CHAPTER TWO

Chemical and Functional Properties of Milk and Milk Components

Chemical composition of milk:

The main constituents of milk are water (87%), solids-not-fat (9% of proteins % + lactose % +Ash%) and total solids (13% of fat% +SNF%). The constituents other than the water are known Total solids (TS).The total solids minus the fat are known as solid not fat (SNF).

Milk plasma or (skim milk): A common term which consists of all the constituents except fat.

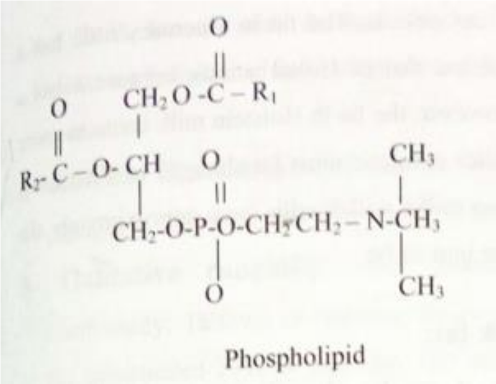
Milk serum or (whey): A common term which consists of all the constituents except the fat and casein. Also defined as the watery product left in cheese making.

"Milk fat "Butter Fat :

It is the most valuable constituents of milk from the standpoint of food value of milk. It contributes the rich flavor of milk and its products, also the smoothness in the mouth and the palatability. It exists in the milk in the form of minute globules in a true emulsion and the diameter of fat globules ranges from 0.001-0.01 millimeter (mm) and a single drop of milk contains about 100,000,000 fat globules. Milk fat is a triglyceride which means one molecule of glycerol that is combined with three molecules of free fatty acid. Each fat globule is surrounded by a very thin film or membrane of protein and phospholipid (Lecithin) which assist in maintaining the stability of the fat emulsion in milk. Agitation as in churning breaks the film and the globules coalesce with a final result of butter. Milk fat contains a large number of the fatty acids than any other fat found in nature. There at least 64 different fatty acids in milk. The saturated fatty acids account for about 60% of the total fatty acids and the unsaturated fatty acids account for about 40% of the total fatty acids, the saturated fatty acids range from butyric acid (with four carbon atoms) to cerotic acid (with 26 carbon atoms). The percentage of butyric acid in milk fat serves as a distinguishing characteristic since no other fats contain this low molecular weight fatty acid. The unsaturated fatty acids in milk present many problems. Oleic acid account for approximately 30% of the total fatty acid content .The major fatty acids of milk may be divided into two groups of volatile and non-volatile. Volatile free fatty acids are butyric (C4), Caproic acid (C6), Caprylic (C8), Capric acid (C10) and Lauric acid (C12). The non- Volatile free fatty acids are Myristic (C14), Palmitic (C16), Stearic (C18), Oleic (C18:1) and Linoleic acid (C18:2). All are saturated except Oleic and Linoleic which are unsaturated free fat acid (FFA). Volatile FFA has considerable influence on the favor and off-flavors in milk. Butyric acid liberated is responsible for the rancid flavor in milk and its products. Normal processing and storage methods do not produce any marked chemical changes in the saturated fatty acids. The unsaturated FFA is of significance in the oxidative deterioration of milk and its products. The melting point of milk fat varies between 32°C to 36°C. The specific gravity of milk fat is 0.93. Milk fat will absorb volatile odors very readily.

Compounds associated with milk fat:

1- Phospholipids: Two of the hydroxyl (OH) groups of the glycerol are joined to the fatty acids but the third hydroxyl group is joined with phpsphoric acid and a choline radical.



Lecithin:

It is the main phospholipid of milk (traces of cephalin phospholipid are also present). It is found on the surface of the fat globules to protect the triglyceride from lipase which splits the triglyceride to 3FFA and glycerol. It is a natural emulsifying agent soluble in fats and water. The trimethyl amine is a breakdown product of choline has a strong fishy odor and is responsible for a fishy off-flavor in butter and dried milk due to break down of this constituent part of lecithin by fat oxidation. Linoleic acid appears to be of prime importance in the development of the flavor defect in butter.

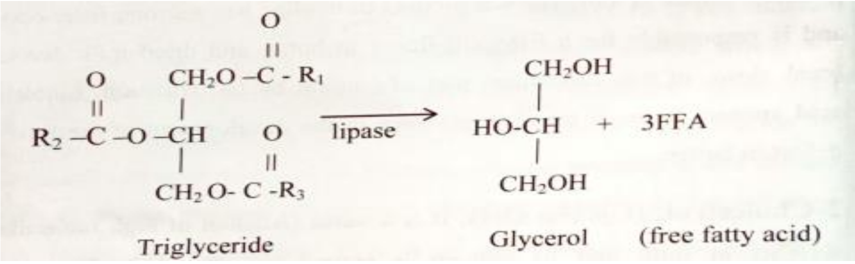
2-Cholestrol: (C27 Has OH): It is a sterol (Alcohol of high molecular weight) in milk and its content is around 0.015% (105-175ppm of cholesterol). One of its forms is 7-dehydrocholestrol which is precursor of Vitamin D being activated by irradiation with ultra violet rays.

3- Fat soluble vitamins: E, D, A and K are the fat soluble vitamins in milk.

4-Carotene: (C40 H56): It exists in green plants as a yellow colored pigment. When taken into the animal body is converted into Vitamin A, therefore it is often called pro Vitamin A. It is a precursor of Vitamin A. The yellow color of the milk fat is due to the presence of carotinoids. The fat in Guernsey milk has a much more golden color than has that of Holstein milk because it has a higher content of carotene. However, the fat in Holstein milk contains more preformed Vitamin A, and since carotene must be changed to Vitamin A before it can be used, the two milks will finally have approximately the same amount of Vitamin A per unit of fat.

Chemical changes in milk fat:

1-Hydrolytic Rancidity: It is also described as bitter or soapy flavor. It is one of the most objectionable flavors found in milk. It is induced by the lipase enzyme which breaks down milk fats (triglyceride) into glycerol and three free fatty acids (FFA). The liberated butyric acid yields such rancid odor and taste.



Lipase is a normal constituent of the milk of cows and present in milk at the time of production, but that fats of normal milk are protected by a membrane (phospholipid) surrounding each fat globule. Excessive agitation breaks the membrane and exposes the triglyceride to the lipase, therefore excessive agitation of milk should be avoided. Pasteurization of milk destroys the enzyme. Rancid flavor is more prevalent in milk obtained from cows in late lactation (less than six weeks before parturition), or from cows producing less than 10 lb (pounds) of milk per day Milk fats are also decomposed by the microbial contamination. Culture inhibitors free fatty acids in rancid milk are caprylic, capric and lauric.

2- Ketonic Rancidity: It is due to decomposition of milk fat and changes of saturated free fatty acids to ketones by the action of fungi.

3-Oxidative rancidity: The oxidized flavor is described as Cardboardy, Tallowy or Metallic flavor. It is due to the oxidation of the unsaturated FFA of milk fat**. The usual causes of oxidized flavor are :**

a- Exposure of milk to copper or iron equipment: such equipment catalyzes such flavor. Because copper and iron increase the incidence of oxidized flavor, so stainless steel, glass, plastic or rubber should be used for all milk handling equipment. The water used for cleaning equipment should contain minimum amount of iron and copper.

b- Excessive exposure of milk to the day-light: Exposure of milk to day light or strong artificial light causes oxidation of unsaturated FFA, for this reason all milk in transparent pipelines or receivers jars and the milk should be protected against day light or strong exposure to artificial light.

c-Poor Nutrition of the cow: The oxidized flavor is more prevalent during winter because the green feeds consumed in spring and summer supply substances (such as Vit E) that delay the oxidation of milk.

d-Vigorous agitation with air: Try to minimize air admission during agitation of the milk which can induce oxidative or rancid flavor in milk.

Proteins of milk :

Nearly all samples of normal milk with a protein content of about 3-4% (3.3%), Casein constitutes about 80% of the total proteins in milk and lactoalbumin about 18% and the rest is lactoglobulin.

Casein: It is the principal protein compound of milk; it contains the elements of carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus, There are at least three separate caseins fractions and designated as α- casein (75%).β- cascin (22%), and γ-casein (3%). The percentage of casein in milk is 2.6%.

α- casein is divided into :

α s casein which is sensitive to the calcium while K-casein (kaba casein) which is not - sensitive to the calcium. K-casein is the stabilizing casein for α and β caseins (it tends to stabilize α s - casein from precipitation in the presence of CaCl). Rennin enzyme destroys its stability property. Casein is a high quality protein as it contains all the essential amino acids (those needed by the body but cannot be synthesized by it). There is no other protein in nature similar to the casein. The casein in its pure state is snow white odorless and tasteless granular substance and contributes to the whiteness of the milk. It exists in milk in combination with calcium as calcium caseinate. It exists in the milk in the form of small gelatinous particles in very fine suspension called the colloidal state (is not in solution). Casein is among the chief constituents of various types of cheese which contain around one-third casein.

Methods of casein precipitation :

Casein is precipitated from milk by dilute acids, rennin, alcohol and heavy.

1-Precipitation of casein by dilute acid:

Proteins being amphoteric and metals containing the amino acid and carboxyl groups, dissociate either into acids or bases. This dissociation leads to positive and negative charges on the protein particles, being positive on the acid side and negative on the basic side. At a certain hydrogen-lon concentration the two dissociations will be equal and thus the positive and negative charges I will be similar, this condition is known as the isoeletric point of a substance. Casein has an isoeletric point of pH 4.7. At this pH casein can be most readily precipitated from milk. So if fresh milk at a pH of 6.6 is allowed to sour to a pH 4.7, the net electric charge will be become zero and the milk will begin show evidence of curdling. The casein precipitated with weak acids is free of calcium because the acid frees it from its union with calcium. If the milk is allowed to stand until sufficient acid is produced, it will curdle naturally. Pure casein is not precipitated by heat. Prolonged heating of fresh milk at high temperature (120°C) under pressure will cause precipitation of casein. On boiling fresh milk, a thin layer of finally precipitated casein together with fat forms a thin layer over the surface of the milk. Heat and acid are interrelated in precipitating casein. The application of heat to milk that is slightly acid will cause the precipitation of casein. The higher the temperature the less acid required for coagulation and conversely, the more acid the lower will be the heating temperature for coagulation.

2- Precipitation of casein by alcohol: The casein that precipitated with alcohol is calcium caseinate, the alcohol acts as a dehydrating agent i.e. it removes the water from the casein and precipitate it.

3-Precipitation of casein by Rennin : When casein precipitated with Rennin, calcium paracasein is formed and it contains more calcium than calcium caseinate. Rennin extract is a liquid containing the enzyme rennin which is obtained from the stomach lining of young calves. The action of Rennent on milk changes the α- casein to α1 paracaseinate and α2-paracaseinate while β casein and γ -casein are co-precipitated but unchanged.

4-Precipitation of casein by heavy metals: Heavy metals such as mercury, silver, lead, copper, zinc, Aluminum and iron will precipitate the casein.

Uses of casein commercially

a- Manufacturing of cheeses.
b- The greatest being in the paper industry.
c- Casein is used in the Manufacturing of plastics (i.e made in rolls sheets and tubes).
d- Casein has a wide application in the manufacturing of paints.
e- Excellent Glues are made from casein which is used also as a clot for suits and dresses.
f-Casein is used as a filler or binder in certain foods as in sausage.
g-Casein is used in Baby-formula

Albumin: chemically known as lactalbumin (the protein of milk). Th albumin content of milk is 0.5%. When albumin dried, it is tasteless powder. It contains the elements Carbon, Hydrogen, Oxygen, Nitrogen an Sulphur but no phosphorus as in the casein. It is present in true solution milk. Albumin is not acted upon by rennet nor coagulated by acids ordinary temperatures. It is precipitated by heat especially at the proper protein value which is around 4.5. Albumin may be separated from milk by firm precipitating the casein by the use of a weak acid, and heating the filtered whey to a temperature of 74°C to 80°C, the turbid appearance of the heated ate shows the presence of coagulated albumin, albumin may be removed by filtration. Albumin cheese may be made from whey. The whey is hosted to 74 to 80°C and then upon adding a little acid to bring it to pH and 4.5, the albumin will precipitate. It can be separated by using saturated Ammonium sulfate or saturated magnesium sulfate. The food value of the whey depends upon the albumin, Lactose, minerals and small amunt of fat.

Lactoglobulin :

It is a third protein in the milk. Milk does not contain more than 0.1% of lactoglobulin. In Cholostrum, it is the chief protein, the amount being higher than that of casein. It is important in providing immunity to certain infections in newborn Calf. It contains the same. elements as in casein It can be separated from albumin by precipitating it with an excess of salts such as magnesium sulfate. Rennin and diluted acids don't affect it, but high temperature will cause it to precipitate along with albumin.

Other proteins :

Some proteose, peptone constituents are present in the mk either naturally occurring or resulting from the degradation of natural protein. There are 8 to 10 enzymes which are native to milk such as:
1-Xanthine oxidase
2-actoperoxidase.
3-Alkaline phosphatase.
4-Trypsin inhibitors.
5-Protease.
6-Amylase.
7-Lipase.
8- Lactenins I and II.
9-Lactose destroying enzyemes.
10- Aldolase.
11- Catalase.

Lactose: It is milk sugar (found only in milk). It is a reducing dissaccharide which upon hydrolysis yields two simple sugars, Glucose and Galactose. Milk contains about 5% of lactose. It is present in true solution in milk. It is less soluble than sucrose and therefore less sweet ie sucrose it about six times as sweet as lactose (i.e lactose is about 1/6 as sweet as sucrose). Prolonged heating milk for 100-130°C results in a brown color development and gives rise to the browning of sterilized milk due to caramelization of lactose. Two form of lactose are known (a and p). B-lactose is much more soluble and therefore, much sweeter than a-lactose Lactose is the major component responsible for maintaining the osmotic pressure of milk in equilibrium with that in blood. Any depression of the synthesis of lactose is accompanied by increases in the concentration of Sodium and Chloride to keep the osmotic pressure of milk in equilibrium with that in blood and therefore the milk tastes salty.

Action of Microorganisms on Lactose:

Lactic acid fermentation is the convertion of lactose to lactic acid by the action of bacteria which gives milk its sour taste; therefore lactose is important for making cultured dairy products (cheese, cultured buttermilk and yoghurt). The fermentation of milk continues until an acid percentage of 0.8% to 1% is present which inhibits further growth of bacteria which produced it. Lactose fermenting yeasts such as *Torula cermoris* are capable of fermenting lactose to alcohol and CO and foamy cream in hot weather is a result of this reaction. Lactose is an important food constituent of milk especially for babies. The pharmaceutical industry uses lactose as filler in medicine and as a coating for pills.

Factors affecting Lactose contents:

1- Breed and species of animal: Some variation in lactose% occurs with individuals within the breed and with species of animal.

2- Disease: Udder infection is largely cause a decline in the lactose content of milk.

3- Lactation period: Milk at the end of lactation period tastes salty due to a decline in the lactose content i.e depression of the synthesis of lactose which is accompanied by increases in the concentration of sodium and chloride to keep the osmotic pressure of milk in equilibrium with that in blood and therefore the milk taste salty.

Lactose Intolerance: It causes abdominal cramps, gaseous distention or diarrhea in severe cases. It is attributed to a deficiency of lactase in the intestinal mucosa of human beings.

Ash: If milk is heated to just evaporate the water, the residue will be solids in milk. Then if the residue burned in Muffle furnace at 450rc white powder which is the ash remains. Ash contains the minerals e inorganic constituents of milk. The percentage of minerals is about 0.7.

Table 3 : Major minerals in milk .

Minerals	Percentage
Calcium	0.112%
Phosphorus	0.095%
Potassium	0.138%
Magnesium	0.013%
Sodium	0.059%
Chlorine	0.109%
Sulphur	0.01%

Table 4 :Minor minerals in milk .

Minerals	mg/liter or ppm
Iron	3 ppm
Zinc	3 ppm
Silicon	2 ppm
Copper	3.0 ppm
Fluorine	0.15 ppm

In milk the trace minerals are Molybdenum, Aluminum, Manganese, Jodine, boron, Titanium, Vanadium, Rubidium, Lithium and strontium. Ash contents of milk vary a little during the season, stage of lactation and location. The potassium, sodium and chlorine are present in solution. Parts of Calcium and phosphorus are combined with the proteins while the remaining part is partly in suspension and partly in solution. Calcium and phosphorus are necessary for bone formation and good teeth. Iron is required in the formation of hemoglobin. Iodine is needed in the production of the hormone thyroxin which prevent goiter. Minerals enter into the heat stability of milk especially at a temperature which is used in the sterilization of evaporated milk .

Vitamins: Milk contains the fat soluble Vitamins (E, D, A & K), also contains the water soluble Vitamins. The B-complex consisting of thiamine (BI), Riboflavin (B2), Niacin (Nicotinic acid), Pantothenic acid, pyridoxine (B6), Biotin, Choline, Inositol, Folic acid, P- aminobenzoic acid and Vit B12. Also contains Vitamin C (Ascorbic acid).

Vitamin A: It is soluble in oils and fat. It is stable at heat, acids and alkalis. Large losses may occur through oxidation and exposure to light.

Vitamin D : It is fat soluble Vitamin. It is stable to heat, acids, alkalis and oxidation

Vitamin E (a –tocopherol): It is fat soluble vitamin stable to heat, light, alkalis and acids. It is destroyed when fats turn rancid.

Vitamin K: It is present in milk only in trace quantities. Stable to heat and destroyed by light and oxidizing agents.

Thiamin or Vitamin B1: It is soluble in water. Pasteurization by holding method, low temperature long time (LTLT) at 62°C/30 minute destroys around 9% of the thiamin content. No loss occurs by HTST pasteurization (High temperature short time) at 73°C/15 seconds Autoclaving milk will destroy all the Vitamin B1. Milk is not a rich source of vitamin B1.

Riboflavine (Vitamin B2): It is water soluble Vitamin and whey is considered a rich source of Vitamin B2. It is stable to heat but fairly stable to oxidation, acids and alkalis but very sensitive to light. It takes up s light readily and uses it to oxidize ascorbic acid, thus destroying this vitamin, therefore. It is important to keep milk out of sunlight.

Niacin: or Nicotinic acid. It is present in the milk to a small extent.

Pantothenic acid: It is found in skim milk and whey. It is unstable to alkalis, acids and prolonged heat. It is stable to light and to oxidizing and reducing agents.

Pyridoxine B6: Unstable in neutral or alkaline solutions. Relatively stable to acid and heat. Light causes its rapid destruction. Milk is a good source of it.

Biotin: It is very stable and milk is a good source of this Vitamin.

Ascorbic acid (Vitamin C): It is water soluble Vitamin. It is lost easily through oxidation. Heat treatment of milk in copper containers activates the speed of oxidation. Vat pasteurization (LTLT) destroys around 18% but HTST pasteurization causes no loss. Most milk does not have a sufficient amount of Vitamin C to fill the need of children's. It is a strong reducing agent.

Enzymes in milk :

Enzymes are organic compounds secreted by living cells and act as catalysts (aid a chemical reaction). The enzymes in milk come either from the cow's udder or from the bacteria. Those enzymes that come from cow's udder are called original enzymes. Bacterial enzymes vary in type and abundance according to the nature and size of the bacterial population. Several enzymes in milk are utilized for quality and control. There are at least 20 enzymes identified as natural constituents of milk.

Lipase :

It acts on fat with liberation of three free fatty acids and Glycerol. It is the main cause of rancidity in dairy products. The rancid flavor is being due mainly to the highly volatile butyric acid. It may be activated by cooling the milk to 4°C then warming to 30°C and finally cooling to below 10°C. Homogenizing raw milk will cause it to turn rancid within a few minutes, also by mixing both homogenized and raw milk. It is inactivate at 40°C and destroyed at 55°C. Milk from cows in late lactation will often have a rancid flavor. Rancidity is detected by taste. Lipolysis occurs at the normal pH of milk. The optimum activity occurs around pH 9. In dairy laboratory, the lipolytic activity is evaluated by determining Acid Degree Value.
The original enzyme (lipase) is inactivated by HTST pasteurization process. Many microorganisms produce lipase which can cause serious problems as the microbial enzyme is very resistant to heat i.e. Heat stable enzyme.

Table 5 : Lipolytic microorganisms in milk

Lipolytic bacteria	Lipolytic molds	Lipolytic yeasts
Pseudomonas	Rhizopus	Candida
Achromobacter	Geotrichum	Rhodotorula
Staphylococcus	Aspergillus	Hansenula
	Penicillium	

Protase :

Proteins are split by protease to proteoses --> peptones--> peptides --> amino acids and therefore, the microbial protease of starter bacteria aids in the ripening of certain cheeses. The original protease that comes from the cow's udder is inactivated by heat at 74°C to 80°C. The common proteolytic bacteria that contaminate milk produce a heat stable protease and these are Bacillus, Clostridium, Pseudomonas, Proteus and Streptococcus liquifaciens.

Catalase:

Fresh milk from a healthy udder contains only insignificant amount of Catalase. In mastitis the bacteria and leucocytes increase the catalase content of milk. If hydrogen peroxide (H2O2) is added to the milk in a test tube, the calatase split H_2O_2 into water and free oxygen. The amount of gas produced will indicate the quality of the milk. This method is used to detect mastitis. It is inactivated by heat at ordinary HTST pasteurization (72°C/ 15 seconds). Its presence does not affect the quality of milk.

$$2\,H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O$$

Peroxidase:

It transfers oxygen from Hydrogen peroxide (H_2O_2) to other readily oxidizable substances. Peroxidase is inactivated if the milk is heated to 80°C for a few seconds. This is a fact which can be utilized to provide whether or not pasteurization temperature above 80°C has been reached. This test is called Storch's peroxidase test. So it indicates whether or not milk has been properly pasteurized by flash method (e.g. 80- 85°C / 5-15 seconds).

Phosphatase:

It has the property of being able to split certain phosphoric acid esters into phosphoric acid and the corresponding phenol (alcohol) and the measurement of this free phenol indicates whether or not the milk has been properly pasteurized because phosphatase is destroyed by regular pasteurization temperature (LTLT) 62°C/30 minutes or(HTST) 72°C/15 seconds. The presence of phosphatase in milk can be detected by adding a phosphoric acid ester and a reagent that change the color when it reacts with the liberated phenol. A change in the color reveals that the milk contains phosphatase (liberating phenol from the alcoholic esters of phosphoric acid). So phosphatase test can be used to determine whether pasteurization has actually been attained. The routine test used in dairies is called the phosphatase test.

Reductase :

The quality of milk is determined by reductase in the Methylene blue reduction test (MBRT) and Resazurine reduction test (RRT). It reduces the Methylene blue in the presence of an aldehyde. A temperature of at least 80°C is required to inactivate it.

Lactase :

Some lactose is broken down to the simple sugars by lactase and then changed to alcohol. However, little of this activity occurs in milk.

Table 6 : Classification of enzymes

Hydrolytic enzymes	Enzymes for efficiency of pasteurization	Keeping quality enzymes
Lipase	Phosphatase	Catalase
Protease	Peroxidase	Reductase
Lactase		

Gases in milk:

Milk contains dissolved gases around 5-9% by volume. These gases consist of Carbon dioxide (CO_2), Nitrogen and Oxygen. Carbon dioxide is present in milk as it comes from the udder, while Nitrogen and Oxygen are taken up by the milk during milking. On standing, the amount of gas becomes less. The oxygen content of milk is of economic importance in its relations to the undesirable oxidized flavor of milk.

Body cells in milk :

Body cells are normally present in the milk The following somatic cells are present in the milk:
1-Leucocytes or white blood cells (WBC): 75% of the somatic cells present in milk are leucocytes.
2-Epithelial cells: Usually the epithelial cells are derived from the outer surfaces of the secreting tissues i.e. shedding of mammary epithelium.

3-Cell debris: i.e. Fragments of epithelial cells.

Notes: In general 25% of the somatic cells in milk are epithelial cells and its fragments

Factors that cause higher somatic cells counts in milk :

1- Colostrum: Body cells are more numerous in the colestral milk than later in the lactation period.
2-Advanced lactation: The numbers of body cells increase again toward the end of lactation period due to shedding of mammary epithelium.

3- High lactation age.

4-Hand milking: It increases the cell counts in milk.

5-Mastitis: Most normal quarters show less than 100,000 cell/ml. Ahigh total counts of 10 cells/ ml and a high proportion of Neutrophiles (up to 90%) is an indication of acute inflammatory affecting much of the quarter.

6- Trauma: Trauma to the mammary gland increases leucocytes count in milk.

7-Brueses: Brueses of the mammary gland increase leucocytes count in milk.
8-Irritation: Irritation to the mammary gland increases leucocytes count in milk.

9- Retention of the milk: Retention of the milk inside the mammary gland increases leucocytes count in milk.

10- Heat period .

Body cell counts in milk are coming to be the most favored method of determining the freedom or otherwise of a cow from mastitis The counts used include the direct microscopic somatic cell count (DMSCC). In chronic mastitis the highest counts are in the strippings. When the foremilk counts are highest the lesion is probably in the teat. Leucocytes breakdown readily in stored milk and for preference smears should be made, fixed and stained within an hour. Counts of less than 250,000 cells ml are considered to be below the limit indicative of inflammation although most normal quarters show less than 100.000 cells/ml .A high total count (10" cells/ml) and a high proportion of neutrophils (e.g 90%) indicate acute inflammation affecting much of the quarter

Significance of leucocytes in milk

It has been always possible to correlate large numbers of leucocytes with pathological conditions in the udder. Formerly, a count of 1,000,000 cells /ml was regarded as indicative of infection. Milk having 1500.000 cells /ml or more of leucocytes are confirmatory test for mastitis.

Red blood cells (RBC) in milk

Udder contains only insignificant number of red blood cells. Any increase in the number of RBC in milk is considered abnormal milk and is necessary to take a decision of rejection of that milk i.e. lead to the rejection of the milk.