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Review Article

Biochemical and Microbiological Aspects of Milk: A Review

BINA RANI[1], RAAZ MAHESHWARI[2]
1. Department of Engineering Chemistry & Environmental Engineering, PCE, Sitapura, Jaipur, Rajasthan
2. Department of Chemistry, SBRMGC, Nagaur, Rajasthan

Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to the baby and can reduce the risk of many diseases in the baby. Milk is an important food with many nutrients. World's dairy farms produced about 730 million tonnes of milk in 2011. India is the world's largest producer and consumer of milk, yet neither exports nor imports milk. New Zealand, the European Union's 27 member states, Australia, and the United States are the world's largest exporters of milk and milk products. China and Russia are the world's largest importers of milk and milk products. Throughout the world, there are more than 6 billion consumers of milk and milk products, the majority of them in developing countries. Over 750 million people live within dairy farming households. Milk is a key contributor to improving nutrition and food security particularly in developing countries. Improvements in livestock and dairy technology offer significant promise in reducing poverty and malnutrition in the world.

MILK: A SOURCE OF DIET

Biologically milk is the normal secretion of mammary glands and as per the dieticians, it’s considered a complete food, but as a chemist milk is considered as a treasure of chemicals. Milk is as ancient as mankind itself, as if the substance created to feed the mammalian infant. All species of mammals, from man to whales, produce milk for this purpose. Many centuries ago, perhaps as early a 6000-8000 BC, ancient man learned to domesticate species of animals for the provision of milk to be consumed by them. These included cows, buffaloes, sheep, goats and camels, all of which are still used in various parts of the world for the production of milk for human consumption. The role of milk in the traditional diet has varied greatly in different regions of the world. The tropical countries have not been traditional milk consumers, whereas the more northern regions of the world. Europe and North America have traditionally consumed far more milk and milk products in their diet. In tropical countries where high temperatures and lack of refrigeration has led to inability to produce and store fresh milk which has traditionally been preserved through means other than refrigeration, including immediate consumption of war milk after milking, by boiling milk or by conversion into more stable products such as fermented milks.

Milk is considered as the most valuable and nutritious product for the human consumption. It is most suitable and elaborate substance provided by the nature. It is the best nutrient medium for the growth of various organisms from microbes to large animals as it contains protein, fat, carbohydrates, inorganic salts and vitamins. Milk cannot be preserved in its fresh conditions for a long time and soon it is fermented or destroyed by the life activities of the different types of microorganisms that gain entrance to the milk from various sources. Milk free from the bacterial contamination can be obtained, if proper care regarding sterility of the milking utensils, hands of the milkman and the proper disinfections of the udder is maintained. Therefore the microorganisms that are present in milk are basically come from the external sources and not from the internal sources. Milk and its products have been around the very long time. Milk is the one food for which there seems to be adequate

Bulletin of Environment, Pharmacology and Life Sciences

substitute. It constitutes almost the entire diet for the young ones of all mammals. Each species produce milk that is specially adapted to the growth of its own young, but milk from one species may be used as food for others. Since animals were domesticated centuries ago, the milk from various species such as cow, buffalo, goat and camel has been used in the diets of people throughout the world.

Freshly drawn milk has a sweet odour. Part of the odour of fresh milk arises from its volatile fatty acids. Milk rapidly absorbs odours from the environment in which it is produced and initially handled. Such absorption results from two actions. Firstly, as it is drawn from the udder, milk contains a quantity of gases which it immediately exchanges with air. If that air contains odours, they will thereby enter into the milk. Secondly, the fat present in milk produced by unhealthy udders and that produced during late lactation may have their own characteristics odours and lack a clean and easily detectable normal odour.

BIOSYNTHESIS OF MILK

Milk is synthesized in mammary glands. Within the mammary gland is the milk producing unit, the alveolus. It contains single layer of epithelial secretary cells surrounding a central storage area called lumen, which is connected to a duct system. The secretary cells are, in turn, surrounded by a layer of epithelial cells and blood capillaries. The raw material for milk production is transported via blood stream to the secretary cells. It takes 400-800 L of blood to deliver components of 1 litre of milk. The milk components are synthesized within the cells, mainly by the endoplasmic reticulum and its attached ribosomes. The energy for the endoplasmic reticulum is supplied by the mitochondria. The components are then passed to Golgi apparatus, which is responsible, which is responsible for their eventual movement out of the cell in the form of vesicles. Both vesicles containing aqueous non fat components, as well as liquid droplets (synthesized by the endoplasmic reticulum) must pass through the cytoplasm and the apical membrane to be deposited in the lumen. It is thought that the milk fat globule membrane is comprised of the apical of the plasma membrane of the secretary cells.

Proteins: Amino acids are building blocks in the blood. Casein micelles or small aggregates thereof begin aggregation in Golgi vesicles

Lipids: C_4 – C_{14} fatty acids are synthesized in the cells, C_{16} and higher fatty acids are performed as a result of rumen hydrogenation and are transported directly in the blood.

Lactose: Milk is in osmotic equilibrium with the blood and is controlled by lactose, K, Na, CL: lactose synthesis regulates the volume of milk secreted.

Milking stimuli such as sucking calf, warm cloth, the regime of parlour, etc. caused the release of hormone called oxytocin which is released from pituitary gland, below the brain, to begin the process of milk led down. As a result of this hormone stimulation, the muscles begin to compress alveoli, causing the pressure in the udder known as leddown reflex and the milk components stored in lumen are released in the duct system. The milk stored forced down in to the teat cistern from which it's milked. The leddown reflex fades as the oxytocin is degraded, within 4 to 7 minutes. It's very difficult to milk after this time.

COMPOSITION OF MILK

An approximate composition of milk is 87.3% water (range of 85.5%-88.7%) 3.9% fat (range of 2.4%-5.5%), 8.8% solids-not-fat (range of 7.9 – 10%), protein 3.25% (3/4 casein), lactose 4.6%, minerals 0.65% Ca, P, Mg, K, Na, Zn, Cl, sulphate, bicarbonate, many acids 0.18% - citrate, acetate, lactate, oxalate,, enzymes – peroxidise, catalase, phosphate, lipase gases – oxygen, nitrogen, vitamins – A, C, D, thiamine, riboflavin, others.

Water

Water constitutes the medium in which the other milk constituents are either dissolved or suspended. Most of it is 'free' and only a small portion is in the 'bound' form, being firmly bound by milk proteins, phospholipids, etc. like all fats, milk fat provides lubrication. Milk fat consists of fatty acids, glycerol (glycerine), phospholipids and other minor materials. As many as 142 fatty acids have been reported to be present in milk fat. Some of the fatty acids of milk fat are volatile, others non-volatile, some are soluble in water, others not. A few the fatty acids in milk fat are not only soluble in water but may be distilled off in stream.

Carbohydrates

The principal carbohydrate of milk is lactose. The amount present in milk depends upon the health of the udder and the breed of the producing animal. Lactose is about 17.8% to 18% soluble in water at 27°C.

Proteins

The proteins of milk are casein, albumin, globulin, protease, peptones and enzymes. Chemical break down of milk proteins can be caused by heat, acid, enzymes, alcohol and other agents. Denaturation of the protein and coagulation or clotting characterizes the early stage of protein change. Recent developments have shown the need for a raid test for the protein content of milk.

Lipids

Several groups of phospholipids are normally present in milk. They are closely associated with both the milk proteins in the milk fat. They are destroyed by heat treatment.

Vitamins

Milk is a good source of vitamin A, vitamin B or thiamine, and vitamin B_2 or lactoflavin. It provides small amount of vitamin C or ascorbic acid, vitamin D and niacin. Other vitamins are present in smaller quantities. Vitamin A, D, E and K are fat soluble, so they tend to be present in milk and milk products in proportion of the fat content. Vitamins C and several vitamins of the B complex are water soluble.

Enzymes

Milk also contains a large number of indigenous enzymes, with differing functions, stability to processing, impact on dairy products, and significance for consumer safety (e. g., antimicrobial enzymes). Some enzymes are of interest for their beneficial activity (e.g. lactoperoxidase), some for use as indices of processing (e.g. alkaline phosphate) and some for effects on the quality of dairy products (e.g. plasmin, lipoprotein lipase), which may be either positive or negative for different products. Children are particularly susceptible to malnutrition because of micronutrient deficiencies, which contributes to poor growth, diminished mental development, and illness. Animal source foods supply not only high-quality and readily digested protein and energy and energy, but are also a compact and efficient source of readily available micronutrients.

Metals

Milk normally contains several metallic elements such as Al, Cu, Fe, Mn, zinc, silicon and others. Most of these, however of these, however, are present only in extremely small concentrations. During processing, milk may pick up additional quantities of these metals and traces of others from utensils and equipment used to handle the milk. Utensils in our villages are made up of earthen ware: zinc plated or galvanized iron, brass or copper. Modern dairy utensils are made of tinned iron, aluminium alloys and stainless steel. The product contact surfaces and other portions of other processing equipment are increasingly being made of stainless steel.The concentration of copper in fresh milk normally varies from 0.050mg/l. this copper is bound to the fat and not normally active chemically. When in contact with copper, milk dissolves this metal and there after contains copper in the form of active ions. These ions, especially in the presence of direct sunlight and/or air, catalyze milk fat hydrolysis. This reaction is irreversible. Eventually several off flavours may appear, particularly in fat products. Milk can develop evidence of such flavours in the absence of free copper ions, but to a smaller degree. Copper salts which are likely to form by the reaction between free ions and milk salts or lactic acid, are greenish in colour, quite bitter to taste and toxic. Products contact surfaces of dairy utensils or equipment should not contain copper.

Traces of iron are also normally present in milk. The average concentration is about 0.420mh/l varying from 0.100 to 0.600mg/l. this iron is also chemically bound. It is largely related to the fat globule membrane and the enzyme proteins. Milk dissolves iron with which it comes in contact, freeing active ions which act much the same as similar ions of copper. However, iron seems to be less active in this respect. The iron reacts with milk salts or lactic acid to give bitter flavours and toxic salts. Iron might also stain product contact surfaces. Apart from the direct effect of the zinc platting, most buckets and cans made of galvanized iron, as are many utensils used in villages, become defective after very little use as the zinc wears off and the iron becomes exposed to the product. Aluminium is chemically rather stable to milk and milk products. Its greatest fault is its vulnerability to attack by alkaline detergents. Alkalis not only corrode aluminium generally, but also pit its surface. A badly aluminium surface cannot be easily cleaned. If at all aluminium alloys, especially those including silicon, are recommended. These alloys are used for cans, tankers, sometimes for butter churns and carts or trollies for bulk butter. Certain components of butter printer heads are often made of an aluminium alloy. Tin is very stable to milk, detergent, and water. Its usefulness is limited principally because it is so soft. Tin is most commonly used as plating on iron, sometimes on copper.

Zinc reacts with milk to produce off flavours. Lead produces salts which are quite toxic, but it is resistant to concentrated sulphuric acid used in Gerber test for milk fat. Nickel and chromium are quite stable to cold milk and detergents, but they are expensive. Their principal use in the dairy-processing plant is as a component of the group of alloys called stainless steels. A variety of alloys are referred to as stainless steels. Most of them contain chromium, manganese and silicon in steel. Several contain nickel. A few have or more other metals. Probably the most widely used members of this group are those containing about 18% chromium and 8% nickel in a low carbon steel. These alloys are not completely about anticorrosive, but are sufficiently so to make them the most satisfactory materials known to the dairy-processing industry for product contact surfaces. Their surfaces are made of an alloy of this group is easily maintained in sanitary condition.

Microbiology of Milk

Nearly all the changes which take place in the flavour and appearance of milk, after it is drawn from the cow, are the result of the activities of micro-organisms. Of these, the important in dairying are bacteria, mould, yeast and virus. Micro-organisms are visible only with the aid of microscope. A few are desirable, while most cause undesirable changes; a relatively small proportions are disease-producing types, and are called “pathogens”. In dairy industry considerable effort is extended in controlling micro-organisms which cause spoilage. The greater the bacterial count in milk, i.e. the greater the number of bacteria per ml, the lower is its bacteriological quality.

Numbers of Microorganisms

When micro-organism find conditions suitable, they reproduce rapidly. A new generation of bacteria may appear in 20-30 minutes. Their method of reproduction is by cell division; consequently their rate of increase is geometric. Under conditions which are less than ideal, their growth rate diminishes proportionally. A definition of milk implies is a very high standard of microbiological quality, states that milk is “the third fluid drawn from a perfectly healthy udder of a perfectly healthy cattle living under the most favourable sanitary conditions”. Even such milk may contain a few viable bacteria. One report showed the standard plate count of aseptically drawn milk averaged from 2,200 for 22 samples in the winter to 3,033 for samples in the monsoon. Another study showed a SPC rangin330 to 4,100/ml in aseptically drawn milk. On the other hand, one group of samples of raw milk from a city market showed an average SPC of 3,000,000 to 3,500,000/ml and an average coliform count of 2,200 to 6,500ml. If milk is not produced and handled with considerable care, the bacterial count may reach several millions within a few hours. Distinct quality defects, practically acid flavour and odour followed by low heat stability, usually accompany such bacterial counts. Freshly drawn milk contains a weak bactericidal factor called lactenin. This factor, however, will not adequately protect milk from bacterial growth. The lactinin content of buffalo milk is slightly higher than that of cow milk. The numbers of bacteria found in milk may depend somewhat upon the method of counting. Quality of milk is considered good when direct microscopic count/ ml is less than 5,00,000 and standard plate count/ ml.

Actions of Microorganisms on Milk Components

Lactose: Lactose seems to be that component of milk most liable to the action of micro-organisms**.** Lactic acid is formed initially by this action. So long as conditions permit, lactic acid formation can continue until the concentration of acid is itself too great for the organisms to remain active. *Streptococcus lactis* can continue functioning in milk until the titratable acidity of the milk, expressed of lactic acid, reaches 1,00% before functioning in milk until the titratable acidity of the milk to as much as 2,25% before it ceases to function. *Torula lactis,* one of the yeasts, can produce a titratable acidity in milk of 1.5% before ceasing to grow. Such levels of acidity do not destroy the organisms they only inhibit their activity.

Proteins: Protein especially the casein, is probably next in frequency and degree of involvement in the action of common micro-organisms in milk. Protein usually coagulates in the presence of adequate acid. Milk will coagulated at room temperature with a titratable acidity of 0.51% to 0.65%. Buffalo milk is reported to be clot on boiling positive or COB+, at an average of 0.21% titratable acidity; cow milk, at 0.25%. protein will coagulate when acted upon by certain bacterial enzymes. Proteolysis results from the action of any of several micro-organisms. The protein is initially altered, freeing some of its amino acids. Finally these break down into amides and ammonia. This process disturbs forces which hold the casein particles or micelles in suspension, converting the protein into soluble substances. Proteolysis usually, but not always, involves coagulation.

Milk fat: the actions of micro-organism do not involve fat as readily as they do lactose and protein. Apart from the lipolytic action of lipase which is normally present in milk, lipolysis results from the action of lipase produced by bacteria such as *Pseudomonas fluorescens,* by some yeasts, and by certain moulds. Lipolysis causes hydrolysis of the fat, breaking it down into glycerol and free fatty acids (FFAs), e.g. organoleptic effect will depend upon which these acids is freed. Free butyric and caproic acids, e.g. give milk products distinctive and usually objectionable flavours and odours.

Ash: The ash or minerals components are seldom attacked directly by micro-organisms. Some are released from combination of casein acid formation and proteolysis for e. g. *Streptococcus citrovorous.*

Actions of Micro-organisms in Milk and Milk Products

Flavours: the acid flavours commonly found in milk is caused by the formation of lactic acid by micro-organisms which attack the lactose, even slightly developed acidity can produce a detectable flavour. A bitter flavour results from certain types of protein breakdown caused by micro-organisms. This defect may appear in milk which acid producers are suppressed by pasteurization. Acid inhibits the activity of many bitter flavour producing micro-organisms. To prevent undesired bitterness in certain types of cheese, acid formers are included in the vulture along with protolytic organisms. The processing product and its handling during storage and curing are the managed to assure a balanced developed development of acid and proteolysis to give the desired blend of acidic and proteolytic flavours.

The unique flavours of several types of cheese are a result of the action of the action of specific mould species. Mould contaminants in products, mainly cream, butter, cheese and khoa, may give undesired mouldy or musty flavours. The Prevention of Food Adulteration Act (PFAA) permits hard cheese to contain 0.1% sorbic acid or its sodium, potassium or calcium salts as sorbic acid which is an antimycotic. The quality of flavour in curd and other fermented milks, in butter and in cheese is improves by use selected micro-organisms. The fine delicate flavour of curd and of cultured butters comes from diacetyl. Diacetyl is an oxidised product of acetylemethyl carbinol, produced by the fermentation of citric acid at a low pH. Milk contains citrates equivalent to between 0.16% and 0.20% calculated as citric acid. A mixture of bacteria which includes lactic acid producing and citrate fermenting types constitutes the microflora of ideal curd or butter cultures. These bacteria include *Streptococcus lactis, Steptococcus cremoris* and *Sacchamoyces cerevisiae* are also known to produce diacetyl.

Odours: An acid can often be detected in milk having slight developed acidity. Many gases formed by micro-organisms have a characteristic odour. Micro0organisms acting upon both the proteins and the fat often produce changes are detected by odour. Milk is frequently graded on the basis of its odour. Organoleptic tests consider not only odours caused by micro-organisms, but also many odours for which the actions of micro-organism are not responsible, such as those odours which carry through the cow from her feed or which enter the milk by absorption from the atmosphere.

Colours: Moulds often discolour the surface of butter and cheese, and may carry their discoloration into the interior of these products. While moulds can be great offenders because they are so difficult to see against a white or light yellow product surface. Discoloration due to mould on old cream or khoa surfaces is sometimes encountered. Surface discoloration may appear on market khoa within 5 days when incubated at 37°C, or 9 days at 22°C. In the absence of proper sanitation, *Pseudomonas* species give rise to a yellowish film on products contact surfaces.

Gas: Many micr-organisms, usually by reacting with the lactose, produce gas in milk and milk products. Yeasts are particularly prone to form gas, mainly CO_2. Some of the *Clostridia* and *Echerichia- Aerobacter* group may be responsible. Gas formation is a common defect of sour cream.

Gas production is essential in certain types of cheese such as Emmanthal, and in one or two fermented milks such as *Kumiss.*

Enzymes: Some enzymes are produced in milk and milk products, but few of the natural enzymes of milk are attacked or changed by the action of micro-organism.

Alkali: A few micro-organisms, such as *Alkaligenes viscolactis. Pseudomomas fluorescens* and *Micrococcus ureae,* produce alkali or neutralize developed acidity in milk. Yeasts and moulds are seldom responsible for this action in milk.

Slime: Sliminess is a common condition caused by the action of specific micro-organisms in butter, in certain types of cheese, and in milk, or cream. When milk or cream is slimy, it is often said to be "ropy". In some areas of the word specify slimy or ropy fermentation are encouraged in milk by using selected organisms and processing procedures. A slimy condition on the surface of butter or on most kind of cheese is considered a serious defect.

Curd: Curd is a fermented milk product. The term"yoghurt" is widely used in other dairy countries for products similar to curd. *Streptococcus thermophlus* and *Lactbcillus bulgaricus* are used together for making yoghurt.; the former produces some acid and a fne aroma, the latter produces high acidity. Bulgarian butter milk is a pure milk culture of *Lactobacillus bulgaricus,* acidophillus milk a pure milk culture of *Lactobacillus acidophilus.* The wide variety of fermented milks includes not only those coagulated by acid formation, but also some characterized by flavours produced by bacteria and yeasts, some containing gas, some of which are alcoholic and one or two with a slimy body. Most fermented milks are made from cow or buffalo milk. However, fermented milks of local importance are also made from ewe, mare or camel milk. *Propionibacterium shermanli* and certain strains of *Streptococcus lactis* and *Streptococcus cremoris* produce folic acid when used as culture organisms in curd.

Cheese: The microflora of cheese may differ greatly from one kind of cheese to another. The starter or inoculums used must vary accordingly. The microflora of modern cheese normally results from systematic inoculation with cultures of one or more micro-organisms. The flowing examples illustrate the variety of bacteria and moulds employed in cheese making.

In Cheddar cheese, the action of rennet is influenced by acidity. The starter used for this cheese, therefore, must contain acid producing bacteria. The most commonly used in this starter are *Straptococcus lactis* or *Streptococcus cremoris.* During curing, however, these species seem to diminish in numbers; this is more important in short cured or mild Cheddar, or during the early stages of long cured of sharp Cheddar/ *Lactobacilli,* which also produce acid, are included in the starter for sharp Cheddar; *Lactobacillus casei* is a coon species of this group of bacteria.

Starters used in Emmanthal or Gruyere types of "Swiss" cheese originating in Switzerland and France respectively but now made in many dairy areas, must include some bacteria capable of tolerating high temperature and others capable of producing both the large gas holes and the special flavour characteristic of these cheeses. *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus helveticus,* or mixtures of these species are used to produce acid; *Propionibacterium shermanii* produces some acid, but is included to produce the large eyes and special flavour required. Gas holes reduce the market quality of many kinds of cheese such as Cheddar and Gouda; large gas holes are essential to good Swiss cheese.

Roquefort or Blue cheese is made with the help of lactic acid starter and rennet; the bacteria are usually *Streptococcus lactis.* As the curd is dipped, spores of *Penicillium roquiforti,* a blue-green mould are added throughout the curd. After being pressed the cheese is punctured to admit air to facilitate mould growth in its interior. Camembert cheese is similar to Roquefort. It is prepared in smaller prints and cured with *Penicilliyum camemberti* which is a white mould.

Ice cream: Types of micro-organisms found in ice cream are primarly those in the raw material used to formulate the mix. Equipment, personnel, and other factors may be source of additional contaminants. The SPC (Special Plate Count) of ice cream not exceed 50,000/g to 1,00,000/g and may be held to less than 20,000/g, even below 1,000/g or so. The coliform test of ice cream should show less than one of these micro-organisms per gram. Neglect or error in the selection of raw materials and/ or in processing can result in counts of several millions. Freezing and hardening ice cream destroys very few viable organisms present in the product. Many can survive for months, which is longer than most ice cream normally held in storage. In fact, the action of the dasher and scraper of the freezer breaks up clumps of groups of micro-organisms if present, giving a higher

SPC, while the individual cell count remains substantially unchanged. Homogenization also breaks up such clumps. Micro-organisms found in ice cream are mainly acid formers and the flora includes *Streptococci, Micrococci* and spore formers. Proper pasteurization of the mix destroys ant pathogens present. Pathogens entering the product thereafter are likely to survive the low temperature of freezing, hardening and prolonged storage.

ANTIMICROBIAL SYSTEM IN MILK

- **Lysozymes:** It is an enzyme that hydrolysis glycisidic bonds in gram positive cell walls. However its effect as a bacteriostatic mechanism in milk is probably in milk is negligible.
- **Lactoferrin:** It is an iron binding proteins that **sequesters iron from micro-organisms, thus taking away one of their growth factors. Its effect as a bacteriostatic mechanism in milk is also probably negligible.**
- **Lactoperoxidase:** It is an enzyme naturally present i raw milk that catalyzes the conversion of hydrogen peroxide to water when hydrogen peroxide and thiocyanate are added to raw milk, the thiocynate is oxidized by the enzyme/ hydrogen peroxide complex producing bacteriostatic compounds that inhibit gram negative bacteria, *E. coli, Salmonella sps* and *Streptococci.* This technique is being used in many parts of the world especially where refrigeration for raw milk is not readily available, as a means of increasing the shelf life of the raw milk.

[Sources: Rani, B. 1988; PhD Thesis "Effect of Different Heat Treatment on the Proteins of Buffalo Milk with added Neutralizers and Additives", National Dairy Research Institute (NDRI), Indian Council of Agricultural Research (ICAR) Karnal, Haryana; Maheshwari, R. K.1989; PhD Thesis "Studies of the Metal Complexes of Buffalo Lactoferrin", NDRI (ICAR) Karnal, Haryana, India]