme β- D-galactosidase that hydrolyses both lactose and a methyl β **-** D- galactopyranoside but not the α anomer. The D-galactose in the lactose molecule is in the beta form was also shown by its synthesis from D-glucose and D-galactose. The point of union of the two monosaccharides was established through products of hydrolysis or methylated lactose.
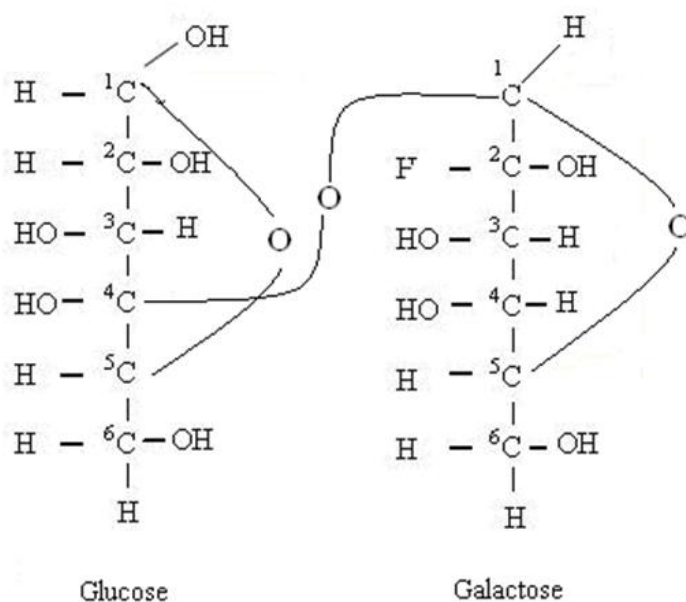
**Fig.17.1: Fischer structure of α lactose**
**(Source Jenness and Patton, Principles of Dairy Chemistry, 1959)**
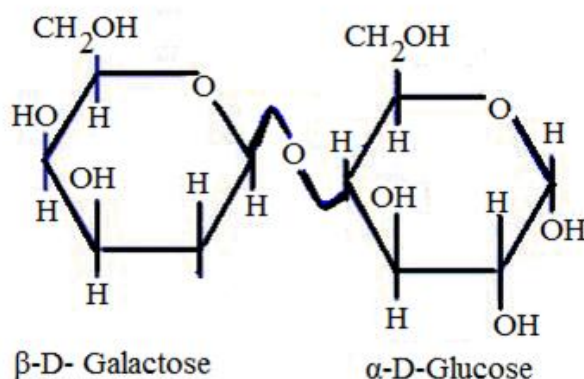
*Lactose, Nomenclature and Molecular Structure*



**Fig.17.2: Haworth structure of α lactose**
**(Source Jenness and Patton, Principles of Dairy Chemistry, 1959)**

The aldehyde and ketone moieties of the carbohydrates with five and six carbons will spontaneously react with alcohol groups present in neighboring carbons to produce intra molecular hemiacetals or hemiketals, respectively. This results in the formation of five- or six-membered rings. Because the five-membered ring structure resembles the organic

molecule **furan**, derivatives with this structure are termed **furanoses**. Those with six-membered rings resemble the organic molecule **pyran** and are termed **pyranoses**

The rings can open and re-close, allowing rotation to occur about the carbon bearing the reactive carbonyl yielding two distinct configurations (α and β) of the hemiacetals. The carbon about which this rotation occurs is the **anomeric carbon** and the two forms are termed anomers. Carbohydrates can change spontaneously between the α and β configurations: a process known as **mutarotation .**

When drawn in the Fischer projection, the αconfiguration places the hydroxyl attached to the anomeric carbon to the right,towards the ring. When drawn in the Haworth projection, the α configuration places the hydroxyl downward. The spatial relationships of the atoms of the furanose and pyranose ring structures are more correctly described by the two conformations identified as the **chair form** and the **boat form**. The chair form is the more stable of the two. Constituents of the ring that project above or below the plane of the ring are axial and those that project parallel to the plane are equatorial. In the chair conformation, the orientation of the hydroxyl group about the anomeric carbon of α-D-glucose is axial and equatorial in β-D-glucose. Covalent bonds between the anomeric hydroxyl of a cyclic sugar and the hydroxyl of a second sugar (or another alcohol containing compound) are termed **glycosidic bonds**, and the resultant molecules are **glycosides**. The linkage of two monosaccharides to form disaccharides involves a glycosidic bond. Several physiologically important disaccharides are sucrose, lactose and maltose.

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Lesson-18

**Physical Properties of Lactose-Part I**

## 18.1. INTRODUCTION

The major constituent present in the soluble portion of the milk is carbohydrates and more specifically the lactose. It influences several properties of milk a thorough study of the various aspects of this carbohydrate will help in proper understanding of various phenomena occurring in milk during the milk processing.

## 18.2 PHYSICAL PROPERTIES

Lactose normally occurs naturally in either of two crystalline form α- monohydrate and anhydrous β or as an amorphous "glass" mixture of α- and β-lactose. Several other forms may be produced under special conditions. These designations refer to the configuration on the number one carbon of the glucose moiety.

## 18.3: PHYSICAL FORMS

The physical forms of lactose are two one is α-lactose and the other is β-lactose. The physical properties of these two forms differ to some extent. The difference is mainly in their solubility, optic rotation and melting point.

**18.3.1.α-Lactose:** Ordinary commercial lactose is α-lactose monohydrate ($C_{12}H_{22}O_{11}$. $H_2O$). It is prepared by concentrating an aqueouslactose solution to super saturation and allow for crystallization to take place at a modern rate below 93.5 $^0$C. That α-hydrate is the stable solid form at ordinary temperatures is indicated by the fact that the other solid forms change to the hydrate in the presence of a small amount of water below 93.5$^0$ C. It has a specific optional rotation of +89.4$^0$ (anhydrous weight basis) and a melting point of 201.6 $^0$C.
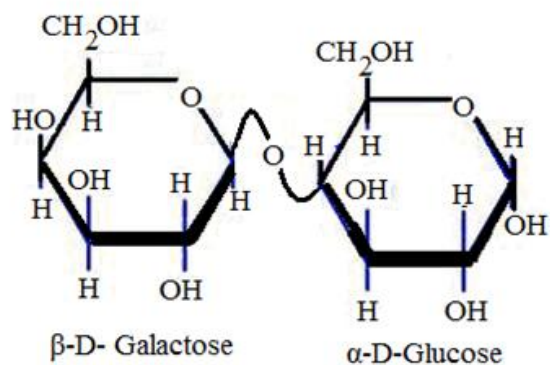
Fig 18.1: Structural Formula for α-Lactose

(Source Harrington, J. Dairy Sci, 1934)

**18.3.2: β-lactose:** This is the other isomeric form lactose. It exhibits a specific rotation of +35.0⁰on anhydrous weight basis. Above 93.5⁰C crystallization or drying of lactose solutions yields β anhydrate.

### 18.4. OPTIC PROPERTY OF LACTOSE

The specific rotation of a chemical compound [α] is defined as the observed angle of optical rotation '$a$' when plane-polarized light is passed through a sample with a path length of one decimeter and a sample concentration of one gram per millilitre. Therefore the specific rotation may be represented by the formula

[α] = 100 a/lc

Where

[α] = specific rotation at 20° C using D-line of sodium

a = degrees of angular rotation

l = length of tube in decimeter

c = concentration of substance in grams per 100 ml of solution

The specific rotation of a pure material is an intrinsic property of that material at a given wavelength and temperature. Values should always be accompanied by the temperature at which the measurement was performed and the solvent in which the material was dissolved. Often the temperature is not specified; in these cases it is assumed to be room temperature. The formal unit for specific rotation values is deg $dm^{-1}cm^{3}$per g but scientific literature uses just degrees. A negative value means levorotatory rotation and a positive value means dextrorotatory rotation.

## 18.5. SOLUBILITY

Lactose is freely soluble in water. However, the solubility of lactose is much lower than that of other common sugars. Solubility increases with increasing temperature. β-lactose dissolves more readily than α-lactose, as is apparent from their very different initial rates of solubility. Final solubility is the same for α- and β-lactose because of the mutarotation equilibrium that is eventually reached in solution. The particle size of the lactose influences its dissolving velocity. Coarselactose crystals dissolve much slower than tiny lactose particles. Dissolving velocity, and hence particle size, does not alter final solubility. Final solubility of lactose depends on temperature. The initial solubility is the true solubility of the form. The increasing solubility with time is due to mutarotation. As some of the α form is converted to β form, the solution becomes unsaturated with respect to *a*, and more α - hydrate dissolves. This process continues until equilibrium is established between α and β in solution and no more α -hydrate can dissolve, thus establishing the final solubility. This solution is saturated with respect to *a*, but a great deal of β-lactose powder can be dissolved in it because of the greater initial solubility of the β form The solution becomes saturated with *a*long before the saturation point of β is reached. However, additional βdissolving in such a solution upsets the equilibrium, and mutarotation takes place. Since the solution was already saturated with *a, a* formed by mutarotation will crystallize to reestablish equilibrium.

Since β -lactose is much more soluble and mutarotation is slow, it is possible to form more highly concentrated solutions by dissolving β rather that *a* -lactose hydrate. In either case, the final solubility of the lactose in solution will be the same. Lactose solubility values at different temperatures are shown in Figure 18.2. The solvent and the presence of salts or sucrose influence the solubility of lactose, as well as the rate of mutarotation. The solubility of lactose increases with increasing concentrations of several calcium salts-chlorides, bromide, or nitrate-and exceedingly stable, concentrated solutions are formed. Acetone also reduces the solubility of lactose based on which a procedure has been developed to recover the lactose from whey.

## 18.6 EQUILIBRIUM IN SOLUTION (MUTAROTATION)

Lactose exists in two forms *viz.,* α and β. By definition, *a* is the form with greater optical rotation in the dextro direction. The specific rotation of a substance is characteristic of that substance. Also important,besides the variables of the equation, are temperature of the solution,wavelength of the light source, and concentration of the solution. Regardless of the form used in the preparation of solution, the specific rotation will continue to change until +55.4 ° is reached at equilibrium. This is equivalent to the 37.3 % in α form and 62.7 % in β form. Since equilibrium rotation is the sum of the individual mix of α andβ forms. The rate of lactose mutarotation is influenced greatly by both temperature and pH. The rate is slow at low temperature but increases as the temperature rises, becoming almost instantaneous at about 75$^0$C.

The presence of sugars and salts can also affect the rate of mutarotation. Although the effect is small in dilute solutions, a combination of salts equal to that found in solution in milk nearly doubles the rate of mutarotation. This catalytic effect is attributed primarily to the citrates and phosphates of milk. The presence of high levels of sucrose, on the other hand, has the opposite effect.
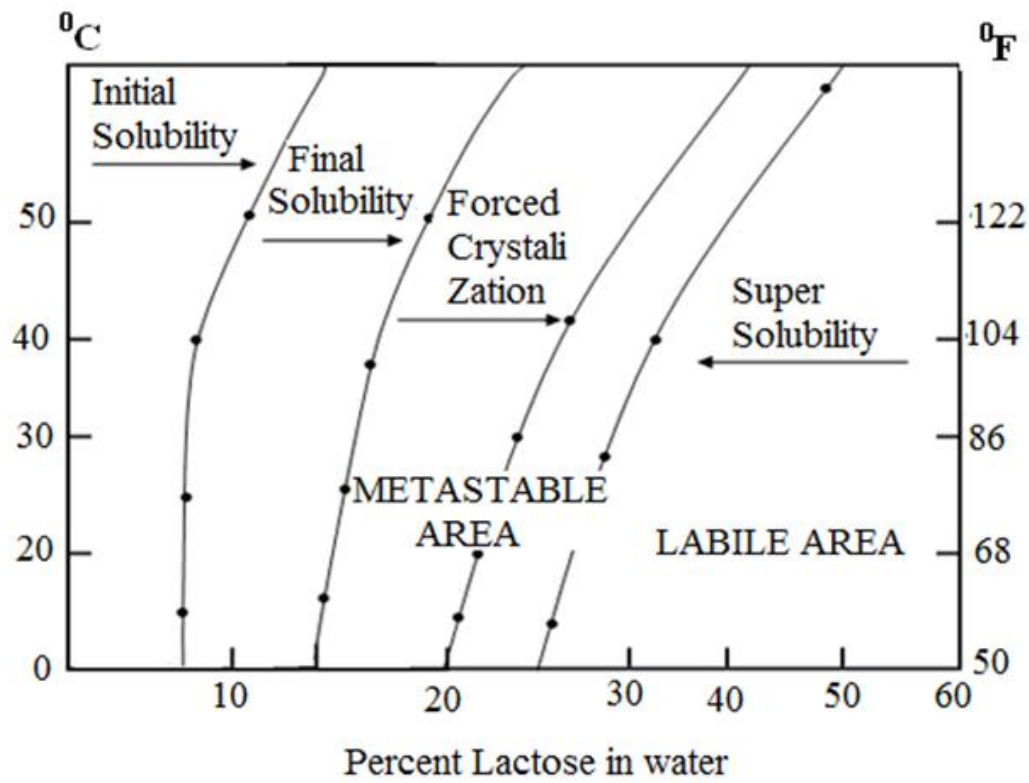
**Fig.18.2 Lactose Solubity curve**

The effect of sucrose is only slight at concentration up to 40% but as concentration increases above this level, mutarotation is rapidly decreased to about half the normal rate of the specific rotation.

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**Lesson-19**

**Physical Properties of Lactose-Part II**

## 19.1. INTRODUCTION

The body and structure of some of the milk products is influenced by the lactose crystallization. The crystallization of lactose will influence the physical properties of the milk product. The knowledge of this physical property helps in obtaining quality products and helps to avoid several defects in the body and texture of these products.

## 19.2. CRYSTALLIZATION OF LACTOSE

The principal factor governing the crystalline habit of lactose is the precipitation pressure, the ratio of actual concentration to solubility. When the pressure is high and crystallization is forced rapidly, only prisms are formed. As precipitation pressure lessens, the dominant crystal form changes to diamond-shape plates, then to pyramids and tomahawks, and finally, in slow crystallization, to the fully developed crystal. Different relative growth rates on the crystal faces account for the various shapes observed. The rate of crystal growth increases rapidly as supersaturation (precipitation pressure) is increased. In dairy products, crystallization is more complex. The impurities(e.g. other milk components), as far as lactose is concerned, may interfere with the crystalline habit. As a result, the crystals tend to be irregularly shaped and clumped, instead of yielding the characteristic crystals obtained from simple lactose solutions. In some instances, the impurities may inhibit the formation of nuclei and thus retard or prevent lactose crystallization. The influence of a number of additives on growth rates has been studied; some additives resulted in marked retardation, whereas others accelerated growth on specific crystal faces. The axes and the faces of the tomahawk crystal is diagrammatically shown in figure 19.1.

The concentration of the additive can influence the relative importance of the acceleration of the crystallization reactions. The tendency toward spontaneous nucleation is also lowered upon repeated re-crystallization. Gelatin is an example of a crystallization inhibitor that reduces the growth rate to 1/3 to 3/4 of normal even at low gelatin concentrations. In highly supersaturated lactose solutions, however,gelatin cannot suppress nucleation, which explains its ineffectiveness in preventing sandiness in ice cream. Consequently various marine and vegetable gums are currently in wide use in ice cream formulations. Both methanol and ethanol accelerate crystallization by as much as 30 to 60% even at low (1%) concentrations, depending on which crystal face is being observed. The rate of lactose crystallization is also markedly increased at low pH (<1). Some carbohydrates actively inhibit the crystallization of lactose, where as others do not. Calcium chloride had the greatest growth-promoting effect; at the 10% impurity level.
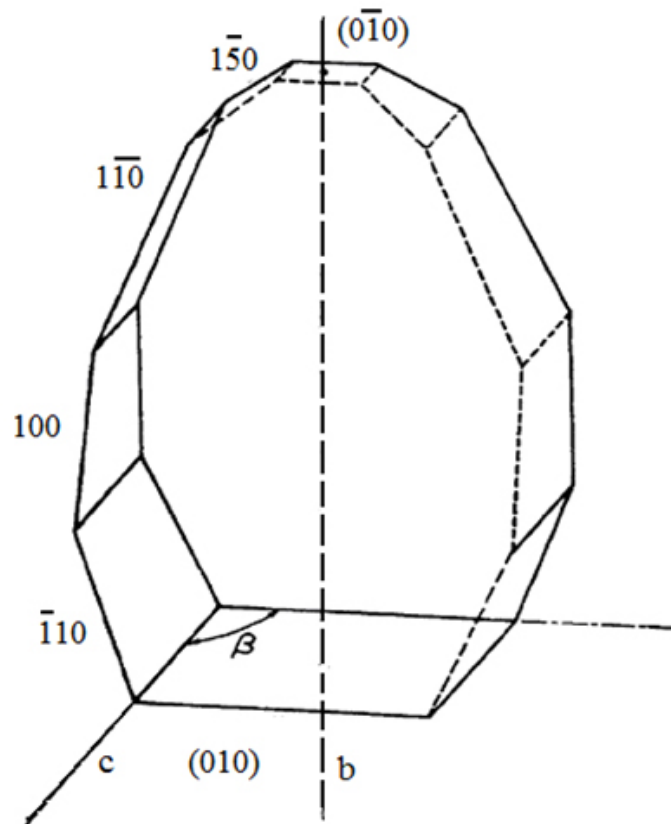
**Fig.19.1 Tomahawk crystal α-lactose monohydrate.**

**(Source: Fundamentals of Dairy chemistry, Wong *et al.*, 1988)**

### 19.3 LACTOSE GLASS

It is also called as amorphous noncrystalline glass. When a lactose solution is dried rapidly, its viscosity increases so quickly that crystallization cannot take place. The dry lactose is essentially in the same condition as it was in solution, except for removal of the water. This is spoken of as "concentrated syrup" or an "amorphous"(noncrystalline) glass. Lactose glass is stable if protected from moisture, but since it is very hygroscopic, it rapidly takes up moisture from the air and becomes sticky. When the moisture content reaches about 8% or a relative vapour pressure near 0.5, the lactose achieves a maximum weight; a discontinuity is observed in the sorption isotherm, and water is desorbed from the lactose

When lactose crystallization occurs above 93.5°C, the crystals formed are anhydrous and have a specific rotation of +35.0⁰ and a melting point of 252.2°C. They are composed of anhydrous. β-lactose, usually, α-lactose crystallizes as a hydrate containing equimolar amounts of lactose and water. The crystals are fairly hard and not hygroscopic. Above 93.5⁰C, anhydrous β-lactose crystallizes.β-lactose dissolves much faster than α-lactose hydrate at room temperature, as its solubility is about 10 times higher and the crystals are usually smaller with a larger surface area.

Amorphous lactose is formed when a solution (e.g. milk) is dried rapidly, as in a spray drier, or frozen. It is a very concentrated solution and it quickly dissolves or, rather, is diluted, on addition of water; but then, α-lactose hydrate may start to crystallize. If the water content of the amorphous lactose is low, say 3%, crystallization may be postponed almost indefinitely; nucleation rate is negligible because of the extremely high viscosity of the 'solution'. The product is, however, very hygroscopic, and when moisture content rises to about 8%, α-lactose hydrate starts to crystallize which helps to make very small crystals. But when crystallization of lactose caused by moisture uptake occurs in milk or whey powder, the result is caking; powder particles are cemented together by crystalline lactose, forming large and stony lumps.

It is almost impossible to obtain pure crystals. For instance, α-hydrate usually contains a few per cent of β-lactose, and vice versa. The different forms mentioned are different crystal polymorphs (i.e., they have different crystal lattices).

## 19.4. DENSITY

The densities of the various lactose crystals differ slightly from each other. α-hydrate form is 1.540, anhydrous β is 1.589, anhydrous α formed by dehydration under vacuum is 1.544 and anhydrous α crystallized from alcohol is 1.575. Densities of lactose solutions are not linear functions of concentration.

## 19.6. RELATIVE SWEETNESS

It has been amply demonstrated that the relatives weetness of sugars changes with the concentration. Therefore it is misleading to say that one sugar is so many times as sweet as another, because this will be true only at certain concentrations. The relative sweetness of some common sugars is presented in table form. It should be noted that lactose is relatively sweeter at higher concentrations than at lower concentrations and is sweeter than is usually reported in reviews of food applications. β- Lactose is sweeter than α-lactose but β form of lactose is not appreciably sweeter than the equilibrium mixture except when the concentration of lactose solution equals or is greater than **7%.** Since there is approximately 62.7% of β form in the equilibrium mixture, a β-lactose solution differs less in sweetness from a solution in equilibrium than does α-lactose solution.

**Table-19.2: Relative Sweetness of Sugars**

| Concentration (% w/v) to give Equivalent Sweetness | | | |
|---|---|---|---|
| Sucrose | Glucose | Fructose | Lactose |
| 0.5 | 0.9 | 0.4 | 1.9 |
| 1.0 | 1.8 | 0.8 | 3.5 |
| 2.0 | 3.6 | 1.7 | 6.5 |
| 2.0 | 3.8 | - | 6.5 |
| 2.0 | 3.2 | - | 6.0 |
| 5.0 | 8.3 | 4.2 | 15.7 |
| 5.0 | 8.3 | 4.6 | 14.9 |
| 5.0 | 7.2 | 4.5 | 13.1 |
| 10.0 | 13.9 | 8.6 | 25.9 |
| 10.0 | 14.6 | - | 0 |
| 10.0 | 12.7 | 8.7 | 20.7 |
| 15.0 | 17.2 | 12.8 | 27.8 |
| 15.0 | 20.0 | 13.0 | 34.6 |
| 20.0 | 21.8 | 16.7 | 33.3 |

(Source: Nickerson, T. A.Fundamentals of dairy chemisty, 1974 )

(Lactose. *In:Fundamentals of Dairy Chemistry,* Webb and Jhonson*)*

Lactose has a clean and sweet taste without any after taste. The sweetness profile resembles that of sucrose. However, the relative sweetness of lactose is small (only 20%) when compared to sucrose(100%). β-lactose appears to be somewhat sweeter than α-lactose, probably due to the fact that β-lactose dissolves somewhat quicker in the saliva of the mouth than α-lactose, hence reaching a higher concentration in the same period of time and thus giving rise to a higher sweetness sensation.

**Lesson-20**

**Chemical Reaction of Lactose-Part I**

## 20.1 INTRODUCTION

It is a well known fact that under rigorous conditions of processing carbohydrates undergoes extensive complex chemical changes. Similarly lactose also undergoes changes even in mild conditions. The chemical reaction of lactose mainly involves four sites.

Ø The hemiacetal linkages between carbon 1 and 5 of the glucose moiety.

Ø β-1,4 glycosidic linkages between the two sugars.

Ø The hydroxyl groups of both the glucose and galactose units

Ø The carbon to carbon bonds

## 20.2 OXIDATION

The extent to which lactose may be oxidized will vary depending up on the particular reagent, its concentration, and other reaction conditions. Thus by selection of conditions it is possible to derive oxidation products from lactose which range from relatively simple alterations of the reducing carbon in the glucose portion of the molecule to a carboxylic acid group to complete degradation with the end products being $CO_2$ and water.

**20.2.1. Mild oxidation**: Mild oxidation of lactose with such reagents as alkaline copper, iodine, or picrate forms lactobionic acid(4-0-β-D-galactopyranosyl-D-gluconic acid), which has a carboxyl group at C-1of the glucose unit. Such oxidation reactions often are used to determine reducing sugars quantitatively. Lactose also can be oxidized to lactobionic acid by mild chemical dehydrogenation and by lactose dehydrogenase produced by certain species of bacteria of the genus Pseudomonas. Lactobionic acid readily forms lactones by esterification of the carboxyl with hydroxyls at C-4 or C-5.

$$Lactose \xrightarrow{\phantom{xx}^-2H\phantom{xx}} Lactobionic\ \partial\ lactone \xrightarrow{\phantom{xx}^+H_2O\phantom{xx}} Lactobionic\ acid$$
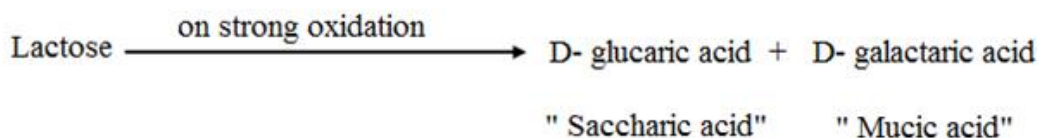
In methods for the quantitative measurement of lactose in which its reducing property serves as the basis of measurement where lactobionic acid is the resultant product. Such compounds as lactobionic acid have a profound tendency to form lactones through inter esterification with hydroxyl groups of the number 4 or 5 carbon.

**20.2.2. Vigorous Oxidation:** Somewhat more vigorous oxidation of lactose with dilute nitric acid ruptures the glycosidic linkage and produces dicarboxylic acid derivatives of the two sugars. The dicarboxyl derivative of galactose, galactaric or mucic acid, was formerly much used as a crystalline derivative for identification of galactose.

**20.2.3 Biological oxidation:** Biological oxidation of lactose to $CO_2$ and water can be brought about by mixed cultures of bacteria and protozoa obtained from sewage sludge. Such processes are useful in decomposing lactose-containing wastes from dairy factories.

### 20.3. LACTULOSE (4-O-β-D-Galactopyranosyl-D-Fructose)

Lactulose is a compound found in heated milk product in which the fructose moiety occurs predominantly in the pyranose and partly in the furanose form.

Lactose $\xrightarrow{\text{on strong oxidation}}$ D- glucaric acid + D- galactaric acid

" Saccharic acid"     " Mucic acid"

Its concentration in commercial evaporated milk will be up to 1%. It is an isomer of lactose that is formed by molecular rearrangement, usually under alkaline conditions where by the terminal aldose residue of lactose is converted into a ketose. Preparation of lactulose with calcium hydroxide has long been known but preparation of ketoses by this method is time-consuming, yields are less than 20%, and the keto sugar must be isolated from un-reacted starting materials, alkaline degradation products, and metal salts. A method used to prepare lactulose in nearly 90% yield is by treatment of lactose with boric acid in an aqueous solution made basic by tertiary amines. Ultra filtration can also be used to obtain lactulose from sweet whey which can be made into a non hygroscopic lactulose by some treatments. Lactulose is extremely soluble in water and polar solvents such as methanol. It is difficult to crystallize, especially when traces of other sugars are present.. Lactulose is unstable in alkaline solution, degrading by alkaline peeling and β -elimination reactions to yield galactose, isosaccharinic acids, and other acid products.Amines can bring about dehydration and degradation reactions. Lactulose is similar to sucrose in humectant's properties. Lactulose has several important uses in the food and drug industries. There is much information on lactulose utilization in infant nutrition. The presence of lactulose in infant feeding encourages the development of *Bifidobacterium bifidum* in the intestinal flora, imitating flora in the guts of breast-fed infants. There has been some concern about the possible laxative effects of lactulose, especially in infants; a low colonic pH might be a contributing factor to this effect. It is currently believed that lactulose cannot be digested by human alimentary enzymes, so even lactose tolerated individuals cannot digest lactulose some of the research workers suggested that lactulose could partially replace sucrose and corn sweeteners in intermediate-moisture foods. Only limited amounts could be tolerated in foods because of its laxative properties.

Lactose may be hydrolyzed to D-glucose and D-galactose with mineral acids, with cation exchange resins in the acid form, and with β-galactosidases (often called lactases). The β-1,4linkage between the two sugar residues is much more resistant to hydrolysis than is the

1,2 linkage between glucose and fructose in sucrose. Treatments such as an hour at $90^0$ C with 1.5 M HCI, or at $150^0$ C with 0.1 M HCI, are required to hydrolyze lactose completely.

## 20.4 ACID HYDROLYSIS

Lactose is resistant to acid hydrolysis compared to other disaccharides such as sucrose. In fact, organic acids, such as citric acid, that easily hydrolyze sucrose are unable to hydrolyze lactose under similar conditions. This is useful in analyzing a mixture of these two sugars, because the quantity of sucrose can be measured by the extent of these changes in the optical rotation of reducing power as a result of mild acid hydrolysis.

The speed of hydrolysis of lactose varies with time, temperature, and concentration of the reactant; some of the research workers have shown that 5 to 40% lactose solutions (w/w)can readily be hydrolyzed with 1 to 3 N hydrochloric acid or sulfuric acid. Ninety percent of the lactose could be hydrolyzed to the constituent monosaccharides at relatively low temperatures (60 $^0$C) and long reaction times (up to 36 hr). Due to degradative side reactions producing high levels of off flavor and color this process could not be used for whey concentrate. Lactose hydrolysis can also be brought about with 0.1 Nhydrochloric acid in short reaction times at $121^0$C. Sulfonic acid-type ion exchange resins have been used to catalyze lactose hydrolysis. The resin was equally effective on lactose solutions and acid whey permeates.

The hydrolysis is carried out at temperatures ranging from $90^0$ to 98°C. The advantages of this method are continuous operation, short reaction times, and no mineral acid to be removed from the hydrolyzed product. High temperature and low pH eliminate problems with microbial contamination. Best reaction rates were achieved with strong acid granular-type cation exchange resins with low degrees of cross linking. The formation of oligosaccharides during acid hydrolysis seem to be much less than during enzymatic hydrolysis

## 20.5 ENZYMATIC HYDROLYSIS

The hydrolysis of lactose using β-galactosidase (lactase) enzymes is most common method available. There has been significant progress in this field, and several processes are almost commercially feasible. There are three major approaches to enzymatic hydrolysis

Ø "Single use" or "throwaway" lactase systems;

Ø Lactase recovery systems based on membranes to retain the lactase for reuse; and

Ø Immobilized systems in which the enzyme is physically or chemically bound to a solid matrix.

Several lactases are available which are suitable for industrial processing of whey or lactose. The enzyme prepared from the yeast *Kluveromyceslactis* has a pH optimum between 6 and 7 and a temperature optimum of about 35°C. The lactase from *K. fragilis* has a pH optimum of 4.8 and a temperature optimum of about 50°C. A batch processing operation is the simplest method of achieving enzymatic lactose hydrolysis but suffers from the disadvantage that a large amount of recoverable enzyme is needed. For small users or manufacture on an irregular basis, the single-use enzyme procedure is probably the method of choice. Membrane reactor systems in which the enzyme is recovered by ultra filtration of the reaction mixture after hydrolysis is complete have been developed.

Lactose hydrolysis with immobilized systems is the method of choice when regular production of hydrolyzed syrups on a large scale is required. The best known of these is the Corning immobilized system, which uses lactase from *Aspergillus niger* covalently bound to a controlled-poresilica carrier. The particle size is 0.4 to 0.8 mm, the wet bulk density is0.6, the activity is near 500 U/g at 50°C, and the optimal pH of operation is between 3.2 and 4.3. The rate of hydrolysis is dependent on the mineral, lactose, and galactose concentrations, as well as on the temperature and pH. Inhibition of hydrolysis can be caused by galactose or sodium and calcium ions, so demineralization is often necessary. Because immobilized systems are designed for long-term use, adequate techniques must be developed to ensure sanitary operations. Common techniques use back flushing with water,acetic acid, milk alkali, and detergents with bactericidal activity. At least 10 di and oligosaccharides have been detected during β-D-galactosidasehydrolysis of lactose Three of the disaccharides have been identified as 3-O-β-galactopyranosyl-D-glucose, 6-O-β- D galactopyranosyl-D-glucose, and 6-β-D-galactopyranosyl- D glucose. Galactose is primarily involved in the formation of the oligosaccharides, which accounts for the lower concentration of free galactose than of free glucose during hydrolysis. The use of hydrolyzed lactose syrups has been proposed as an alternative sweetener to corn syrup solids.

## 20.6 Fermentation of Lactose

Lactose is metabolized by various microorganisms to compounds of lower molecular weight. In dairy field the most important is lactic acid fermentation. Lactic acid bacteria are present everywhere. Their activity in milk intended for liquid consumption is not desirable while it is important in the preparation of cultured products, butter, and cheese. Some microorganism can produce as much as 1.5% lactic acid in milk. These bacterial are classified as homofermentative if they produce only lactic acid and heterofermentative if they produce acetic acid, alcohol,and carbon dioxide along with lactic acid.

Homofermentative bacteria have phosphotransferase system in the cell membrane that phosphorylates lactoseat C-6 of the galactose moiety as it enters the cell. A β-D-phosphogalactosidase then hydrolyses the lactose-P to glucose and galactose-6-P. The glucose is phosphorylated at C-6 and metabolized to pyruvate via Embden-Meyerh of pathway of glycolysis. The galactose-Pconverted via a fructose isomer, tagatose-P to the triose-P stage of glycolysis and then to pyruvate.

Other homofermentative bacteria such as *S. thermophilus* and *Lactobacillusbulgaricus* do not phosphorylate entering lactose but rather hydrolyse it withan intracellular β - galactosidase. Heterofermentative organisms lack aldolase enzyme to cleave the six carbon atom to two three carbon units in the Embden-Meyerhof pathway. They use a pathway known as hexose-monophophateshunt pathway in which 6-phosphogluconic acid is formed from glucose and thenit is decarboxylated and the resulting five carbon unit, ribose- 5-phosphate is split to acetyl phosphate and glyceraldehyde-3- phosphate. The former yields acetic acid and / or ethanol and the latter yields pyruvic acid and then lacticacid.

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Lesson-21

## Chemical Reaction of Lactose-Part II

### 21.1 INTRODUCTION

Milk products are especially sensitive to the effects of heat treatment encountered under conventional process and storage conditions because of an abundance of reactive functional groups: aldehyde group of lactose, ε -amino group of lysine and other reactive N-containing groups (e.g. indolyl group of tryptophan, imidazole group of histidine, guanidine group of arginine and theα-amino group of proteins and free amino acids). The most important heat induced changes in dairy products that involve lactose are the changes associated with browning. Milk is the only important naturally occurring protein food with a high content of reducing sugar. Lactose may isomerize or it may react with protein in milk. The reaction of lactose with the caseins and whey proteins of milk systems will be *via* the Maillard or non-enzymatic browning reaction. It is also referred to as glycosylation of proteins for e.g. in case of lactose it is known as lactosylation ofproteins.

### 21.2 MAILLARD TYPE BROWNING

Generally Maillard type browning, is detrimental to the organoleptic, nutritional and functional qualities of the product and are therefore undesirable. However this reaction is being utilized favourably in the preparation of products like khoa where milk is heated in the presence of sucrose to produce brown product with a pleasant flavour. Maillard reaction also plays an important role in the generation of flavour during the manufacture of ghee or clarified butter and milk chocolate.

**21.2.1 Reaction Mechanism and Pathway of Maillard Reaction** : The first step in the Maillard reaction involves the nitrogen atom of an amino compound and carbonyl group of an aldehyde, or ketone in food systems and the reactants are predominantly protein and reducing sugars. In some situations the carbonyl products of lipid peroxidation, vitamin C, free amino acids and ammonia are also important reactants. The reaction proceeds with the elimination of a molecule of water to form a Schiff's base which subsequently rearranges to for man N- substituted glycosylamine intermediate. The amino acid carboxyl group plays an important role in the catalysis of the Amadori rearrangement. Thus the N-substituted glycosylamines derived from the amino acids are inherently unstable and they are either hydrolysed to the parent amino acid and reducing sugar or react via a spontaneous rearrangement to form the corresponding keto – (α-1-amino-1-deoxy-2-ketose)or aldo (α-2-amino-2-deoxyaldose)derivative depending on whether the parent sugar is an aldose or a ketose respectively. The aldo to keto transformation is referred to as the Amadori rearrangement and the corresponding keto to aldo rearrangement as the Heynes rearrangement.

The Maillard-type browning, sugar-amino type is the most prevalent, since it requires relatively low energy of activation and is auto catalytic. Direct caramelization, on the other hand, has a rather high energy of activation and therefore is less important. Lactose and

casein are the two principal reactants in the browning of milk products, but dried whey products containing lactose also undergo browning. Roller-dried products showed significant losses inlysine content, due to the contact with the drums with high temperatures. The protein-carbohydrate complex or its decomposition products result in the production of reducing substances, fluorescent substances, and disagreeable flavour materials. For example, 40 compounds were isolated and identified from a model system of casein and lactose that a product which had been stored at 80°C and 75% relative humidity for 8 days to accelerate browning. Substances which are isolated are furans, lactones, pyrazines, pyridines, acetylpyrrole,amines, pyrrolidinone, succinamide, glutarimide, dicarboxylic acid, acetone, 2-heptanone, and maltol were identified in the brown mixture, along with D-galactose, D-tagatose, and lactulose.

In milk products, active sulfhydryl groups serve as natural inhibitors in retarding heat-induced browning, but their mechanism is not understood. Sodium bisulfite,sulfur dioxide, and formaldehyde also inhibit browning in milk systems as well as in simpler mixture of amino acid and sugar solution. In actual practice, browning in dairy products is controlled by limiting heat treatments, moisture content, and time and temperature of storage. Browning has detrimental effect on the nutritive value of food products through interaction of the free €-amino group of lysine in the proteins with carbohydrates and the resultant rearranged product. Destruction of essential amino acids, particularly lysine and probably histidine, has been shown to occur during the storage and browning of non fat dry milk with high moisture (7.6%) content. Similar powders of low moisture (3.0%)did not deteriorate in nutritive value during storage. Reaction ofβ-lactoglobulin with lactose in the "dry state" (10% moisture) resulted in various degrees of lysine destruction, depending upon temperature and heating times. Arginine, histidine, acidic and neutral amino acids were not damaged by the thermal treatments (0 to 90°C) in the presence of lactose.

### 21.2.2 Factors that influence Maillard reactions

The overall rate and product profile of Maillard reaction in foods are highly dependent on number of parameters, however the most important are listed below:

Ø Reactants

Ø pH

Ø Temperature

Ø Moisture content

Ø Water activity

**21.2.2.1** Reactants: The nature and the molar concentration of the reacting species have considerable influence on the rate and mechanism of the Maillard reaction. Low molecular weight reactants tend to react more readily than high molecular weight reactants. Glucose is more reactive than lactose and contribute to the increased rate of browning In lactose hydrolysed milks

**21.2.2.2. pH:** The rate of Maillard reaction increases with increasing pH,maximum upto 9-10 depending upon the type of amino acids involved. Bases can catalyse the initial steps of the carbonyl amine reactions by removing the proton from the nucleophile increasing the nucleophilicity. The browning of pure Amadori product is also accelerated at alkaline pH

values. pH exerts a considerable influence on the mechanism of the Maillard reaction by determining the type of enolization favoured(1,2 or 2,3-enolization) and hence the pattern of the Amadori compound degradation.

**21.2.2.3 Moisture content and $a_w$** : Both moisture content and the $a_w$ of a food system exert a major influence on the Maillard reaction. Water my influence the rate of reaction by controlling the viscosity of the liquid phase and by dissolution concentration or dilution of reactants. At a very low $a_w$ value the proportion of the total reactants in solution is negligible and therefore the reaction rate is minimal. As the $a_w$ increases the concentration of remains constant provided excess solute is available to maintain saturated solution. However the total volume in which the reaction takes place increases.
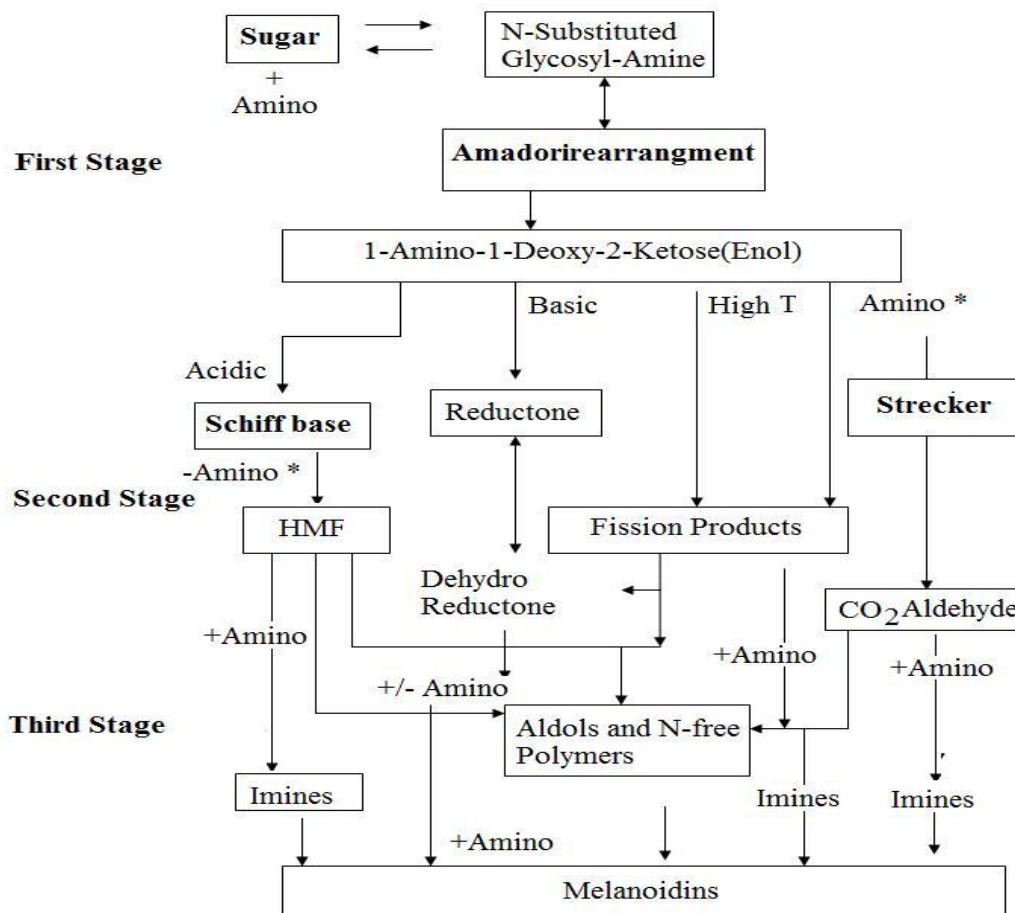
**Fig.21.1 Maillard Browning Pathway**

(Source:Hodge, J.Agricultural and Food Chemistry, 1953)

**21.2.2.4 Miscellaneous factors:** Phosphate, citrate, and phthalate buffers have been shown to accelerate the Maillard reaction. In the 5-7 pH range, phosphate has a dramatic effect on reaction rate (increasing up to 15 fold) as compared to that of phosphate free system.

## Lesson-22

## Caramelization and its Significance

### 22.1 INTRODUCTION

Caramelization is a type of non-amino browning reaction. This process consists of heating sugar slowly to around 170°C.As the carbohydrates are heated, the molecules break down and reform into compounds with a characteristic brown colour and flavour. It is a chemical decomposition of non-protein substances that occurs spontaneously at high temperatures and the reaction is known as pyrolysis. Caramelization is a complex, poorly understood process that produces hundreds of chemical products. Caramelization by heat during baking contributes to flavour and colour. However, it occurs to limited extent in milk and milk products and hence has limited significance.

### 22.2 DEFINITION

Caramelization is the removal of water from a sugar, by heating. This process results in is omerization and polymerization of the sugars into various high molecular weight compounds. Compounds such as difructose anhydride may be created from the monosaccharides after water loss. The fragmentation reaction of sugars during heating would result in low molecular weight compounds that may be volatile and may contribute to flavour. Polymerization reactions lead to large molecular weight compounds that contribute to the dark brown colour.

Caramelization type of browning may be defined as the heat decomposition of sugars as a function of pH and buffers in the absence of amino compounds. It requires high order of activational energy.

Caramelization is the oxidation of sugar by heating resulting in the production of nutty flavour and changing to brown colour. During the process, volatile chemicals are released, producing the characteristic caramel flavour.

The types of reactions that occur during the process of caramelization are

- Sucrose inversion to fructose and glucose
- Condensation reactions
- Intramolecular bonding
- Isomerization of aldoses to ketoses
- Dehydration reactions
- Fragmentation reactions
- Unsaturated polymer formation.

### 22.3. PYROLYSIS

Pyrolysis is the chemical decomposition of condensed substances that occurs spontaneously at high temperatures.

### 22.4. CARAMEL

Caramel colouring is a dark, rather bitter-tasting liquid. It is the highly concentrated product of near total caramelization, which can be used as food colouring and apart from its addition in beverages such as cola.

**22.4.1 Components of Caramel** : Three main components of caramel called caramelan, caramelen, and caramelin were isolated by using dialysis. Caramelan was described as a brown brittle, deliquescent solid having a bitter taste.

Ø Caramelan melts between 136 °to 144 °C and it is readily water soluble.

Ø Caramelen is a brown substance much darker than caramelan and not deliquescent it melts at 153.5°-154 °C

Caramelin exists in three modifications' namely, soluble in cold water, soluble only in boiling water and insoluble in all ordinary solvents. They are infusible materials that are much darker than caramelan and carmelen. Three high molecular weight components have been separated from caramel prepared from sucrose. They are melanoidin found in ammonia processed caramels seem to be the sole polymeric compound in caramel. The pH values of caramel constitute an important property of caramels. A high pH may indicate an incomplete burn or alkali present. Above pH 6.0 caramel is susceptible to the attack by molds and below pH 2.5 it quite readily resinifies.

A good caramel should contain colloidal particles which do not precipitate during storage or to the products to which caramel is added. Though the control of viscosity of caramel is difficult manipulation of the temperature on the period of its contact with reagent, it is possible to obtain caramel of desired viscosity. The rate of dehydration is one of the most important parameter to influence the properties of caramel in general. The time of mutual contact of reagents and manipulation of the temperature may lead to caramel of desired viscosity.

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**Lesson-23**

**Nomenclature and Structure of Glycerides**

### 23.1 INTRODUCTION

Lipids are better energy sources than sugars because they are less oxidized than carbohydrates. These lipids are being synthesized in adipose tissue, mammary gland and liver. Lipids are compounds that are soluble in ether, benzene, hot alcohols, etc. They are not soluble in water. The lipids in bovine milk are present in microscopic globules as an oil-in-water emulsion. The primary purpose of these lipids is to provide a source of energy to the newborn calf. Both the fat content of the milk and the fatty acid composition of the lipids can vary considerably as a result of changes in factors like breed of cow, diet and stage of lactation. The fat content can vary from about 3.0 to 6.0%, but typically is in the range 3.5 to 4.7%. Changes in the composition of the fatty acids (e.g.: 16:0 and 18:1) can be quite marked and can lead to changes in physical properties of the fat. These changes make comparison difficult between different samples of milk fat and ideally comparisons should be made between cows in mid-lactation and fed on similar diets. From a practical viewpoint, milk lipids are very important as they confer distinctive nutritional, textural and organoleptic properties on dairy products, such as cream, butter, whole milk powder and cheese. The common property of all lipids is their hydrophobic nature. Lipids includes fats and oils, waxes, phospholipids, steroids (like cholesterol), and some other related compounds.

### 23.2 NOMENCLATURE

The fats and oils are made from two kinds of molecules: glycerol (a trihydroxy alcohol) and fatty acids attached to it by ester linkage. The ester of glycerol containing fatty acids are formerly known as triglycerides and now termed as triacylglycerol.

The practice of using common names for various chemical compounds was prevailing earlier but these names often caused confusion in correct identification of these compounds. An example is the use of caprylic to describe 1-octanol and 2-octanol and attempts to qualify the name with "primary" and secondary were not useful in identifying the compounds. The IUPAC names have been given for the chemical compounds to overcome the difficulty in identification of the fatty acids.

### 23.3 NOMENCLATURE OF FATTY ACIDS

There are different types of nomenclature for the fatty acids. In one method they are classified depending upon their saturation and unsaturation as shown in Table 23.1

**Table-23.1: Nomenclature of fatty acids on the basis of saturation**

| Saturated | Parent hydrocarbon + oic | C18:0- Octadecanoicacid |
|---|---|---|
| Unsaturated | With one double bond + en | C18:1- Octadecenoic acid |
| | With two double bonds + dienoic | C18:2- Octadecadienoic acid |
| | With three double bonds + trienoic | C18:3- Octadecatrienoic acid |

Another type of nomenclature is by using empirical formula and the name of the fatty acids as shown in Table 23.2.

**Table-23.2: Systemic nomenclature for the fatty acids and their empirical formula**

| | Common name | Systematic name | Empirical formula |
|---|---|---|---|
| **1) Saturated Fatty Acids** | | | |
| C16:0 | Palmitic acid | n-Hexadecanoic acid | $CH_3(CH_2)_{14}COOH$ |
| C18:0 | Stearic acid | n-Octadecanoic acid | $CH_3(CH_2)_{16}COOH$ |
| **2) Unsaturated Fatty Acids** | | | |
| C16:1(9) | Palmitoleic acid | cis-9-Hexadecenoic acid | $CH_3(CH_2)_5CH=CH(CH_2)_7COOH$ |
| C18:1(9) | Oleic acid | Octadecenoic acid | $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ |
| C18:2(9,12) | Linoleic acid | All cis-9,12-Octadecadienoic acid | $CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COOH$ |
| C18:3(9,12,15) | Linolenic acid | All cis-9,12,15-Octadecatrienoic acid | $CH_3CH_2(CH=CHCH_2)_3(CH_2)_6COOH$ |
| C20:4(5,8,11,14) | Arachidonic acid | All cis-5,8,11,14-Eicosatetraenoic acid | $CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$ |

## 23.4. GENERAL STRUCTURE

General structure of Triglyceride (triacylglycerol, TAG or triacylglyceride) is an ester composed of a glycerol bound to three fatty acids. It is the main constituent of vegetable oil and animal fats.

Triglycerides are formed from a single molecule of glycerol, combined with three molecules of fatty acid. The glycerol molecule has three hydroxyl (OH-)groups. Each fatty acid has a carboxyl group (COOH-). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds.
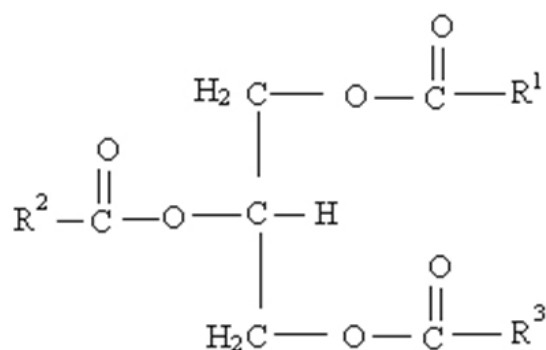
Fig-23.1: Structure of triacylglycerol

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

For understanding the composition of various lipid classes it is necessary to have an idea about the terminology used for explaining the position of the various fatty acids. An abbreviation of fatty acids is used for such purpose. According to this the first figure is the number of carbon atoms and the second number of double bonds. As for example, stearic acid is referred as C18:0 while oleic acid is denoted as C18:1. To locates the fatty acid in acylglycerols stereospecific numbering (Sn) is used. If a glycerol molecule is drawn with the secondary hydroxyl to the left, the hydroxyl above it is Sn-1 and that below is Sn-3. Although milk fat contains more than 400 fatty acids most these fatty acids exist in traces while only 20 fatty acids are found in larger concentration. The recent studies carried out on the whole milk fat composition revealed that the short chain acids are selectively associated with the sn-3 position. With increase in chain length of the acid there is greater tendency to be esterfied at position Sn-1.

There are three types of TG The first type of TG are with 48- 54 carbon atoms composed of long chain 1,2 DGs containing 18:0,18:1 and 18:2. In type 2 TG are containing 36-46 carbon atoms and the Sn-3 position acids composed of 4:0,6:0 and 8:0, these tri glycerides are enantiomers. Type 3 TG the carbon 26- 34 the 1,2 DG containing medium chain fatty acids and the 3 position acids are short and medium chain acids in sn-3 position that are different from those in Sn-1 are also enantiomers

Chain lengths of the fatty acids in naturally occurring triglycerides can be of varying lengths, but 16, 18 and 20 carbons are the most common. Natural fatty acids found in plants and animals are typically composed only of even numbers of carbon atoms due to the way they are bio-synthesized from acetyl CoA. Bacteria, however, possess the ability to synthesize odd- and branched-chain fatty acids. As a result, ruminant animal fat contains odd-numbered fatty acids, such as 15, due to the action of bacteria in the rumen.

Most natural fats contain a complex mixture of individual triglycerides. Because of this, they melt over a broad range of temperatures. Cocoa butter is unusual in that it is composed of only a few triglycerides, one of which contains palmitic, oleic, and stearic acids in order of concentration.

<div align="center">**Lesson-24**</div>

<div align="center">**Composition, Classification and Distribution of Lipids in Milk**</div>

## 24.1 INTRODUCTION

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 importance of milk fat cannot be over emphasized.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. The rich pleasing flavour contributed by the milk fat to the milk products render them acceptable by all the consumers and further no other fat can be used to duplicate it. The body and texture of the milk products is largely being influenced by this milk component.

## 24.2 COMPOSITION OF MILK FAT

Knowledge about the physical state of milk lipids is essential before proceeding to the detailed study of its composition. The bulk of the milk fat exists in the form of small globules with a average size of approximately 2 to 5µm . This is water in oil type of emulsion; the surface of these globules is covered by a adsorbed layer of material commonly known as fat globule membrane. Small quantities of milk fat also occur in the milk serum in combination with proteins.

## 24.3 COMPOSITION OF LIPIDS IN BOVINE MILK

The bulk of 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. The Diacylglycerols(DGs), monoacylglycerols (MGs) and free fatty acids are the products of lipolysis and are more in concentration as compared to the components which are present in traces. The lipid composition of the bovine milk fat is presented in Table 24.1

**Table-24.1: Lipid composition of bovine milk fat**

| LIPID | WEIGHT (%) |
|---|---|
| Triacylglycerols | 97-98 |
| Diacylglycerols | 0.28 – 0.59 |
| Monoacylglycerols | 0.016 – 0.038 |
| Free Sterols | 0.22 – 041 |
| Free Fatty acids | 0.10 – 0.44 |
| Phospholipids | 0.2 -1.0 |
| Hydrocarbons | Traces |
| Sterol esters | traces |

**(SOURCE: Patton and Jensen,** *Biomedical Aspects of Lactation,* **1976).**

## 24.4 CLASSIFICATION OF LIPIDS

**24.4.1Simple Lipids: The lipids which yields only fatty acids and glycerol upon hydrolysis.**

**a) Neutral fats:** Found in adipose tissue, butterfat, lard, fish oils, olive oil, cornoil, etc. These are esters of three molecules of fatty acids with one molecule of glycerol. This includes triacylglyerol, diacylglyerol and monoacylglyerol.

The structure of a triacylglyerol is shown in figure 24.1.



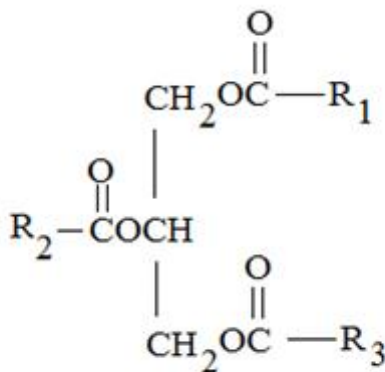**Fig.24.1 Structure of Triacylglycerol**

**b) Waxes:** Composed of esters of fatty acids with long chain monohydric alcohol. It has industrial and medicinal importance.

### 24.4.2. Compound Lipids

**24.4.2.1. Phospholipids(phosphatides):** Found chiefly in animal tissues. Substituted fats,consisting of phosphatidic acid; composed of glycerol, fatty acids, and phosphoric acid bound in ester linkage to a nitrogenous base.
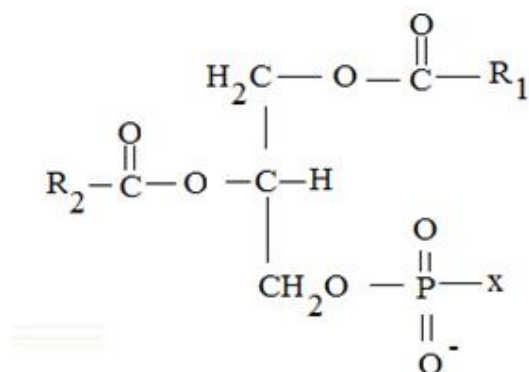


Fig.24.2: General structure of phospholipids

**Lecithin:** Found in brain, egg yolk, and organ meats. Phosphatidyl choline or serine; phosphatide linked to choline; a lipotropic agent; important in fat metabolism and transport; used as emulsifying agent in the food industry.
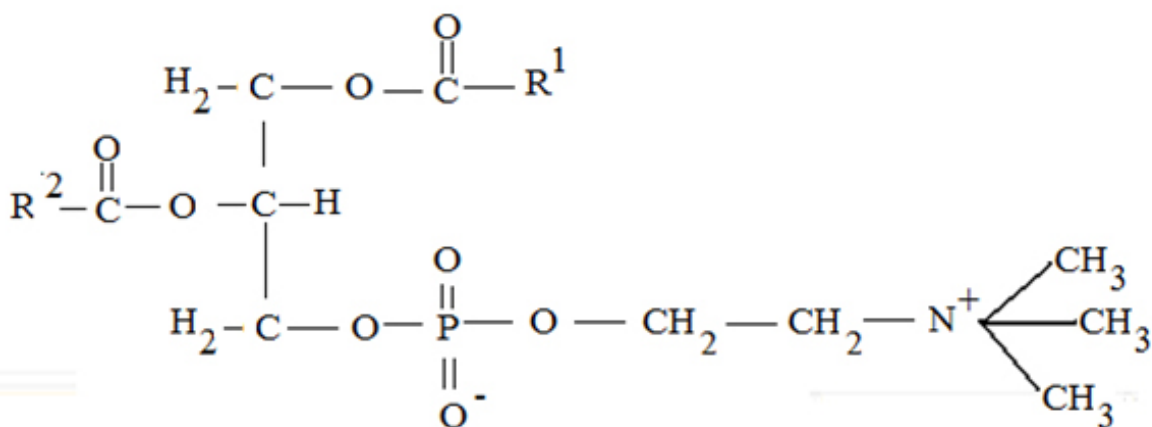


Fig.24.3: Structure of phosphatidylcholine (lecithin)

**Cephalin:** It occurs predominantly in nervous tissue. Phosphatidyl ethanolamine; Phosphatidyl serine where phosphatide linkage to ethanolamine or serine; plays a role in blood clotting.
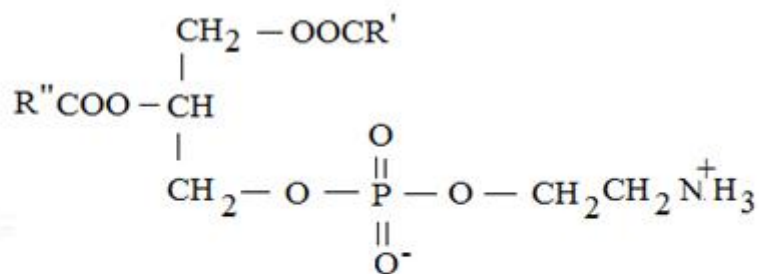
$$CH_2-OOCR'$$
$$R''COO-CH$$
$$CH_2-O-\overset{\overset{O}{||}}{\underset{\underset{O^-}{||}}{P}}-O-CH_2CH_2\overset{+}{N}H_3$$

Fig: 24.4 Structure of ethanolamine

**Plasmalogen:** Plasmalogen is a predominant component of the membrane phospholipids of many animal and microbial species. The vinyl ether bonds in position $sn$ -1 In animal tissues, the highest proportion of the plasmalogen form is usually in the phosphatidylethanolamine class with rather less in phosphatidylcholine, and commonly little or none is in other phospholipids such as phosphatidylinositol. In phosphatidylcholine of most tissues, a higher proportion is often of the $O$ -alkyl rather than the $O$ -alkenyl form. Found in brain, heart, and muscle

$$CH_2-O-CH{=}CH-R'$$
$$R''COO-CH$$
$$CH_2-O-\overset{\overset{O}{||}}{\underset{\underset{O^-}{||}}{P}}-O-CH_2CH_2\overset{+}{N}H_3$$
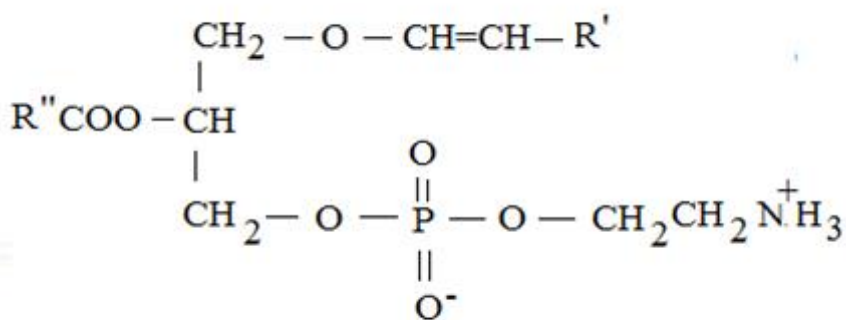
Fig: 24.5: Structure of Plasmalogen

**a) Lipositol:** Found in brain, heart, kidneys,and plant tissues together with phytic acid. Phosphatidyl inositol; phosphatide linked to inositol; rapid synthesis and degradation in brain; evidence for rolein cell transport processes.

**Inositol(Phosphatidylinositol)** : Inositol ( Phosphatidylinositol ) is a negatively charged phosphor lipid and a minor component in the cytosolic side of eukaryotic cell membrane. Inositol can be phosporylated to form phosphatidylinoistol phosphate, phosphatidylinoistolbisphosphate, phosphatidylinoistoltrisphosphate,all these are collectively

called phosphoinositides. phosphoinositides play important roles in lipid signaling, cell signaling and membrane trafficking.
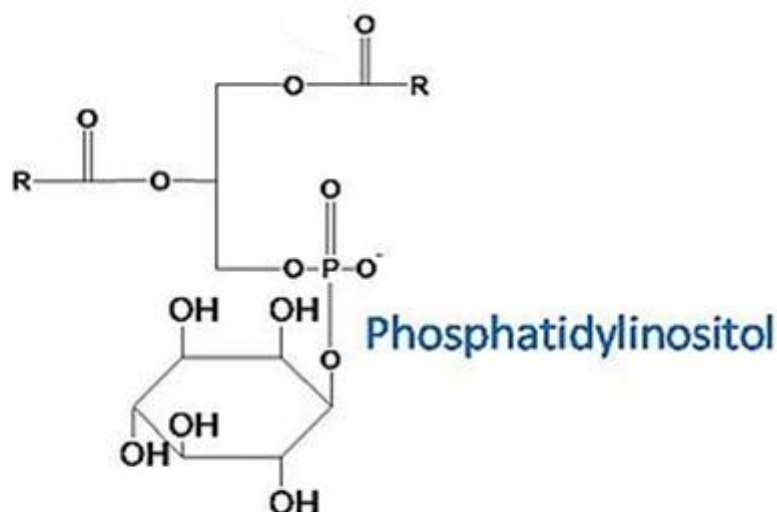
Fig: 24.6: Structure of Phosphatidylinositol

**24.4.2.6: Sphingomyelin:**Found in nervous tissue, brain, and red blood cells. Sphingosine-containingphosphatide; upon hydrolysis it yields fatty acids, choline, sphingosine,phosphoric acid, and no glycerol; source of phosphoric acid in body tissue.
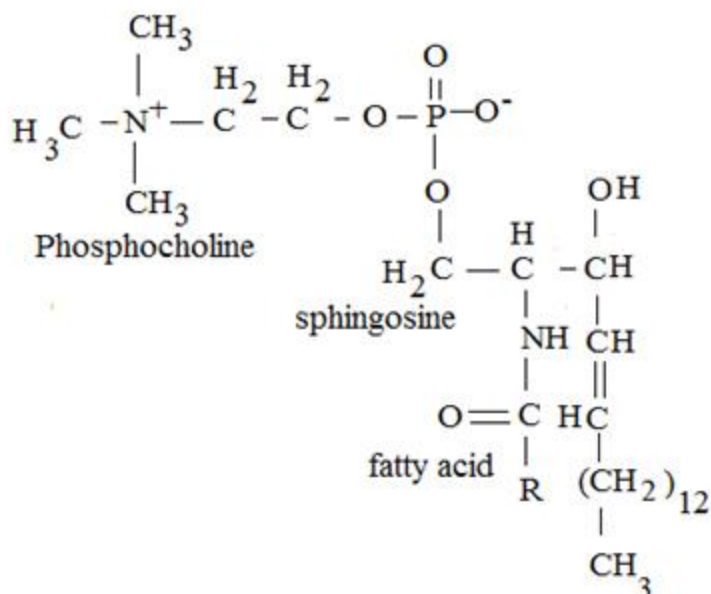
Fig. 24.7: Structure of Sphingomyelin

(Source: www. lipidlibrary.aocs.org)

## 24.4.2.7: Glycolipids:

Cerebroside:Cerebrosidesis a common name for a group of glycosphingolipds called monglycosylceramides which are an important component of muscle and nerve cell membranes. Theyconsists of a cerimide with a single sugar residue at the 1 – hydroxyl moiety.The sugar residue can be either glucose or galactose. Accordingly these are named as glucocerbrosides and alactocerebrosides. Galactocerebrosides are typically found in neural tissue while glucocerebrosides are found in other tissues Myelin sheaths of nerves, brain, and othertissues. Yields on hydrolysis of fatty acids, sphingosine, galactose (orglucose), but not fatty acids; includes kerasin and phrenosin or cerebronicacid
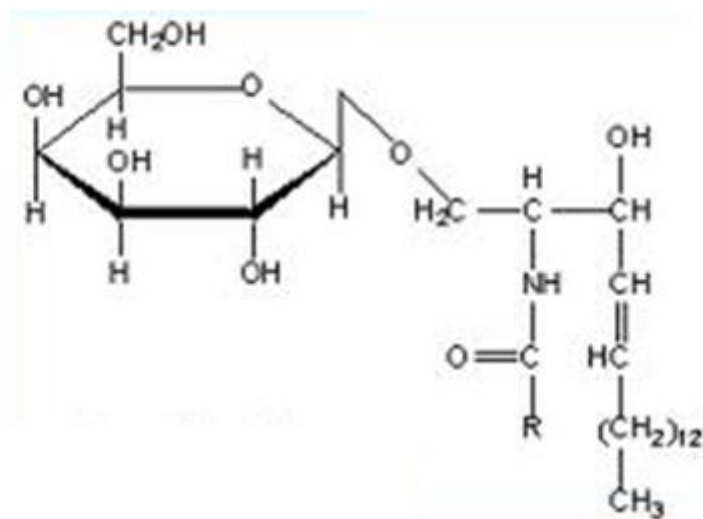


Fig.24.8: Structure of Cerebroside

Ø **Ganglioside:** Brain, nerve tissue, and other selected tissues, notably spleen; contains aceramide linked to hexose (glucose or galactose), neuraminic acid, sphingosine,and fatty acids.

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

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

## 24.4.3: Steroids

### a) Sterols

**Cholesterol:** found in egg yolk, dairy products, and animal tissues. It is a precursor of bile acids and Vitamin $D_3$ .
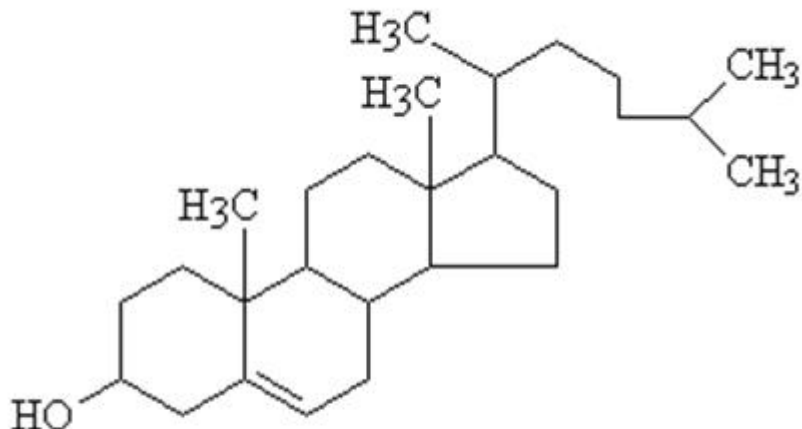
Fig. 24.9: Structure of Cholesterol

(Source: www. lipidlibrary.aocs.org)

### 24.4.4. Derived Lipids

**a) Fattyacids:** They occur in plant and animal foods; also exhibit in complex forms with other substances. Obtained from hydrolysis of fats; usually contains an even number of carbon atoms and are straight chain derivatives. Classification of fatty acids is based on the length of the carbon chain (short, medium, orlong); the number of double bonds (unsaturated, mono, or polyunsaturated); or essentiality in the diet (essential or non essential). A current designation is based on the position of the double bond, counting from the methyl ($-CH_3$)group, called the omega end. The most important omega fatty acids are: Omega-6 fatty acid and Omega-3 fatty acid, eicosapentaenoic, and docosahexaenoic acids.

### 24.5 DISTRIBUTION OF LIPIDS IN MILK

In milk different classes of lipids are distributed in various phases and the Table 24.2 will give us an idea about their range of occurrence and their location in milk. It must be remembered here that the lipids mentioned in this represent a group of compounds and they should not be considered as a single constituent.

**Table-24.2: Distribution of lipids in milk**

| Constituent | Range of occurrence (%) | Location in milk |
|---|---|---|
| Tri glycerides | 98-99 | Fat globule |
| Phospho lipids (Lecithin,cephalin,sphingomyelin) | 0.2 – 1.0 | Fat globule membrane and milk serum |
| Sterols (cholesterol,lanosterol) | 0.25 – 0.40 | Fat globule, Fat globule membrane and milk serum |
| Free Fatty Acids | Traces | Fat globule and milk serum |
| Waxes | Traces | Fat globule |
| Squalene | Traces | Fat globule |
| Fat soluble vitamins | Traces | Fat globule |
| Vitamin A | 7.0 -8.5 µg/g of fat | - |
| Carotenoids | 8.0 – 10.0 µg/g of fat | - |
| Vitamin E | 2-50 µg/g of fat | - |
| Vitamin D | Traces | - |
| Vitamin K | Traces | - |

**(Source: Principles of Dairy Chemistry Jenness and Patton, 1969)**

From the table 24.2 it is evident that milk lipids are found in three distinctly different phases of milk namely fatglobule, the membrane surrounding these globules and the milk serum.

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**Lesson-25**

**Fatty Acid Composition of Milk Lipids and Structure of Fat Globule**

## 25.1. INTRODUCTION

Milk and milk products occupy a significant position in the human diet which is primarily attributed to 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. As such a study of the fatty acid composition and factors which influence them helps us in getting the desired type of fatty acid composition of the milk fat to some extent, alternatively how we can modify the composition to get the desirable attribute to the products prepared from it.

## 25.2. FATTY ACID PROFILE OF MILK FAT

Bovine 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. Nearly 50 of the total 80 fatty acids which are commonly encountered in the milk fat would make only 1% of the total fatty 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.

Polyunsaturated fatty acids are further classified as either *cis* or *trans* (geometric isomerism) or conjugated or non conjugated positional isomerism. Depending upon the number of carbon atoms present in the fat can also be grouped as even or odd numbered fatty acids. A convenient method of notation of fatty acids two numbers designating the carbon chain length and the unsaturation (Number of double bonds) Thus $C_{4:0}$ denotes butyric acid which has 4 carbon atom and is saturated without any double bond. Similarly $C_{18:1}$ or C18-2 denotes oleic acid or linoleic acid having a carbon 18 with 1 and 2 double bonds respectively. The chain length of fatty acids in milk fat varies from $C_4$ to C26. Normally the fatty acids percentage is not expressed on the weight basis but on molar percentage.

**Table- 25.1: Fatty acid profile of bovine milk fat**

| S.No | Fatty acid | Notation | Mole per cent | |
|---|---|---|---|---|
| | | | cow | Buffalo |
| 1 | Butyric acid | 4:0 | 8.8 | 11.4 |
| 2 | Caproic | 6:0 | 3.5 | 3.1 |
| 3 | Caprylic | 8:0 | 2.0 | 1.0 |
| 4 | Capric | 10:0 | 3.0 | 1.6 |
| 5 | Lauric | 12:0 | 3.8 | 2.6 |
| 6 | Myristic | 14:0 | 9.9 | 10.6 |
| 7 | Palmitic | 16:0 | 26.1 | 30.3 |
| 8 | Stearic | 18:0 | 9.1 | 10.5 |
| 9 | Long chain saturated fatty acids | 20:0 to 26:0 | 1.0 | 0.7 |
| 10 | Monounsaturated | 10:1 to 14:1 | 1.8 | 1.0 |
| 11 | Oleic acid | 16:1 | 3.6 | 3.6 |

(Source: Text book of Dairy Chemistry, Mathur *et al.*, 2005)

## 25.3. FACTORS AFFECTING FATTY ACID COMPOSITION

The composition of milk fat is not constant and is very variable. The important cause for variation is feed and both lipid and non lipid components affect milk fat composition. Consequently it will vary with the year region farming practice and so on. Therefore it makes little sense to give information on the variations unless we give detailed account of the effect of several factors. Since the fatty acids which are preformed from food 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. Some of the fatty acids are being synthesized by the gland from the acetate and β hydroxyl butyrate produce by rumen bacteria.

The fatty acids synthesized by utilizing the fatty acids producing rumen bacteria will have short or medium chain length $C_4$ to $C_{14}$ and part of the $C_{16}$. Acetate contribute to the increments to all of the $C_4$ to $C_{14}$ acids and β hydroxyl butyrate is used primarily for the initial four carbon "primer" units of the most fatty acids synthesized. Low roughage diets diminish acetate and increase propionate production in the rumen. 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. To a considerable extent the rumen microorganism hydrogenates the dietary fatty acids. Feeding highly unsaturated oil such as safflower oil increases the C18:2 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), all had similar variations; the

values increased during the first 4 to 8 weeks of lactation, remained relatively constant until the fifth or sixth month, and then decreased again until the end of lactation. There was little variation in 16:0 throughout lactation. Stearic (18:0) and oleic (18:l) acid contents were high in early lactation, then declining throughout the mid lactation and again increasing at the end of lactation.

## 25.4 STRUCTURE 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 1-8 µm diameter. The size distribution found may depend greatly on the measurement method employed. Globule size can considerably altered by various treatments particularly homogenization. The fat globules of milk differ in composition. The size of the fat globule will also alter the composition. This is because the quantity of membrane lipids (predominantly phospholipids) per unit mass of fat is higher for smaller globules. The fat globules are enveloped by a layer called the fat globule membrane.

The membrane consists of a well organized sequence of different components arranged according to their polarity and hydrophobicity. This layer is predominantly proteinaceous in nature.

The composition of the MFGM often varies with the method of isolation or preparation. Protein constitutes nearly 25 to 60 per cent of the total dry weight. 25 different enzymes have been reported and the isolated 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.

Lipids are the next major constituent of the MFGM and their content could vary from 0.5 to 1.2 mg per gram of protein. The lipid portion comprises of neutral lipids (50 to 80%of total lipid) or phospholipids. Due to their amphiphilic nature, the phospholipids have an important role in maintaining the stability of fatemulsion. Glycerides constitute a major portion of the neutral lipids of the membrane. Other constituents like cerebrosides, gangliosides sialic acid,cytochrome and hexoses have been reported.

The constituents of the MFGM are specifically oriented on the fat surface. The inner most layer consists of the high melting triglycerides in contact with the fat layer. The next layer consists of the phospholipids with their hydrophobic portion oriented towards inwards while the hydrophilic portion oriented outwards. The phospholipids are interspaced with cholesterol, vitamin A etc. The outermost layer is made up of protein enzymes etc absorbed on to the surface. These proteins are in contact with plasma and are even capable of binding water to a some extent. The membrane constituents are very thinly spread over the surface of the fat globule and stabilize the fat in water emulsion. The breaking or disruption of the membrane also makes the lipids more susceptible to enzymatic action due to greater accessibility.

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**Lesson-26**

**Properties of Milk Lipids**

### 26.1 INTRODUCTION

In bovine milk fat the triacylglycerols account for about 98% of the total milk lipids. The diacylglycerols, monoacylglycerol and free fatty acids (FFA) are mostly products of lipolysis, and the cholesterol and phospholipids are cellular membrane material which accompanies the fat globule during extrusion from the secreting cell.

### 26.2 PROPERTIES OF LIPIDS

The detailed distribution of the lipid material associated with the fat globule is given here under. The properties of the individual lipids will be discussed here under. During the discussion of the lipid properties the short connotation of fatty acids have been used. Accordingly, for a fatty acid i.e., 18:0, stearic acid; l8:1, oleic acid;etc. The first figure is the number of carbons, the second the number of double bonds.

The composition of lipids in milk is presented in Table 26.1.

**Table-26.1: Composition of Bovine Milk Lipids**

| Lipid | Weight (%) |
|---|---|
| Hydrocarbons | Trace |
| Sterol esters | Trace |
| Triacylglycerols | 97 -98 |
| Diacylglycerols | 0.28 - 0.59 |
| Monoacylglycerols | 0.016 - 0.038 |
| Free fatty acids | 0.10 - 0.44 |
| Free sterols | 0.22 -0.41 |
| Phospholipids | 0.2 - 1.0 |

**(Source: Patton and Jensen, Biomedical Aspects Of Lacttion, 1976)**

# Chemistry of Milk

## 26.3 TRIGLYCERIDES

Triglycerides are the major lipid class accounting for 97 to 98% of the total lipids in many species. Triglycerides can be defined as esters of glycerol and three moles of fatty acids. These triglycerides can be hydrolysed under suitable conditions to yield component fatty acids and glycerol .It is observed that distribution of the fatty acids or groups of residues over the three positions is not random. Particularly the preference of $C_4:0$ and $C_6:0$ for the 3 position is not random. Position of the fatty acid residuesin the triglyceride molecules affects physical properties particularly crystallization and it determines which fatty acids are predominantly liberated by enzymatic hydrolysis. However the position of these fatty acids has least effect on the chemical reactivity. Triglycerides are not very reactive at room temperature. At higher temperature oxidative attack of the double bonds may occur. The Keto and hydroxyl groups may react.

The triglycerides are very apolar and not surface active. They act as a solvent for many other apolar substances. A small amount of water dissolves in liquid triglycerides (about 0.15% at room temperature in liquid milk fat) but triglycerides do not dissolve in aqueous media at all. They may be a part of some lipoproteins. A kind of mixed micelles largely consisting of protein and compound lipids and thus be present in trace quantities in plasma.

The triglyceride composition in several milk products will be affected as in the case of butter milk which is obtained by churning of cream have a lower melting triglyceride than those of the whole milk fat which is reflected in a higher degree of unsaturated fatty acid residues. The fat can be fractioned by partial crystallization and separation resulting in obtaining fractions with different properties.

**26.3.1 Milk fat constants:** 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. These constants would also enable to detect the adulteration qualitatively and some instances quantitatively.

**26.3.1.1: Refractive index:** This property of milk fat is based on the principle that the degree to which the light waves pass through the liquid fat, which will be the characteristic feature of that milk fat. This physical constant for milk fat is determined by using the Abbey refractometer. The reading is usually obtained at $40^0C$. This instrument directly gives the refractive index. Butyro refractometer is also useful for determining the refractive index. The reading obtained with this instrument has to be converted in to refractive index or to be used as butyro refractometer reading (B.R. Reading). The reading for the milk fat ranges between 1.4527 and 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. There fractive indeed of a fat is influenced by the both the molecular weight and the degree of unsaturation of the component fatty acids.

**26.3.1.2:Saponification Number:** The saponification number is equivalent to the number of milligrams of potassiumhydroxide required to saponify one gram of fat. This value may range from 210 to 233 for milk fat. This value of a lipid indicates the average molecular weight of fatty acids present in it. The average molecular weight of a triacylglycerol is equals 168,300 / SV. The formula useful for calculating the average molecular weight is:

SV =56,100(45+14 x )

where x average number of C atoms per fatty acid residue

This value for milk fat is much higher for most other fats oils with the exception of coconut and palm kernel oil. Saponification value is more useful in detecting the mineral values such as paraffin in ghee as the are not acted upon by alkali and such a sample does not form a homogenous solution upon saponification.

**26.3.1.3: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 chlore (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 to 42

**26.3.1.4:Reichert-Meissal Number:** It is the number of milliliters of 0.1N sodiumhydroxide or aqueous alkali solution required to neutralize the steam volatile water soluble fatty acids distilled from 5g of fat under the precise conditions specified in the method. This value is quite significant for milk fat, 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. R.M for milk fat ranges between 18 to 30

**26.3.1.5:Polenske number:** It is the number of milliliters of 0.1N sodium hydroxide or aqueousalkali solution required to neutralize the steam volatile water insoluble fatty acids distilled from 5g of fat under the precise conditions specified in the method. 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. This value for milk fat ranges between 1.0 to 3.3

**26.3.1.6: Kirschner value:** It is the number of milliliters of 0.1 N aqueous alkali solution required to neutralize steam volatile water soluble fatty acids distilled from 5g of fat which forms water soluble silver salts distilled from 5g fat under specified conditions

**26.3.1.7: Melting point:** Triacyl glycerol molecule of a milk Fats are mixture of mixed fatty acids forming. 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 following methods are used for the determination of melting point of milk fat:

1. Mettler dropping point method

2. Falling ball method (softening point)

3. Open capillary tube method

4. Slip point method

The research work conducted by De Man *et.al.*(1983) the Mettle dropping method and softening point methods give reproducible results where as slip point method the results are poor. The average softening point for milk fat milk fats were 30.4$^{0}$C for soft butter and 38.4$^{0}$C for hard butter(Parodi,1973).

**26.3.2: Crystallization Behavior:** Milk fat is liquid above $40^0$C and completely solid below

$-40^0$. Between these extremes milk fat is a mixture of crystals and oil, where oil is the continous phase. Milk fat contains a large number of triacylglyerols, making the process of crystallization complex. The properties of milk fat are the average of the properties of its triacylglycerols. Crystals in fat globules generally cannot grow larger than the globule diameter.Most crystals are much smaller than globule size and they flocculate into a network, giving the globules rigidity. Crystallization starts with the formation of crystal nuclei in the molten fat as few molecules gather in molecular aggregates where the potential energy is reduced to a minimum.

**26.4.LIPOLYSIS AND RANCIDITY OF MILK FAT**

Market milk and some products manufactured from milk may have a flavor which is described as "rancid". Development of this flvour 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.

**26.4.1. Hydrolytic rancidity:** The flavor defect commonly referred as "rancidity" or more specifically "hydrolytic rancidity" is caused primarily by the presence in milk of a single enzyme "milk lipoproteinlipase". Presence of this enzyme in milk is ascribed to leakage from blood through mammary tissues rather than true secretion. Although, most of the milks contain sufficient amounts to cause rancidity, in practice it will not occur because the substrate (triacyl glycerol) and the enzyme are well partitioned.In addition to this there are several factors which affect the enzyme activity. Lipolysis takes place at an oil water interface. The factors which influence enzyme activity are, surface area available , 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.

**26.4.2. Autoxidation:**Lipid oxidation in fluid milk and number of its 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. 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 tend to influence. These factors would also influence composition and percentage of products formed due to autoxidation.

**26.4.2.1.Mechanism:** Chemical reactions involved autoxidation of milk fat are grouped in to three categories. *viz.* intiation, propogation and termination. The initial step in the autoxidation of unsaturated fatt acid and their ester is the formation of free radical. In the case of monounsaturated and non conjugated polyene fatty acids the reaction is initiated by the removal of hydrogen atom from the methylene group of adjacent to the double bond. The resulting free radical stabilized by resonance adds oxygen to form peroxide containing free radicals these in turn react with another mole of unsaturated compound to produce two isomerichydroperoxides in addition to free radicals capable of continuing the chain reaction.

Oleic acid, having two methylene groups, gives rise to four isomeric hydroperoxides. The preferential points of attack in a polyenenon conjugated systems are the methylene groups located between the double bonds. Autoxidation of linoleic and linolenic acids can lead to the formationof three and six isomeric hydroperoxides respectively by reacting with $C_{11}$methylene group linoleic acid and $C_{11}$ and $C_{14}$methylene groups of linolenic acids. In a polyunsaturated fatty acid methylenegroups located other than those located between the double bonds can alsoinvolve in these reactions but to a lesser degree. In addition to formation ofpolyperoxides, carbon to carbon polymerization, and the formation of epoxides andcyclic peroxides are also possible in these reactions.

Hydroperoxides formed due to autoxidation beingunstable they readily decompose forming the saturated and unsaturatedaldehydes. The mechanism suggested forthis is that it involves in cleavage of the isomeric hydroperoxide to thealkoxyl radical which undergoes carbon- to-carbon fission to form aldehyde. Formation of other productssuch as unsaturated ketones saturated and unsaturated alcohols saturated andunsaturated hydrocarbons and semialdehydes are also being observed. Saturatedand unsaturated Aldehydes impart characteristic off-flavours in the products.The terms often used to characterize the flavor are "painty", nutty, melon-like, grassy,tallow,oily, card board, fishy, cucumber etc.,

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**Lesson-27**

**General Properties of Compound Lipids**

## 27.1 INTRODUCTION

Although the compound lipids are less in their concentration but they play a very important role in the stabilization of the fat globule being an important constituent of the fat globule membrane.

## 27.2 DEFINITION

The lipids which on hydrolysis yield phosphates, carbohydrates and nitrogenous compounds like sphingosine, ethanolamine, serine, carbohydrates, choline, etc. in addition to the glycerol and fatty acids are known as compound lipids

## 27.3 PHOSPHOLIPIDS

The phospholipids comprise approximately 1% of the total lipid in bovine milk. Although phospholipids are quantitatively less but they play a very important role in the formation and secretion of the milk fat apart from forming a stable colloidal suspension or emulsions in aqueous solution. These lipids play an important role during storage and processing of milk due to their susceptibility to oxidation of the polyunsaturated fatty acids Their bipolar molecules and the relatively high concentration of unsaturated fatty acids

Phospholipids are also found as lipoprotein complexes in skim milk. The skim milk phase may containing 30-50% of the phospholipids in milk. The phospholipids in milk are synthesized by the mammary cells viapathways that are common to other mammalian cells. The major glycerophospholipids are phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol.

The composition of the phospholipids in milk is presented in the Table 27.1. In milk the glycerophospholipids are found predominantly in the diacyl form. However small amounts of plasmalogen which are the vinylether form of glycerophospholipid are also being reported. The studies carried out by some research workers revealed that 4.0% of the phosphatidylethanolamine was in the ether form and 1.3% of phosphatidylcholine was in this form. It was also observed that 1.3 to 2.5% of bovine phospholipids are plasmalogens. Some compounds that are being found as phospholipids in milk lipids which are suspected to be the degradation products or remnants of biosynthesis of lipid material in the mammary glands. Phosphotidic acid is a product formed by the action of phospholipase D on the phosphatidyl choline. The quantity of this product in milk is very low.

**Table-27.1: Phospholipid Composition of Bovine Milk**

| 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 |

(Source: Fundamentals of Dairy chemistry, Wong *et al.*,1988)

The most abundant and best-known compound lipids in milk are the phospholipids or phosphatides, of which the phosphoglycerides makes up the major portion.phospholipids or phosphatides are lipids which contain phosphorus as esterified phosphoric acid.

In other phosphoglycerids, the choline residue is replaced by ethanolamine (-CH2-CH2-NH$_3^+$),serine, or inositol (a cyclitol).

The lipid becomes highly polar due to the two charged groups in the molecule where as the rest is very apolar, so that the molecule is amphiphilic and does not dissolve well in either water or fat. The phospholipids, however, form micelles in water and in fat, with the polar ends at the outside and inside, respectively. They are difficult to transform from the hydrated into the other form, and vice versa. Their amphiphilic nature makes the phospholipids very surface active. In milk, they are in contact with water and with proteins as "lipo-proteins."Little is known about the nature of the bonds in such complexes. The phospholipids, particularly the cephalins, have many unsaturated fatty acid residues compared to the neutral glycerides. They include polyenoic fatty acids. This is important for lipid autoxidation.

**27.4 SPHINGOLIPIDS**

In addition to phosphoglyceride, milk contains sphingolipids. Their common residue is sphingosine. In all of them, a fatty acid residue is bound to the.-NH group; on average these lipids contain very long-chain fatty acids. In sphingomyelin, aphosphocholine group (as in lecithin) is bound to the terminal oxygen; it is thus a phospholipid.

## 27.5 CEREBROSIDES

Glucosyl and lactosyl (cerebrosides) compounds have been isolated from milk by some research workers. They found 1.7 mg / 100ml in the globule membrane and 0.8 mg / 10 ml in skim milk. The membrane bound cerebrosides contained mainly acids of 20 to 25 carbons and those in skim milk contained 18 carbon or less In the cerebrosides or glycosyl ceramides a sugar group is bound. This is mostly glucose, and in milk lipids also lactose. The gangliosides are only a minor fraction of milk fat; they have several ketoses,including N-acetylneuraminic acid bound to the terminal oxygen. The properties of the sphingolipids are comparable to those of the phosphoglycerides. The entire compound lipids are found mostly in the same places. The distribution between fat globule membrane and milk plasma (presumably in lipoprotein particles) is for all classes similar, but not exactly equal. Compound lipid are absent in the core of milk fat globule.

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Lesson-28

**Unsaponifiable Lipids : Cholesterol and Its Properties**

## 28.1 INTRODUCTION

The lipid compounds which are not saponifiable in the milk fat are considered under this fraction. The compounds in this fraction have unique properties hence the properties of these compounds needs a special mention.

## 28.2 UNSAPONIFIABLE MATTER

When the milk fat is being saponified with an alcoholic base and ether extract obtained is known as unsaponifiable matter unsaponifiable lipids. The actual procedure for the saponification is as follows:

When a weighed amount of fat in an excess of approximately 0.5N alcoholic base for 1 hour would result in the decomposition of triglycerides and other ester materials decompose to fatty acid, salt, 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 unsapoinifiable matter which for milk fat generally falls within the range of 0.30 to 0.45 % by weight. As the above procedure involves heating for one hour in an alcoholicbase , there is possibility of having some artifacts during the process along with the saponifiable matter.

Shifting of double bonds of some of the unsaturated fatty acids is likely to occur during this process. The structure and properties of the components of the unsaponifiable matter is taken into consideration. These constituents are usually measured direct under mild conditions than those involved in the isolation of the unsaponifiable matter.

The principal known compounds are cholesterol and related sterols, the fat soluble vitamins A,D,E and K, traces of squalene, unidentified waxes, and homologus series of n-alkylmethyl ketones containing odd number of carbons $C_3$ to $C_{15}$

## 28.3 STEROLS

These compounds are found in the unsaponifiable fraction of milk lipids consists mostly of cholesterol with some lanosterol. Research workers who have come with dry saponifiaction have reported that the content of sterols were 0.33 to 0.36% of butter oil. Further investigations in this field have revealed the presence of two new constituents named dihydrolanosterol and β sitosterol. Although researchers were able to identify 17 sterols from bovine milk as trimethylsilylethers. Among these sterols five were identified and reported

their content as a percent of the total sterol.

Table-28.1: 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 | $\Delta^5$Avenasterol | 0.03 |

These analyses were done by GLC mass spectrometry. In the previous years the reliable data on cholesterol content was not available but with the advancement of various analytical techniques it is now made available by the research work carried out by several researchers in the last tow to three decades. The amount of cholesterol in whole milk fat was 13.49 ± 1.01mg per 100 g of milk which contained 3.47± 0.74 g of fat

**28.3.1:Cholesterol** : The principal sterol of milk is cholesterol ($C_{27}H_{45}OH$),the formula for cholesterol is presented in Figure28.1
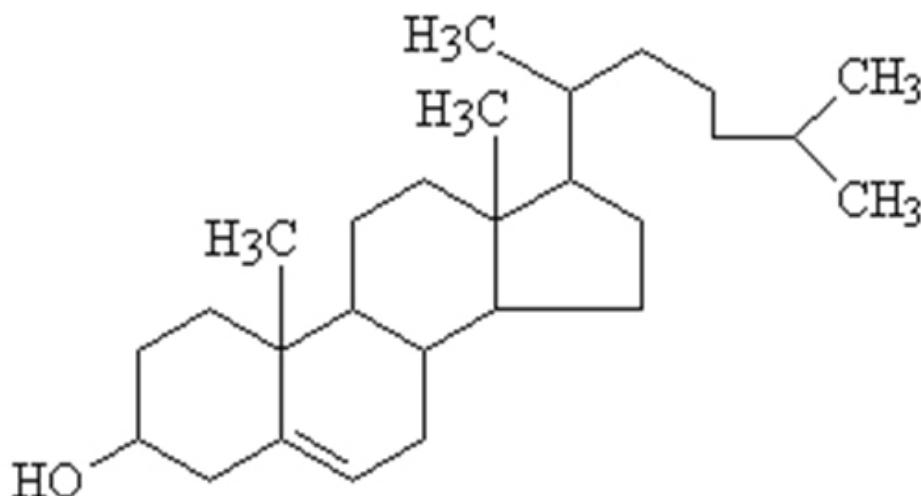
Fig.28.1: Structure of Cholesterol

The cholesterol content of milk ranges from approximately 0.25 to 0.4% by weight of the fat. Since the total unsponifiable 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.

Many of the methods used for the analysis of cholesterol, in milk are not specific for this sterol alone. 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 is, in fact, saponifiable. There are also conflicting results on the other, minor, sterols in milk.

**28.3.1.1. 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

**28.3.1.2. 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.

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

**Lesson-29**

**Vitamins in Milk**

## 29.1 DEFINITION

Vitamins may be defined as carbon compounds of diverse structures, which are not used for energy or fixed into the tissue frame work but which are required in minute amounts for the normal functioning of the living organisms. Vitamins are also recognized as nutrients for animals obtained from different foods etc. These can not be synthesized in the body. Vitamins are also synthesized by several other living organisms such as microorganisms like yeast and molds. Sun light also helps in the synthesis of vitamins like Vitamin D. Since milk being considered to be nearly complete food it is consumed by human beings of all ages.

## 29.2 VITAMINS IN MILK

Milk contains various fat soluble vitamins like vitamin A, D, E, K, along with water soluble vitamins like vitamin C and B complex vitamins and provitamins. However, concentration of some of these vitamins is very less. The concentration of the vitamins in the milk is shown in Table.29.1

**Table-29.1: Concentration of the vitamins in milk**

| Vitamins | Class | Concentration (per litre) |
|---|---|---|
| Vitamin A | Fat soluble | 1590 I.U.* |
| Vitamin D | Fat soluble | 22.1 I.U. |
| Vitamin E | Fat soluble | 1.0 mg |
| Vitamin K | Fat soluble | 0.04 mg |
| Vitamin $B_1$ | Water Soluble | 0.4 mg |
| Vitamin $B_{12}$ | Water Soluble | 1.5 mg |
| Pantothenic acid | Water Soluble | 3.0 mg |
| Biotin | Water Soluble | 50 µg |
| Niacin | Water Soluble | 0.2-1.2 mg |
| Pyridoxine $B_6$ | Water Soluble | 0.7 µg |
| Folic acid | Water Soluble | 1.0 µg |
| $B_{12}$ | Water Soluble | 7.0 µg |
| Vitamin C | Water Soluble | 20 mg |
| Inositol | Water Soluble | 180 mg |
| Choline | Water Soluble | 150 mg |

**\*** Aretinol equivalent (RE) is equal to 3.33 IU retinol or 10 IU β-carotene
**(Source: Text book of Dairy Chemistry by Mathur/et.al., 2005)**

The abbreviation IU stands for the international units which is the measurement for some vitamins. It is the same idea as milligrams, though theyare not equivalent. These units exist in order to account for the fact that certain preparation of the same vitamin is not bioequivalent. Regardless of the preparation 1 IU will provide the same biologica activity.Eg. 1 IU of vitamin E equals the biological equivalent of about 0.667 mg d-alpha-tocaopherol or 1 mg of dialpha to copherol acetate. Though the mass of each preparation is different, the biological activity is the same.

## 29.3 CLASSES OF VITAMINS

On the basis of their solubility vitamins are distinguished into two:

1. Class A: Fat soluble vitamins A, D, E and K,

2. Class B: Water soluble vitamins, the B-complex and vitamin C (Ascorbic acid).

The B-complex vitamins includes thiamine,riboflavin, niacin, pantothenic acid, pyridoxine biotin, choline, inositol, folic acid, p-aminobenzoic acid. $B_{12}$ All the above vitamins are present in milk, however, vitamin A and $B_2$ group vitamins are present in appreciable amounts

**29.4 VITAMIN-A (Retinol)** It is a primary alcohol derivative of a polymer consisting of four isoprene units. It is formed by the cleavage of provitamin A carotenoids, polymers of eight isoprenes, of these βcarotene has the highest vitamin A potency because it consists of identical halves and thus yields two retinol molecules, while other carotenoids yield only one. Plants synthesize carotenoids but not retinol, animals cannot synthesize carotenoids but can split them to retinol (vitamin A). Vitamin A levels are frequently expressed in International Units (I.U.), although this unit is officially no longer accepted. One I.U. equals 0.344μg of crystalline vitamin A acetate, or 0.300 μg vitamin A alcohol; or 0.600 μg β-carotene. Current usage is to express vitaminA potency as retinol equivalents (RE)

1 RE = 1 μg Retinol

= 6 μg β carotene

=12 μg other provitamin A carotenoids
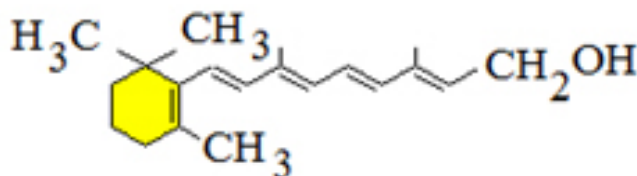
= 3.33 IU from retinol

= 10.00 IU from β carotene



**Fig.29.1: Structure of Retinol**

## Chemistry of Milk

Vitamin A is present in cow's milk as retinol, retinol ester and carotene. The content depends strongly on the amount of carotenoids in the feed. The ratio of retinol to carotene in milk varies with breed and species of animals. Vitamin A is relatively stable to heat in the absence of oxygen, because of the highly unsaturated character of the molecule, It is quite susceptible to oxidation,especially in the presence of sunlight or artificial light.

VitaminA is unstable in the presence of mineral acids but stable in alkali Vitamin A and the carotenoids have good stability during various food processing operations. Losses may occur at high temperatures in the presence of oxygen. These compounds are also susceptible to oxidation by lipid peroxides, and conditions favouring lipid oxidation will also result in vitamin A breakdown. Pasteurization of milk does not result in vitamin A loss but exposure to light will result in loss. It is essential,therefore, that sterilized milk be packed in light-impervious containers. Possible losses during storage of foods are more affected by duration of storage than by storage temperature. There are several forms of provitamin A,belonging to the carotenoid pigments. The most important one is β-carotene and some of the pigments that can be derived from it are of practical importance. These are β-apo-8' carotenal and β-apo-8'-carotenoic acidethyl ester. For example, Apo carotenal (R = CHO) and apo-carotenoic acidester (R=COOC$_2$H$_5$). Other provitamins are α-and γ-carotene s and cryproxanthin .
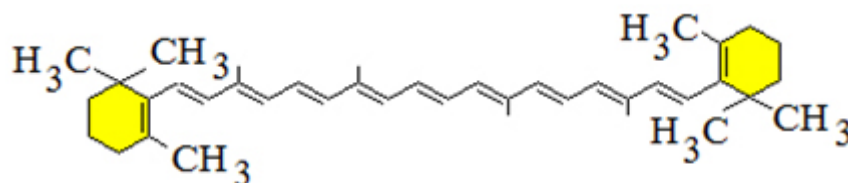
**Fig.29.2: Structure of β-Carotene**

It has been found that vitamin A added to milk, is more easily destroyed by light than the native vitamin A. This is not because natural and synthetic vitamin A is different, but because these two types of vitamin A are dispersed differently in the milk. The form in which vitamin A is added to food products may influence its stability. Vitamin A in beadlets form is more stable than that added as a solution in oil. The beadlets are stabilized by a protective coating. If this coating is damaged by wetting with water, the stability of the vitamin is greatly reduced.

**29.4.1 Importance** : Vitamin A is important in the diet as it is necessary for growth, health and reproduction. It keeps the epithelial tissues healthy and thus aids in preventing infections. Lack of this vitamin will cause night blindness. Vitamin A aids in maintaining normal glandular functions such as maintenance of adrenal cortex and steroid hormones synthesis. It acts to initiate vision from light energy. Vitamin A is present as carotene in plant and must be converted to vitamin A for its function. Vitamin A is a growth factor. It has an important role in the vision of mammalian organisms.

**Table- 29.2: Vitamin A and carotene content of some foods**

| Product | Vitamin A (I.U./100 g) | Carotene (mg/100g) |
|---|---|---|
| Beef (grilled sirloin) | 37 | 0.04 |
| Butter | 2363-3452 | 0.43-0.77 |
| Cheddar cheese | 553-1078 | 0.07-0.71 |
| Eggs (boiled) | 165-488 | 0.01-0.15 |
| Herring (canned) | 178 | 0.07 |
| Milk | 110-307 | 0.01-0.06 |
| Tomato (canned) | 0 | 0.5 |
| Peach | 0 | 0.34 |
| Cabbage | 0 | 0.3 |
| Broccoli (boiled) | 0 | 2.5 |
| Spinach (boiled) | 0 | 6.0 |

**(Source: Text book of Dairy Chemistry, Mathur *et al.*, 2005)**

## 29.5 VITAMIN D

Vitamin D includes a number of compounds with antirachitic activity. Ergocalciferol ($D_2$)occurs in plant material and is formed by UV irradiation of ergosterol. Cholecalciferol ($D_3$) is formed by UV irradiation of7-dehydrocholesterol in animals particularly in the skin. Both forms of this vitamin occur in milk, $D_2$ coming from the feed and $D_3$ from their radiated skin of the cow. The recent research shows, that the active form of vitamin D is identified as 1,25-dihydroxycholicalciferol which triggers the synthesis of calcium binding proteins for the calcification of bones. Thus this vitamin prevents disease called rickets in children and osteomalacia in pregnant women and osteoporesis in old. The potency is sometimes expressed as international units where, 1 IU is 0.25ng of cholecalciferol. Average value of this vitamin in bovine milk is 13.0 and 31.2, I.U. for winter and summer milk respectively.
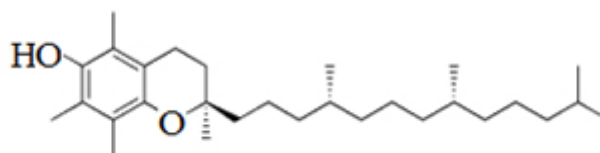


**Fig. 29.3 Structure of Vitamin E**

## Chemistry of Milk

The potency of thevitamin D in milk can be increased by

Ø Exposure of cow to sunlight

Ø Feeding higher levels of irradiated plantmaterial

Ø Irradiating milk

Ø Fortification in milk with vitamin D.

Itis stable in milk, not affected by pasteurization, boiling or sterilization.Storage of fluid milk for 30 months at -17.8°C, irradiated evaporated milk for2 to 3 years or of frozen butter for over 2 years resulted in little or notloss of the vitamin.

**29.5.1 Importance:** Vitamin D is necessary to aid in calciumand phosphorus retention so as to make strong bones and prevent rickets. It increases tubular preabsorption, and citrate blood levels. It maintains and activates alkaline phosphatase in bones and serum calcium and phosphorus level. Vitamin D is sometimes called the sunshine vitamin because the ultraviolet radiation of the sun can convert an active precursor of the skin(7-dehydrocholesterol) to vitamin D. Children need vitamin D for their growth and to a lesser extent mothers require this vitamin during pregnancy and lactation.

## 29.6 VITAMIN E

Vitamin E consists of a group of to copherols,the most potent of these and the principal one in milk is α-tocopherol $C_{29}H_{50}O_2$ this is strong reductant and serves as an antioxidant protecting lipids from oxidation. The vitamin E content is rather low. It depends to some extent on feed. Summer milk has higher concentration than winter milk. It is stable to heat but may be partly destroyed by intensive illumination in the presence of $O_2$ the vitamin E content of human milk is about ten times that of cow milk.
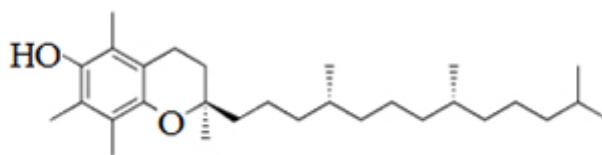


**Fig. 29.3 Structure of Vitamin E**

**29.6.1 Importance:** It is astrong antioxidant, prevents oxidation of unsaturated fatty acids and vitamin A in intestinal tract and body tissues. It acts in oxidation reduction reactions, plays role in human nutrition and is associated with reproductive factor, necessary for nutrition of muscles and better utilization of vitamin A. It maintains integrity of vascular system and central nervous system. Vitamin E is a detoxifying agent and maintains kidney, tubules, lungs, genital structure,liver and red blood cell membranes.

## 29.7 VITAMIN K

Vitamin K is present only in traces in milk if at all. Human needs for this vitamin are supplied from consumption of plant materials containing it and by microbial synthesis in the digestive track .

$$
\begin{array}{l}
\text{CH}_2 - \text{OOCR}' \\
\quad | \\
\text{R}''\text{COO} - \text{CH} \\
\quad | \qquad\qquad\quad \text{O} \\
\quad\qquad\qquad\qquad || \\
\text{CH}_2 - \text{O} - \overset{\text{O}}{\underset{\text{O}^-}{\overset{||}{\underset{||}{\text{P}}}}} - \text{O} - \text{CH}_2\text{CH}_2 \overset{+}{\text{N}}\text{H}_3
\end{array}
$$
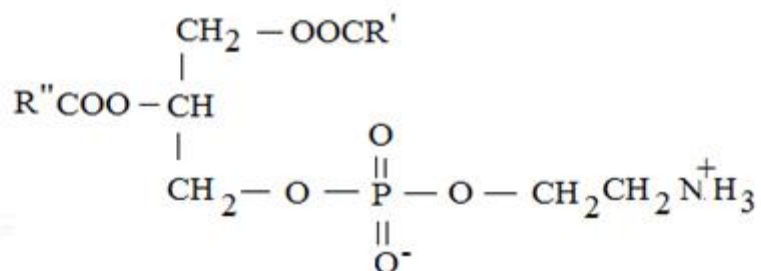
Fig: 24.4 Structure of ethanolamine

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**Lesson-30**

**Salts in Milk**

**30.1 INTRODUCTION**

The salts of the milk include those constituents that are present as ions or in equilibrium with ions, except hydrogen and hydroxyl ions. Salts are important from nutritional point of view. They also largely determine the physicochemical state of the casein micelles, which in turn influences heat stability of the milk. Some metals acts catalysts in oxidation of milk fat leading to oxidative rancidity. Certain metals like Ca, P, Mg, Mo,Co, Fe, etc. are components of metallo proteins such as lactoferrin,lactoperoxidase, xanthine oxidase and parts of phospholipids and certain vitamins.

The salt composition in milk cannot be expressed either as an equivalent to inorganic or organic substance because of the fact that some salts exist as organic substances also. It is also not possible to express them as ionic substances as a considerable part of these minerals are present in non ionized form. The ash content of the milk also does not represent the salt composition since during ashing of milk, some of the salts are lost due to volatilization. It is also possible that some non salt substances get included in ash. The minerals in human, cow and buffalo milk plays an important role in the availability of these minerals for the young ones. They also play an important role in the digestibility of milk proteins.

**30.2 OVERALL COMPOSITION OF MINERALS**

The most important salts of bovine milk are given in a Table 30.1.

**Table-30.1: Overall Composition of Minerals in Bovine milk**

| Constituent | Concentration | |
|---|---|---|
| | **Mean (mg/100 g)** | **Range (mg/100 g)** |
| **Cationic** | | |
| Sodium | 58 | 47-77 |
| Potassium | 140 | 113-171 |
| Calcium | 118 | 111-120 |
| Magnesium | 12 | 11-13 |
| **Anionic** | | |
| Total phosphorus | 74 | 61-79 |
| Inorganic phosphorus | 63 | 52-70 |
| Ester phosphorus | 11 | 8-13 |
| Chloride | 104 | 90-127 |

Not all of the salt constituents are found in the dissolved state in milk. Calcium, magnesium, phosphate, and citrate are partitioned between the solution phase and the colloidal casein micelles. For analytical purposes, partition of the salt constituents can be achieved by equilibrium dialysis or by pressure ultra filtration.

Table 30.1 presented above shows the proportion of the several constituents found in the dissolved and diffusible state. Actually,phosphate is present in five classes of compounds: inorganic dissolved,inorganic colloidal, water soluble, esters, ester bound in caseins, and lipid.These can be determined by making the following analyses:

Ø Total P in the dry or wet ashed sample-I

Ø Lipid Pin digested Rose-Gottlieb extract-II

Ø Dissolved P in digested ultrafiltrate-III

Ø In organic dissolved P in undigested ultrafiltrate-IV

Ø Acid soluble P in undigested 12.5% trichloroacetic acid filtrate-V

Then

Inorganic dissolved P = IV

Inorganic colloidal P = V - IV

Water soluble ester P = III- IV

Casein P = I - (II + V)

Lipid P = II

The total inorganic phosphate (Pi) is, of course, V. The salt constituents in the dissolved state (ultra filterable or diffusible) interact with each other to form various complexes. The concentrations of each of these constituents can be calculated (with suitable computer programs) from a knowledge of their several interaction or association constants. Na, K, andC1 are primarily present as free ions but Ca, Mg, phosphate, and citrate are distributed throughout many complexes; those in the highest concentration are CaCit$^-$, Mg Cit$^-$, $H_2PO_4^-$ HPO$_4^-$ and CaHPO$_4$. The calculation yields Ca$^{2+}$and Mg$^{2+}$ concentrations of 2.0 and 0.8 mm respectively.

## 30.3 TRACE ELEMENTS

A large number of trace elements are found in milk.Although these elements are very less quantitatively they have significant influence in the various properties of milk and also play a significant role inhuman nutrition. In addition to the major salt constituents discussed up to this point, the elements listed in the Table 30.2 are given here under. These elements have been detected in normal bovine milk by spectroscopic and chemical analyses. They include a large number of metals, the metalloids such as, B, and Si, and the halogens F, Br, and I. The reported concentrations of the trace elements exhibit large variation. (e.g., I, Mo, Zn), The concentration of elements like I, Mo, and Zn in the milk depends markedly on the diet

consumed by the cow. The concentrations of some of them are increased by contamination withutensils and equipment to which milk is exposed while handling and processing.

**Table-30.2: Concentration of trace minerals in bovine milk**

| Concentration (µg/liter) | | |
|---|---|---|
| Element | Range | Typical Value |
| Aluminum (Al) | 150-1000 | 500 |
| Arsenic (As) | 30-60 | - |
| Barium (Ba) | - | Trace |
| Boron (B) | 100-1000 | 300 |
| Bromine (Br) | 500-20,000 | - |
| Cadmium (Cd) | 1-30 | - |
| Cesium (Cs) | - | Trace |
| Chromium (Cr) | 5-80 | 15 |
| Cobalt (Co) | 0.4-1.0 | 0.5 |
| Copper (Cu) | 10-200 | 75 |
| Fluorine (F) | 70-220 | - |
| Iodine (I) | 10-1000 | - |
| Iron (Fe) | 100-1500 | 300 |
| Lead (Pb) | 20-80 | 40 |
| Lithium (Li) | - | Trace |
| Manganese(Mn) | 20-100 | 50 |
| Mercury (Hg) | - | Trace |
| Molybdenum (Mo) | 20-120 | 70 |
| Nickel (Ni) | 0.30 | - |
| Rubidium (Rb) | 100-3400 | - |
| Selenium (Se) | 4-1200 | 12 |
| Silicon (Si) | - | ~1400 |
| Silver (Ag) | 15-50 | 45 |
| Strontium (Sr) | 40-500 | 170 |
| Tin (Sn) | - | Trace |
| Titanium (Ti) | - | Trace |
| Vanadium (V) | - | Trace |
| Zinc (Zn) | 2000-5000 | 3300 |

Some high values for the concentrations of certain trace elements may have resulted from contamination during laboratory analysis. Molybdenum appears to be found exclusively in xanthine oxidase and Co in vitamin $B_{12}$. Iron is an essential component of xanthine oxidase,lactoperoxidase, and catalase. About half of the total Fe and 10% of the Cu are in the fat globule membrane. Copper has been studied extensively in relation to oxidation of milk lipids. The trace metal present in highest concentration in milk is Zn; its concentration of 3.5 mg/liter is about 3% of that of Mg, the major salt constituent present in lowest concentration. About 85% of the Zn is associated with casein micelles. Alkaline phosphatase, a Zn-containing enzyme, is located primarily in the fat globule membrane but accounts for only a small fraction of the total Zn. Manganese is required for fermentation of citrate by certain lactic acid bacteria, and with some milks the bacterial formation of diacetyl in cultures is inhibited by lack of sufficient Mn. Apparently, iodine is present in milk solely in the form of iodide ion; its concentration depends markedly on the amount consumed by the cow.

The casein micelles contain undissolved calcium phosphate and a little citrate; it is often called "colloidal calcium phosphate". Some cations, notably $Ca^{2+}$ and $Mg^{2+}$, are associated with the negatively charged proteins. Small quantities of other ions (e.g. $Cl^-$) also may be bound to the proteins. Almost all of the salt is in the serum or in the casein micelles, and very little is bound to the fat globules. The distribution of salt between various casein micelles in serum is presented in the Table 30.2. The salt in the casein micelles can be dissolved by lowering the pH to about 4.6 or lower,and also, though slowly and incompletely, by dialysis against water or a calcium free solution.

## 30.5 DISTRIBUTION OF SOME SALTS BETWEEN CASEIN MICELLES AND SERUM

Milk contains phosphorus in many forms. It is present as orthophosphate, but part of it is bound to organic components like serine and threonine residues of casein, molecules of hexoses and glycerol,phospholipids, etc. Phosphorus is also found in the hydroxyapatite structure of the colloidal calcium phosphate in the micellar structure of casein. Table 30.3gives particulars of the distribution of salts between casein micelles and serum. The sulfur content of milk is about 0.36 g per kg, but most of it is in the amino acid residues methionine and cysteine of the proteins. About 10% is present as inorganic sulfate. There is,of course, a significant variation between lots of milk.

### Table- 30.3: Distribution of salt between casein micelles and serum

| Compound | Milk (mg/100g) | Serum (mg/100g) | Dry Casein (mg/g) |
|---|---|---|---|
| Na | 48 | 49 | 0.9 |
| K | 143 | 145 | 3.3 |
| Mg | 11 | 8 | 1.5 |
| Ca | 117 | 40 | 31.0 |
| Inorganic phosphate | 203 | 116 | 37.0 |
| Citrate | 175 | 173 | 5.6 |

(Source: Davies and white, J.Dairy.Res,1960)

## Chemistry of Milk

The electrical conductivity is largely determined by the salt solution. The pH of milk dialysate is a function of its salt composition and temperature. The presence of the proteins in milk does affect this pH, of course, as it affects the salt composition. The titratable acidity of milk depends both on its salt composition (largely phosphate content) and its protein content. The freezing point of milk follows from the total concentration of dissolved substances especially chloride and lactose.

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**Lesson-31**

**Physical Equilibrium among Milk Salts**

## 31.1 INTRODUCTION

Milk contains several elements but all of them are not entirely in a soluble state. Some minerals exist in colloidal and ionic state at the normal pH of milk. The compounds which are in soluble condition play an important role in keeping various milk constituents in stable condition. A balance exists between the components which are in soluble state and those which are in colloidal state.

## 31.2 SALT SOLUTION IN MILK

The dissolved salts of milk are phosphate, citrate, chloride, sulphate, bicarbonate, sodium,potassium, magnesium calcium. Sodium and potassium in milk are present entirely in soluble state. It is necessary have physical equilibria among the milk salts for stability of milk, especially during heat processing. Colloidal particles of casein contain predominantly calcium, magnesium, phosphate and citrate. The chloride and sulfate are entirely present as the free ions $Cl^-$ and $SO_4^{2-}$ at the pH of milk The salts of the various weak acids (phosphates, citrates, and carbonates) are distributed among various ionic forms. Calcium and magnesium form soluble ions with citrate, phosphate and bicarbonates.

- $H©^{-2} \rightarrow ©^{-2} + H^+$

- $©^{-2} + Ca^{+2} \rightarrow Ca©^-$

- $Ca©^- + H^+ \rightarrow CaH©$

- $2\ Ca©^- + Ca^{+2} \rightarrow Ca_3©_2$

Where © =Citrate

A considerable portion of the calcium is held in the form of complex soluble ions such as Ca©- Similar complex ions are also formed with phosphate $CaPO_4^-$ and with bicarbonate $CaHCO_3^+$.The formation of such complex ions has the effect of reducing the concentration of calcium and magnesium ions in the solution. Only calcium salts are included but a similar scheme would apply to the magnesium salts.

## 31.3 SALT BALANCE

Salts in milk exist in colloidal and soluble form. A large amount of salt is present in casein micelles structure and is in equilibrium with salts in the soluble phase of milk. During acidification/fermentation of milk some salts gets solubilized from the colloidal state and come in to the soluble state. This destabilizes the casein micelle structure and tends to precipitate out as happens during curd setting of milk and preparation of acid casein. It also

# Chemistry of Milk

happens that the casein micelle stability is lost during heating of milk at high temperature such as in UHT processing, sterilization and boiling of milk. This is due to the in solubilization of colloidal calcium phosphate and final loss in the stability of milk, this in turn affects the quality of dairy products. Such changes in the salt balance also take place during manufacture of condensed milk,evaporated milk and milk powder.

The salt balance in milk is defined by the following equation

$$\text{Salt balance} = \frac{Ca^{+2} + Mg^{+2}}{Citrate^{-3} + PO_4^{-3}}$$

It has been observed that the balance between certain salts such as $Ca^{+2}$ and $Mg^{+2}$with citrate and phosphate in the colloidal and soluble phase plays apivotal role in the stability of casein micelles structure. It appears adelicate balance cations and anions in the colloidal and soluble state is important in determining the stability of milk. The monovalent cations such as $Na^+$ have a dispersing effect, while divalent and trivalent cations such as $Ca^{+2}$ and $Mg^{+2}$ have an aggregating effect. So during heat processing of cow milk and buffalo milk the heat stability would be governed by the concentration of above ions in addition to other factors. Therefore,addition of sodium citrates and phosphate improves the heat stability of milk.

Colloidal salts remain in equilibrium with the dissolved salt (diffusate form). Various treatments of milk may cause transfer of salts from one phase to the other. Various methods have been used to study the partition of salts between dissolved and colloidal phases. Two thirds of the calcium and one third of the magnesium and on half of the phosphorus are colloidal in normal milk. It is presumed that all the colloidal phosphorus is present in the micellar calcium phosphate and it appears that all the colloidal citrate is similarly located where as colloidal calcium and magnesium are partly in corporate in milk colloidalphosphate and partly bound in a more direct manner to casein. A small portion of colloidal calcium is bound to α lactalbumin. B lactoglobulin can also bind calcium and magnesium and there is other minor calcium binding protein. Nearly all the sodium, potassium and chlorine are diffusible.

The principle dissolved salt constituents or milk consists of phosphate, citrate, chloride,sulphate, bicarbonate, sodium, potassium, magnesium and calcium. At pH 6.6 of milk sodium and potassium are not found in any combination with other constituents and are present as the cations $Na^+$ and $K^+$ while the chloride and sulfate being anions of strong acids are present as freeion $Cl^-$ and $SO_4^-$. However the salts of the weak acids phosphate citrates and carbonates are distributed among various ionic forms. Calcium and magnesium form soluble complex ions with citrate, phosphate, and bicarbonates. Figure 31.3 shows the changes in salt balance due to various treatments of milk. Various factors effect salt equilibria. These are

1. Temperature
2. Variation in acidity
3. Variation in carbon dioxide content
4. Concentration

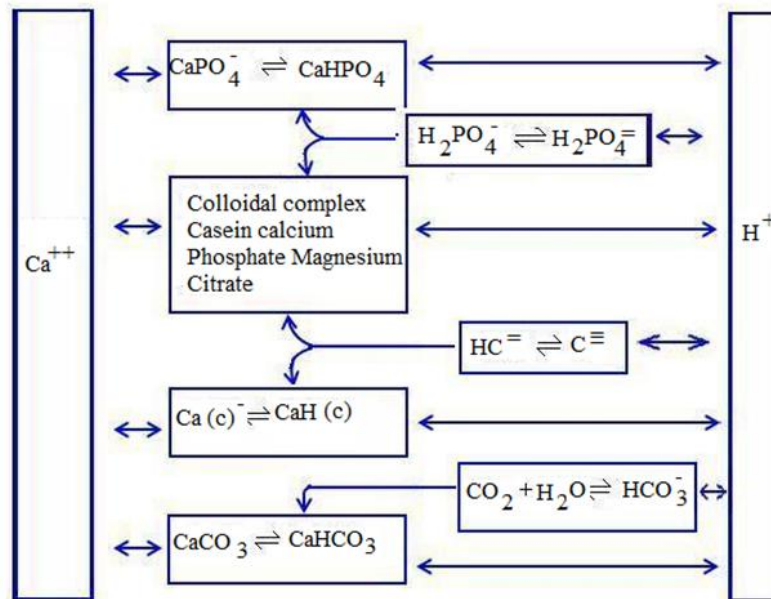Sequestering agents and ion exchangers

**Fig 31.1 Equilibrium among Milk Salts**

(Source: Jenness and Patton Principles of Dairy Chemistry, 1959)

**31.3.1: Temperature:** The temperature will shifts the balance among the various forms as milk is subjected to various cooling and heating treatments after it is drawn from the cow at $37^0$C. With the rise in the temperature the dissolved will be transferred to colloidal phase. Similarly lowering the temperature below that at which the milk is drawn would cause a transfer of calcium and phosphate from the colloidal particles to the dissolved state.

**31.3.2: Acidity:** There will be pronounced shift in the salt equilibria with the addition of acid to milk wither directly or indirectly by bacterial action. Decrease in the pH withdraws the calcium and phosphate from colloidal particles until at about pH5.2 all the calcium and phosphate is in the dissolved state.

**31.3.3: Carbon dioxide content:** Milk as secreted by the cow contains about20mg of $CO_2$2 from milk is accelerated by heating and agitation. Removal of $CO_2$ would affect the balance in the rest of the system. It would be expected that the removal of $CO_2$ and the consequent rise in pH would be reflected in a shift in calcium phosphate from the dissolved to the colloidal state and probably also in a shift of calcium ions activity. per 100ml or about 10% by volume. This gas is rapidly lost from milk owing to the low content in the air. The loss is irreversible under ordinary conditions of handling. The loss of CO

**31.3.4: Concentration:** As the milk is concentrated there is a tendency for calcium phosphate and calcium citrate to accumulate in the colloidal particles because the solubility is exceeded. As these materials are insolubilized , hydrogen ions are liberated, lowering the pH. The net result is an increase in concentration of citrate and phosphate in both the dissolved and colloidal state.

**31.3..5: Sequestering agents and ionex changers:** In order to stabilize the milk or even to improve the utility of milk for a particular purpose it is desirable to treat milk so as to alter its ionic balance. The best known example for this is the addition of phosphate or citrate to

stabilize milk against subsequent heat coagulation. This practice is common during the manufacture of evaporated milk, Addition of phosphate or citrate result in the binding of more of the calcium in the form of soluble complexes and decreasing activity of the calcium ions.

Soluble reagents which thus tie up a particular ions are called sequestering agents and are said to sequester that ion. Ethylenediamine tetra acetic acid is on such sequestering reagent. It is very good reagent for divalent and polyvalent cations. Treatment of milk with ion exchange column will remove both anions and cations from milk by a process called "monobed resin" treatments. It is mixture of cation exchange resine in the hydrogen form and an anion exchange resin in the hydroxyl form. When a salt containing solution is passed through such a mixture of resins both anions and cations are absorbed by the resin and hydrogen and hydroxyl ions are released which immediately combine to form water. By such treatments milk can be deionized to any extent desirable in a single pass through a resin bed or by batch wise treatment with a mixture of the two resins.

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**Lesson-32**

**Milk Contact Surface and Metallic Contamination**

**32.1 INTRODUCTION**

Action of Milk on certain metals would dissolve small amount of the metal and form metallic salts which would give rise to a 'metallic' taste to the milk. Some of the salts in milk have a catalytic action on the oxidation of milk fat resulting in the development of an oxidized flavour. These metals are said to taint milk.

**32.2 FACTORS INFLUENCING THE ACTION OF MILK ON METALS**

The factors which influence the degree of action by milk on the metal are:

Ø Temperature of milk.

Ø Period of contact.

Ø Cleanliness and polish of metal.

Ø Amount of free air in milk.

Ø Acidity of milk etc.

**32.3 SELECTION OF METALS FOR DAIRY EQUIPMENT**

During the processing of milk the metallic surface will definitely come in contact milk as such the interaction between the two cannot be avoided. As such it is necessary to take in to consideration this aspect while choosing the metals for designing various equipments with which milk will be in direct contact. The following aspects need to be considered in such situations.

Ø Non-tainting

Ø Non-toxic

Ø Insoluble (in milk or its products)

Ø Highly resistant to corrosion (by milk, cleaning and sanitizing agents, etc.)

Ø Easy to clean and keep bright

Ø Light yet strong

Ø Good agents of heat transfer

Ø Good in appearance throughout its use

Ø Low In cost

Ø Non-absorbent

Ø Durable

Since it is not possible to get any single metal which can meet the entire above requirement,alloys of different metals like stainless steel is recommended for use in the dairy industry. The most satisfactory combination of metal that could be used is 18:8 stainless steel and aluminum alloy presently.

## 32.4 CHARACTERISTICS OF DIFFERENT METALS AND THEIR ALLOYS USED FOR DAIRY EQUIPMENT

32.4.1. Copper and its alloys: This metal is mostly used for the preparation of Milk pails, coolers, vats, strainers,pipe fittings, milk pumps, pasteurizer coils, etc. (used for tinning only). Due to the action of milk it will cause taints in the milk or the dairy products. For preventing such effect the surface is coated with tin. Due to the action of milk on this metal a Green corrosion product is formed which is toxic for human consumption. The greatest advantage of this metal is that it is a Soft metal and it is easy to work with this metal. In order to increase the usefulness of this metal it could be used by having tin coating on the surface and this makes the metal more durable. Retinning of the metal is cheap and it will increase the makes it reusable for a considerable period. It high conductivity promotes rapid heat transfer and its cost is also reasonable

32.4.2. Aluminum and its alloys: This metal and its alloys are often use in the preparation of milk cans, milk pails, linings for tanks and tankers, etc. Aluminum will not taint the milk and being a Soft metal it is very easy to work with this metal. If the metal is impure its durability is reduced. However if aluminum alloyed or anodized it is much more durable. The greatest advantage of this metal is its light weight. The disadvantage of this metal is difficulty in cleaning due to its porosity, corrosion when alkaline dairy detergents and sanitizers are used. It is difficult to solder this metal.

32.4.3. Low-carbon steel: This low carbon steel is widely used for bodies of tanks, vats, bottle-washers, conveyors, etc.

32.4.4. Stainless steel (18:8): This alloy of iron is most widely used for all milk/ dairy product contact surfaces. The pure iron taints milk/dairy product while stainless steel does not taint. The 'Rust' caused to this metal in its pure state is slightly toxic. As this is a tough metal it will presents problems in fabrication.

Ø Stainless steel is highly durable in the preparation of various dairy equipment.

Ø The advantages in the use of stainless steel are highly resistant to corrosion by common acids and alkalis

Ø Takes high polish and therefore easy to clean

Ø Corroded by brine and chlorine solution

Ø Welding has to be done to repair cracks etc

32.4.5. Tin: This metal is used mainly as a 'coating' for milk/dairy product contact surfaces of cans, vats, etc. It will not taint milk although quite soluble in it. This metal is too soft to be used for any kind of equipment. Tin coating is not durable as it wears off readily by corrosion, abrasion, etc. However re-tinning process not at all difficult

32.4.6. Nickel and its alloys: This metal is used as a coating for milk/dairy product contact surfaces of pasteurizing vats coolers etc. Ni-alloy used in freezing chamber of ice-cream freezers, cylinders and plungers of homogenizers, etc. It has very slight effect on milk flavour although the most soluble in milk among dairy metals. It is mildly toxic. An alloy with iron makes it very tough and is quite difficult to handle during fabrication. However this metal is much more durable than tin coating, but it is more expensive. Lactic acid causes corrosion of this metal however it is not corroded by the alkaline washing powders. The highest disadvantage in the use of this metal is that it is more costly when compared with the chromium and tin

32.4.7. Chromium and its alloys: This metal is more useful for the coating on various types of equipment. It is mostly used for the milk/dairy product contact surfaces. This metal is non tainting and is resistant to corrosive action by acid and alkaline cleaners. The most important characteristic of this metal is that it is very expensive

## 32.5 CORROSION CONTROL

From the practical point of view corrosion cannot be entirely prevented in dairy equipment; however its rate can be controlled to a large extent. The following measures will help in preventing corrosion of stainless steel surface:

Ø Keep the surface clean

Ø Permits surface to air dry, whenever possible.

Ø Use cleaners and sanitizers in the lowest concentration and for the shortest duration that will do the desired cleaning job.

It is the common observation in dairy industry that the stainless steel which is used extensively in the dairy industry also will form an invisible film of chromiumoxide forms on its surface when the stainless steel is dry and exposed to the atmosphere. This film protects the surface forms corrosion. But when the film breaks or wears away, and the active metal gets exposed and corrode more easily. As the chlorine and its compounds are very corrosive it is advisable that equipment should be sanitized with chlorine solutions, preferably just before it is to be used, so as to avoid pro-longed contacts, and thus corrosion (pitting).

## Chemistry of Milk

Milk contact surface and Metallic contamination: it is an established fact that milk normally contains metallic elements like aluminum, copper, iron, manganese, silicon, zinc and others. Although concentration of these metals is very low in normal milk they may be added to milk during processing due to the action of milk with the contact surfaces on these metals.

Milk utensils in our villages are commonly made of earthenware, zinc plated or galvanized iron, brass or copper. The product contact surfaces and other portions of modern processing equipment are increasingly being made of stainless steel. In the normal milk copper is bound with the fat as such this metal is not active chemically. Milk when comes in contact with copper it will dissolve in milk and would exist in active form as copper ions. In the presence of direct sunlight and /or air the copper ions catalyze the hydrolysis of milk fat which an irreversible reaction resulting in off flavor development. This flavor defect is more in high fat products. Copper salts formed due to the reaction of itsions with milk slats or lactic acid are greenish in colour, quite bitter to taste and is toxic.

In normal milk iron is present in a chemically bound form. It is largely related to the fat globule membrane and the enzyme proteins. Similar to the copper, milk also dissolves iron when it comes in contact with it and will be forming free ions resulting in the development of off flavors and toxic compounds. Iron will also stain the product contact surface. A common practice prevailing when utensils, cans or buckets made of galvanized iron are to be used for handling milk and milk products, to coat the contact surface with zinc (Zinc plating) but upon long usage these vessels become defective due to wearing off the plating exposing the iron to be in contact with the milk directly. Special consideration is to be given to the reaction of these metals with milk when they are used for preparing the equipment and containers for handling milk and milk products. Development of off flavours, discoloration of the products, corrosion of the vessels, formation of toxic compounds, are some of the consequences when milk contact surface is made with these metals. Stainless steel is an alloys containing chromium, managanese silicon and nickel. The most satisfactory materials for the product surfaces is the stainless steel containing 18% chromium, 8% nickel in a low carbon steel. The processing equipment made with surfaces with this alloy are easily cleaned.

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