REVIEW OF LITERATURE
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The domestication of cattle occurred between 6000 and 10,000 years ago. Not much is known about the history of this period, but men probably hunted cattle as wild animals prior to the time that they were domesticated. The oldest written records of the human race are found in the Sanskrit literature of ancient India. These records date back nearly 6000 years, but milk had already become an important food item to these early peoples of central Asia and their wealth was measured in terms of number of cattle. Later the cow was made a sacred animal and is still so considered by a major population of India. Over 50 references to cows and milk are found in the old testament and the promised land was described as "a land flowing with milk and honey".

The soldiers of Genghis Khan, the Mongol Emperor who conquered Asia and a large part of Europe in the thirteenth century, carried dried milk as a part of their ration. Cheese was an important part of the food supply carried by the Vikings in their voyages.

Before 1850, most milk produced was necessarily consumed within a few miles of its production because of the lack of suitable means of transportation and refrigeration. Gradually farmers within easy driving distance began delivering milk over regular routes in the cities. This was the beginning of the fluid milk sheds which surround over large cities today. After 1850 and the middle of the nineteenth century is a convenient point to set as marking the beginning of modern dairying. More changes have been made during the past 100 years than in all the previous centuries.
Among the many factors which have played important roles in the evolution of the modern dairy industry, some points to be mentioned are 1) Faculty system, 2) Improved machinery, 3) Transportation, 4) Improved livestock, 5) Research and Scientific investigation and 6) Economic factors.

**MILK**

Nutritionally milk is considered as one of the most complete foods for man and microorganisms (Sinha and Nambudripad, 1973). In fact milk is the only source of subsistence during the period of early infancy. Moreover, milk and milk products constitute a very important part of our daily food intake in view of their extremely high nutritive value and health attributes. It is an important and indispensable food in well balanced diets and contributes a great number of nutrients in relation to our needs for good nutrition and health. Besides being nutritive, milk is delicious and easily digested, adding to beauty and satiety. It is good for brain development, reproductive faculties, longevity and general metabolism. A meal supplemented with milk and fed to poorly nourished children improves the body health, mental vigour and blood haemoglobin. However, milk and milk products are extremely vulnerable to microbial contamination during the course of their production and processing if not handled properly (Grover et al. 1993). Its flexibility is understood with the fact that milk can be separated into fat and other constituents and converted into various milk products. The extended shelf-life and flavour improvement by processing, aging and culturing enhances the scope of milk utilization.
MILK CONSTITUENTS

Milk is a complete fluid food comprising all the nutrients required by a neo-natal for growth and sustenance. The role of the major constituents of milk like fat, protein, lactose and vitamins in nutrition has long been elucidated. Besides these macromolecules, milk also contains certain micro-molecules whose influence on human and animal health has been the focus of research in the past decade or so. Many of these constituents of milk have specific bioprotective role, most of which are now clinically proved (Sharma et al. 1999).

PROTEINS

Milk proteins contain all the essential aminoacids in fairly high amounts. In addition, casein and albumin of milk are associated with certain biologically important minerals like calcium and phosphorus, as well as certain vitamins. Though milk contains only 3-4 percent proteins it is regarded as an excellent source at relatively cheaper rate compared with other animal proteins such as eggs, meal and fish (Badshah and Prasad, 1992).

Milk proteins are said to be of “high biological value” as they contain all the essential aminoacids in amounts and proportions required to support growth and perform numerous vital functions within the body. Proteins contribute to building and repair of body tissue, acting as buffer to keep acid-base balance of the body, participating in muscular contraction, acting as antibodies and as body's immunological defence mechanism and supplying energy. Casein makes up approximately 80 percent of milk proteins, the remaining being whey proteins. Casein the major protein, leading to the formation of micelles by entrapping calcium and phosphate ions, is of physiological significance since casein micelles are good carriers
of these two important elements as well as a good source of amino acids by themselves.

The major whey proteins of bovine milk are β-lactoglobulin and α-lactoalbumin, the former representing half the total whey proteins. It functions as binder and transporter of retinol and significantly enhances retinol uptake. It is also a carrier of antibodies, possessing immunological properties. α-lactoalbumin is important from the nutritional point of view, as it is readily digestible (Whitney, 1988).

**MILK FAT**

The average of fat content in cow’s and buffalo’s milk are 4 percent and 6 percent respectively. Fat is utilized mainly as a source of energy. In terms of composition, milk consists of primarily triglycerides with small amount of di and monoglycerides, phospholipids, sterols (such as cholesterol), carotenoids, fat soluble vitamins and some traces of free fatty acids. The composition of fatty acid in milk fat is characterized by a high proportion of saturated fatty acids (60-70 percent) an appreciable amount of mono unsaturated fatty acids (25-30 percent) and a small amount of polyunsaturated fatty acids (4 percent). Milk fat has relatively a high content of short and medium chain fatty acids with 4 to 8 carbon atoms which are arranged in such a fashion that butyric and caproic acids occur in the outer position and long chain fatty acids such as myristic acid are found at position 2. All this makes it easily digestible. Milk fat also provides the body with essential fatty acids which otherwise are not synthesized in the body. These are important for the proper functioning of cell walls and membranes (Sharma et al. 1999).
Several low molecular fatty acids (C₄ to C₁₀) of milk fat help in the digestion of stearates and proteins. Ghee, made-up of fully saturated, mono-oleo-di-saturated and di-oleo-mono-saturated glycerides improves the digestive process (Badshah and Prasad, 1992).

**CARBOHYDRATES (Lactose)**

The major carbohydrate of milk is lactose, which varies from 4.4 to 5.2 percent in bovine milk. Lactose, apart from providing energy inhibits putrefaction by promoting the growth of aciduric bacteria in the intestine. This reduces the intestinal pH and facilitates the absorption of minerals like calcium, magnesium, zinc and phosphorus. It is assumed that galactose, one of the monosaccharides in lactose, has a role in the early development of the infant's brain and spinal column. Other minor carbohydrates called micro nutrients such as galactose, fucose N-acetyl glucosamine, N-acetyl nuraminic are also present. These have physiological significance and act as bifidus factor (Huria and Achaya, 1997).

Inability to digest lactose is mainly due to the lactase deficiency. With this deficiency, the lactose in milk remains largely undigested and the excess lactose increases gut osmolarity. As a result water is drawn into the intestine, causing fullness and watery diarrhoea. The excess lactose also leads to increased fermentation by intestinal bacteria with consequent effects of flatulence, stool acidity and bloating. Since lactase deficiency is usually rare, there is no justification for excluding milk from the diet. A milk-free diet leads to a lack of essential nutrients and would make the nutritional situation much worse in developing countries (Mishra et al. 2000).
MINERALS IN MILK

Minerals usually exert their biological efforts through enzyme systems. The roles they play in the body are many and diverse with each mineral having specific functions. In general, minerals function as components of skeletal tissues, as constituents of organic compounds of soft tissues, as activators and components of enzymes, as acid-base regulator, as osmotic pressure regulators, as promotores of neuro-transmission and cell transformation and division and as controllers of muscle excitability. All the minerals now known to be needed for nutrition of human beings and which must be supplied by diet are present in milk in varying quantities.

Though minerals form a very minor part of the total nutrients present in milk, they are very important in human nutrition. Calcium and phosphorus, which form a major bulk of minerals present in milk, play a very important part in bone formation and body growth of the consumer. They are also important in coagulation of blood and activities of muscles and nerve tissues. A slight variation of these minerals in milk greatly affects the quality of milk products like cheese, evaporated milk etc. On account of such vital importance, many workers have studied the mineral contents in milk and the factors which affect their concentrations (Jacobson et al. 1972).

Milk is considered as a very good source of certain minerals required for our body. The ratio of calcium and potassium to magnesium and sodium in milk resemble very closely to the ratio of same elements in the body of man. Milk and its products are important sources of calcium which is essential in building the body skeleton as well as affecting muscle action including that of heart. Cheese is particularly a rich source of calcium, containing approximately three times that of wheat and greatly exceeds that of
corn. Milk also contains trace elements such as manganese, zinc, aluminium, copper, iron and cobalt (Archibald, 1958).

The mineral content of cow-milk is about 7.3 gram per liter. It is considered higher than that of human milk (2.0 gram per liter). In cow milk the minerals especially sodium, potassium, chloride, calcium, phosphorus and magnesium are higher. Milk is an excellent source of calcium and a good source of phosphorus, two major bone-building minerals, but is relatively poor in iron and magnesium. The calcium in milk is well used by human body. Its beneficial effects of calcium have been particularly recorded in preventing osteoporosis, stroke and providing protection against colon cancer and kidney stone (Patel and Schauen, 1998).

In the absence of milk and dairy products in the diet, calcium intake in excess of 300 milligram per day is difficult to achieve. Moreover, milk is one of the dietary sources of calcium, not only because of its significant quantity, but also because of its calcium to phosphorus ratio. The presence of nutrients such as lactose and vitamin D enhances the absorption of calcium.

The amino acids lysine and arginine present in generous amounts in milk increase the intestinal absorption of calcium. Citric acid and vitamin A also encourage calcium uptake and are present in milk. Fortification of milk with vitamin D is helpful in increasing calcium utilization. It is also an established fact that calcium is solubilized in acidic environments and also that calcium as present in milk is highly absorbable in the intestine, although the pH of the intestinal contents is near neutral or slightly alkaline. It has been suggested that moderate and exchangeable binding of calcium to caseino phospho-peptides (break down of peptides of casein) present in the intestine is responsible for the high absorbability of calcium from milk. In essence, to obtain the optimum dose of calcium, a
wide range of factors are important, including a balanced diet, which is not easy to achieve without milk and its products.

Sodium is a principal cation in extracellular fluid and primary regulator of extracellular fluid. It is also important in the regulation of molarity, acid-base balance and the membrane potential of cell as well as in active transport across all membranes. Potassium is a principal intracellular cation. It also exists in extracellular fluid and contributes to the transmission of nerve impulses, to control the skeletal muscle contraction and to the maintenance of blood pressure. Chloride is an essential cellular anion and is necessary for the maintenance of fluid and electrolyte balance (Gehrke et al. 1954).

Iron plays a vital role in human nutrition as a component of several metalloproteins such as haemoglobin, myoglobin and cytochromes. It serves as a carrier of oxygen and as an activator for all vital functions. Although the quantity of iron in milk is less, it is present in a readily soluble form and, therefore, is completely absorbed from the intestine better than in iron from other iron-rich foods (Imamura et al. 1961).

VITAMINS

In addition to the above food constituents, minor nutrients required for growth, health and reproduction are also present. These essential substances for life are termed as vitamins and are over twenty five in number. The fat-soluble vitamins present are A, D, E and K where as thiamine (vitamin B\textsubscript{1}), riboflavin (vitamin B\textsubscript{2}), pyridoxine (vitamin B\textsubscript{6}), pantothenic acid, niacin, biotin, folic acid, cyanocobalamine (vitamin B\textsubscript{12}) and ascorbic acid (vitamin C) are water soluble.
RAW MILK QUALITY

Raw milk quality basically depends on the bacterial load. The type and number of bacteria present in milk will be responsible for acidity development, flavour changes and sourage of the milk. Based on bacterial load, raw milk is graded into three major classes as per International Standards (Ranada, 1998). Some people consume raw milk directly at the production site, a practice still prevalent in the village believing it to be safe. A more stringent standard is required to curtail such practice.

There is a need for better understanding of the current prevalence of bacterial pathogens in raw milk. The information could be assessed the public health value of the public concerning the probability of exposure to bacterial pathogens when consuming raw milk. Raw (unpasteurized) milk can be a source of food-borne pathogens. Raw milk consumption results in sporadic disease outbreaks.

Primarily the quality of market milk was judged mainly on the basis of its fat content and density. But at present the presence of microbes in milk plays a vital role in judging its quality. A milk sample having proper fat and other contents may be discarded, if it shows a higher microbial counts, while a sample with a low or prescribed microbial counts may be accepted cheerfully (Rai et al. 1990).

In recent years, milk production has tremendously increased in our country and it is very necessary that every drop of milk has to be preserved well. Production of milk in many farms, however, appears to be most unhygienic and particularly, those places where chilling facilities for milk are inadequate, a substantially high bacterial load will be found in milk due to loading for 2-3 hours at elevated atmospheric temperature prevailing in most
parts of our country. Unhygienically produced milk will contain not only potential pathogenic organisms but also toxic metabolites.

**PRESENCE OF MICROBES IN MILK**

Milk and milk products provide ideal environment for various bacteria (Bryan *et al.* 1981). Different organisms have been incriminated by various workers for their role in contamination of milk and milk products. Most microbiological protocols that are routinely used to assess the quality of raw milk were not designed to detect specific pathogens. Raw milk with low aerobic plate counts or low somatic cells counts may or may not contain pathogenic bacteria capable of causing illness. Conversely an elevated total bacterial count may or may not coexist with the presence of human pathogens.

Generally, the microorganisms occur as post processing contaminants. Aerobic spore formers are of considerable importance in food industry because of their ability to produce enzymes and the resultant undesirable textural and flavour defects. *Bacillus cereus*, the toxin producing species of aerobic spore formers has been implicated in many food poisoning cases (Johnson, 1984; Raja Kowaski and Mikolajcik, 1987; Wong *et al.* 1988; Ramaraju and Kirankumar, 1988; Eapen *et al.* 1983).

Spore forming bacteria deserve consideration because they bring about spoilage in pasteurized, boiled and ultrahigh temperature-treated milk. The aerobic spore forming bacteria gain entry into the milk from a variety of sources during production and handling and constitute the major flora in market milk supplies in India. It has been reported that the keeping quality of heat treated milk is also affected (Jayachandran *et al.* 1985).
Milk is exposed to heavy bacterial contamination under village conditions where the concept of hygienic practices in milk handling is yet to be introduced. Due to long time, the distances involved in the collection of milk from villages and its transportation to the chilling centers at ambient temperatures there is a great scope for the rapid growth of bacteria in milk under tropical temperatures leading to its early spoilage of food. The frequent spoilage of milk results in heavy economic losses and use of such milk may also result in health hazards. Thus the initial quality of milk at the time of its production and collection has a tremendous effect on the quality of milk during the subsequent operations of transport, processing and distribution.

Gill et al. (1994) reported that *Staphylococcus aureus* of food poisoning which is a milk-borne disease. *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* have also been isolated from milk (Wouafo et al. 1996). *Streptococcus* and *Salmonella* species have similarly been associated with contamination of milk products (Gill et al. 1994; Gazzar and North, 1992). Serious health hazards due to the presence of pathogenic microbes in food can lead the food poisoning outbreaks (Frazier and Westhoff, 1997). Food-borne infections and intoxications are increasing in both industrial and developing countries today. In United States 6 to 81 million people experience food-borne infection each year (Wu et al. 2001).

In India, the chances of transmission of diseases through milk and milk products are much higher due to unsatisfactory milk hygiene, adulteration practices, poor health conditions of animals and ignorance of dairy workers (Sharma and Joshi, 1992). The detection of pathogenic bacteria in food helps in controlling food-borne infections; the estimation of the level of bacterial contamination in food allows assessing the shelf-life of food,
which is important from the health point of view and economic point of view (Veeraraju and Rengarao, 1990).

In this country, microorganisms have replaced adulterants as main agents of food poisoning which has become a major concern world-wide. About 1000 millions cases of acute childhood diarrhoea reported annually in the third world, are attributed mainly due to contaminated food and water (Malik and Nageshwar Rao, 1998).

Milk refrigeration at the farm reduces the growth rate of mesophilic bacteria, extending the time that milk can be stored before processing. However, it does not prevent the growth of psychrotrophic bacteria present as normal contaminants in raw milk. Although most of these psychrotrophic are killed by the individual heat treatment of milk, they can produce exocellular enzymes (proteinases and lipases) that are not completely inactivated by the heat treatment. These enzyme are capable of degrading various components affecting storage life of heat processed milk and the quality of dairy processing. Pasteurization is designed to destroy all bacterial pathogens common to raw milk excluding spore forming bacteria (Meer et al. 1991) and possibly mycobacterium paratuberculosis (Grant et al. 1996).

**BUTTER**

Milk fat plays an important part in infantile diet. It contributes almost 40-50 percent of the total energy in human milk. It is also a source of essential fatty acids and fat soluble micronutrients (Thompkinson and Mathur, 1987).

Butter is a smooth, fatty substance separated from the milk. It is defined as the food product exclusively made from milk or cream or both with or without common salt and with or without
additional colouring matter and contains not less than eighty percent by weight of milk fat. Butter was originally made direct from milk on a small scale and factory production became a common practice when gravity separation of cream was possible (Varnam et al. 1994). Its composition varies according to the method of manufacture and whether it was made from sweet or sour cream. Although butter is usually made from cow's milk, it can also be made from milk of other animals such as buffaloes, goats, donkeys, horses and camel. The names of the butter are specifically named after the source as peanut butter, cocoa butter, almond butter and coconut butter (Visual food, 1996).

Milk is a very perishable food product with outstanding importance as a food but dangerous if not in proper condition. Butter serves as the balance wheel of the dairy industry. Surplus milk is converted into butter during flush season (De, 1982).

**COMPOSITION OF BUTTER**

The variation in the composition of butter are slight because of the uniform methods of manufacture and the effort to keep the fat content near the legal minimum requirement. Salt is added to butter in order to improve its keeping quality as well as to meet the market demand. The amount of salt used varies with the region; most markets prefer 1.5-2.5 percent but in the south about 3 percent salt is added. Sweet butter contains no added salt. Diacetyl may be added as a flavouring agent but, if so used, the total diacetyl content must not exceed 4 ppm (Shivekumar et al. 1993).

**NUTRITIONAL VALUE OF BUTTER**

The nutritional value of butter depends almost entirely upon its content of fat and vitamins. The food value of butter is rated to its high milk fat content. It contributes a concentrated
source of energy, provides essential fatty acids and acts as a carrier of fat soluble vitamins. One pound of butter contained an average of 18000 International Units of vitamin A. The amount of vitamin D varies greatly. Butter fat is easily digested and it can be absorbed without producing digestive disturbances in larger amount than any other common fat. The digestibility of butter usually is stated to be 97.8 percent of the value found for milk (Lampert, 1974).

**PHYSICAL CHARACTERISTICS OF BUTTER**

The flavour of butter is difficult to describe as desirable or undesirable. This can not be measured chemically and can be described only by comparison with some well known flavour possessed by other substances. The colouring of the butter either too much or too little is objectionable. In general, the market requires a butter of a light straw colour (Downey, 1980; Deeth and Fitz-gerald, 1976).

**MICROBES IN BUTTER**

Presence of bacteria, yeasts and molds are undesirable in butter. When made from unpasturized cream, butter may contain any of the organisms present in the original cream or milk. Pasteurization of the cream destroys all pathogenic bacteria but some harmless organisms generally survive. Some of these are retained in the butter milk but others find their way into the butter. Pathogenic organisms are able to survive in butter and the bacteria of tuberculosis and typhoid fever have been isolated from butter made from contaminated cream (Ray, 1987).

There is no evidence to show that the keeping quality of butter is related in any direct way to the number of bacteria, yeast or molds that may be present. In the fresh butter of good quality, the predominating organisms usually are *Streptococci* and *Micrococci*. 
During storage, the Micrococci generally grow more rapidly than Streptococci.

The presence of rod shaped organisms in butter is undesirable because they often are proteolytic and produce cheesy, putrid and unclean flavours and odours (Woo and Lindsay, 1984). Proteolytic bacteria may be greatly outnumbered by harmless species and yet be present in sufficient number to produce surface taint. Under favourable conditions of temperature, salt concentration and moisture, Pseudomonas putrefaciens grow and cause surface taint but undoubtedly other organisms (Wagenaar, 1952). Lipolytic activities of yeast and molds are recognized to be generally responsible for the development of hydrolytic rancidity due to liberation of free fatty acids caused by lipolysis of fat through production of certain enzymes (Downess, 1959; Kaul et al. 1979). Butter wrapped in papers impregnated with sorbate or propionate could limit the mold growth on the surface when stored at slightly higher temperature.

In tropical countries like India spoilage of butter occur commonly in summer and especially when the transportation is done to far off places. Due to limited refrigeration facilities in rural areas, butter gets separated into fat and dahi, with the result the product no longer resembles butter in smell or taste. The ideal temperature for storage of butter of good keeping quality is -12°C to 18°C. Refrigerated storage of foods is a universally accepted method for prolonging their shelf life. One limiting factor of such storage is the presence of psychrotrophic bacteria (Anderson, 1980) which spoil food stuffs at low temperature. In chilled stored foods the lipolytic activity of psychrotrophic microorganisms can give rise to quality changes (Alford and Pierce, 1961).
FERMENTED MILK PRODUCT – DAHI OR CURD

Dahi or curd is the product obtained from pasteurized or boiled milk by souring, natural or otherwise by a harmless lactic acid or other bacterial culture (PFA, 1976). Dahi is commonly known as dahi in northern part of India, closely resembles yoghurt. This product possesses a characteristic type of flavour and taste. The popularity of dahi is not only due to its refreshing taste and palatability but also due to its scientifically proven nutritious quality as a milk product (Sharma et al. 1993). Dahi contains enough protein, carbohydrates, fat, minerals as well as vitamins which are useful for the well being of the human body. Over 40 percent of the total milk produced in India is converted into dahi.

Great interest exists in the research, commercial and consuming communities on the healthy effects of lactic acid bacteria. The term “lactic acid bacteria” applies to a functional grouping of friendly bacteria (non-pathogenic) that produce lactic acid and are traditionally used in food fermentation. The lactic acid bacteria used as a starter culture converts some protein into amino acids which is easily absorbed by the body and its digestion requires less energy. Dahi also contains more B-complex vitamins as compared to milk. Hence, fermented milk plays a useful role in preventing the gastrointestinal infections which cause diarrhoea. Studies have shown that fermented milk suppresses the growth of tumour in animals and decreases chances of cancer (Singh, 1996). Dahi is used as a part of daily diet and as a refreshing beverage. Dahi also forms an intermediate production in the preparation of several commercially important materials such as ghee and shrikhand (Rathi et al. 1990).

Two types of dahi are popular. One which is mildly sour wherein mostly Streptococci dominate and the other is highly acidic wherein large population of Lactobacilli dominate. Based on the
acidity (lactic acid percentage) dahi has been classified as sweet aroma dahi with the maximum acidity 0.7 percent and sour dahi with > 1.0 percent acidity.

It is assumed that fermented milk products are commonly free of pathogens because of the presence of lactic acid bacteria which produce lactic acid and anti-microbial compounds (Schaack and Marth, 1988). The special advantage of consuming dahi is for the lactose intolerant people who can not digest milk because the excess lactose in milk gives them cramps and other digestive discomforts. The bacteria in dahi convert a part of lactose into glucose and galactose and thus it can be easily digested by those people who are intolerant to lactose in milk.

Better growth and increased nutrient utilization efficiency associated with the consumption of fermented milk products. This beneficial effect has been attributed to a more digestible protein, enhanced bio-availability of minerals particularly iron and synthesis of B-group vitamins especially folic acid. It has been claimed that the fat is more digestible in dahi than in milk, because a certain degree of pre-digestion takes place during fermentation. The chief sources of energy in milk are fat and lactose. Fermented milk may be fortified with skim milk powders, caseinates, ultra-filtered concentrates, fruit pulp etc. They may, therefore provide the consumer with a higher intake of protein, carbohydrate, calcium and certain B-group vitamins than milk.

*Lactobacillus acidophiles* and *lactobacillus casei* play important roles. These organisms get implanted in the large intestine of human beings through regular consumption of the product thereby providing therapeutic benefits. Balasubramanyam and Varadaraj (1994) reported that dahi as a potential source of lactic acid bacteria is against food-borne pathogenic and spoilage bacteria. Among the pathogenic bacteria *Bacillus cereus* causes
food-borne diseases in human beings. In such circumstances bacteria present in the dahi increase the immunity of a person and increase the disease fighting ability. It increases the number of lymphocytes in the blood that fight back disease causing bacteria. This property controls various intestinal disorders such as diarrhoea, constipation and vomiting which are caused by the activity of the pathogenic bacteria (Gandhi and Nambudripad, 1975). Although a large number of reports have been published, only few seem to contribute convincingly to our knowledge of health effects of fermented milk on humans (Singh, 1996).

KHOA

Khoa is a popular milk product of Indian subcontinent and forms the base for several other products of significant economic value. It is a perishable product and has very limited shelf-life under ambient conditions.

Khoa means the product obtained from cow or buffalo or mixed milk by rapid drying. This is one of the most important of the unfermented milk products (Mathur, 1991). During its preparation heat is applied to evaporate the moisture in a rapid manner, therefore, the keeping quality of the milk is increased by the elimination of most of the bacteria (Rao et al. 1977). Khoa is generally used in the preparation of sweets like pedhas, milk cake, kalakand, gulabjamun, burfi and sometime in ice cream also.

Available data indicates that about 6.5 to 7.0 percent of the milk produced in India is utilized for khoa production which amounts to 3,22,000 tonnes (Mathur, 1991 and Alam, 1999). The total Indian sweet market is around 16,000 crore rupees in terms of annual sales and is by and large handled by the unorganized sector.
The existing largest market offers a good potential for the dairy plants to convert this surplus milk to khoa.

The shelf-life of khoa is about 2-4 days under ambient conditions and 3 weeks under refrigerator conditions. Khoa made by adapting the roller dryer process displayed a shelf-life of less than 5 days at 30°C, and 15 days under refrigerated storage. Addition of sugar to it further increased its keeping quality and this can be stored for a longer period than any of the other milk product having practically all the constituents of milk in concentrated form.

The effect of packaging materials on the keeping quality of khoa was studied by Rao et al. (1977). Investigations carried out on the preservation of khoa had shown that an initial moisture content of 20-25 percent and a temperature of 80-90°C at the time of packing in cans ensured minimum shelf-life of 14 days at 37±1°C. Packing at 25-30°C affected the acceptability adversely, thereby rendering the canned product unmarketable (Rudreshappa and De, 1971).

ICE CREAM

Ice cream may be defined as a frozen dairy product made by suitable blending and processing of cream and other milk products, together with sugar and flavour, with or without stabilizer or colour, and with the incorporation of air during the freezing process (Goyal et al. 1987). Fresh ice cream may be prepared from raw milk or pasteurized milk. Colouring agents are added to make it more attractive to the consumer.

Ice cream is one of the most popular dairy products relished by all classes of people. It is a palatable, highly nutritious, delightfully sweet and refreshing milk product. The preparation
needs costly ingredients and complex procedures which make it difficult to prepare at home. (Ramasamy et al. 2001).

**QUALITY OF THE ICE CREAM**

The quality of ice cream is determined by many factors like the composition of its raw materials, body and texture, flavouring and colouring agents added, processing and storage. The body and texture of ice cream made from buffalo milk may be slightly better than that from cow milk. Most of the ice cream consumed in the homes is vanilla flavoured. Vanilla ice cream combined with other flavours rates second followed by chocolate and strawberry ice cream (Rajalakshmi, 1983).

Ice cream as an industry in India is of comparatively recent origin. Today ice cream may be considered as a luxury food item, although its popularity is increasing rapidly. Consumption of ice cream in India is seasonal. It has yet to be accepted as a regular item in India as in many western countries. Ice cream making is a lucrative business in many countries, but the indiscriminate way and manner the products are produced calls for concern due to the inherent danger involved. The demand for ice cream has doubled in the past few years.

In eighties the great success of the ice cream industry appears due to the change in perception, encouragement of new ideas and products by the society. The eighties have seen the Indian ice cream industry growing faster than ever before in its five decades history. New flavours, exotic product combinations, attractive packaging, sophisticated advertising and an aggressive marketing have all helped the ice cream industry speed along the growth road at a pace closer to 25 percent annually.

Ice cream market is estimated around 150 crore rupees per year and is believed to be growing at the rate of 25 to 35 percent
annually. The accurate statistics in this wholly privately owned industry are hard to come by and unorganized sector is known to be several times larger than the organized one. It is felt that the present demand for ice cream can grow much more rapidly, given reasonable consumer prices, adequate production facilities, better quality product and wider retailer network (Moorthy and Balachandran, 1993).

ICE CREAM AND HEALTH HAZARDS

The bacteriological quality of ice cream in the market and the conditions of manufacture and sale, except in the case of few modern ice cream plants are unsatisfactory. The practices of hygiene and sanitation prevailing in ice cream industry give ample scope for the entry of bacterial contaminants which pose serious health hazards.

Ice cream is a good medium for the growth of microorganisms both pathogenic and non-pathogenic. The presence of enterococci in ice cream signifies the fecal contamination of the products and tends to reflect the sanitation of the production plant and the distribution agencies.

Investigations have shown that ice cream could support the growth of certain food poisoning organisms, having been in the past responsible for staphylococcal food poisoning and out-breaks of typhoid and paratyphoid fevers (Parry and Powsey, 1979). Further more, the source of water, storage condition, utensils used and repeated hand contact during preparation may contaminate the products which could result in various health hazards (Wachukwu et al. 2000) have shown that ice cream ranks next to milk among dairy products, as a cause of epidemics.

Ice cream is widely consumed in our country and may be subjected to contamination at various stages of preparation,
packaging and handling. The ingredients used in the ice cream also contribute to the microflora of the product and occasionally ice cream are implicated in food poisoning or gastroenteritis in human beings (Yadav et al. 1989).

In India ice cream produced, stored, transported and distributed is far from satisfactory and as such the situation warrants a strict vigilance and exposes the need for suitable sanitary standards for its manufacture and sale. (Prabhakar Rao et al. 1977). Turantas (2002) analysed fifty-three ice cream for total coliform, fecal coliform and fecal streptococci so that the value of fecal streptococci could be used as an indicator of fecal contamination and sanitation of ice cream. These results indicate that there is no direct relationship between the presence of fecal coliform and fecal streptococci and the high occurrence of fecal streptococci in ice cream suggests that fecal streptococci is a better sanitary indicator of ice cream.

Ice cream is likely to be contaminated with Salmonella sp. and other enteric pathogens. A small-scale producer provides further chances of contamination due to negligible and unhygienic methods, opening the ice cream container frequently during the sale and keeping it open over longer periods. The practice followed by the small-scale manufacturers for the sale of ice cream bars, without paper wrapper is yet another factor responsible for the poor quality of ice cream bars. Small-scale producers are not preparing ice cream of standard quality because of inadequate and unclean equipment as well as lack of scientific knowledge regarding the production of ice cream.
MICROBES INVOLVED IN THE CONTAMINATION OF MILK AND MILK PRODUCTS

BACTERIAL POPULATION

Total bacterial population commonly referred to as Standard Plate Count (SPC) can be a reliable parameter of food quality since the number of bacteria present depends upon the degree of contamination. Although they do not give any direct indication of the presence of pathogens, increased bacterial concentration is directly related to the outbreak of food-borne diseases. This is an empirical measurement because bacteria occur singly, in pairs, chains, clusters, or pockets, and no single growth medium or set of physical and chemical conditions can satisfy the physiological requirements of all bacteria in a sample. Consequently the number of colonies may be substantially lower than the actual number of viable bacteria present. To facilitate the collection of reliable data for milk and milk products, a standardized plate count to estimate the total bacterial population is essential.

COLIFORM ORGANISMS

The coliform group includes both fecal and non-fecal organisms. There are several reasons why coliform organisms are chosen as indicators of fecal contamination rather than food-borne pathogens directly.

The coliform organisms are constantly present in great abundance in the human intestine. It is estimated that an average person excretes 200-400 billion of these organisms per day. These organisms are foreign to milk products; hence, their presence in milk products is looked upon as evidence of fecal contamination.

They are easily detected by culture methods- as small as one bacteria in 100 milliliter of sample, whereas the methods for
detecting the pathogenic organisms are complicated and time consuming.

They survive longer than the pathogens which tend to die out more rapidly than coliform bacilli.

The coliform bacilli have greater resistance to the forces of natural purification than food-borne pathogens. If the coliform organisms are present in milk products, the assumption is the probable presence of intestinal pathogens.

The coliform group of bacteria includes all the aerobic and facultatively anaerobic, gram-negative, non-sporulating bacilli that produce acid and gas from the fermentation of lactose. The classical species of this group are *Escherichia coli* and *Enterobacter aerogenes*. *E.coli* is a normal inhabitant of the intestinal tract of man and other animals. These species bear a very close resemblance to each other in their morphological and cultural characteristics.

The importance of coliform organisms in the dairy industry lies in the fact that any of its members may find its way to the milk from the alimentary canal of vertebrates, thus indicating fecal contamination of milk and its products. Indeed all those engaged in dairy science look upon the presence of coliform organisms in milk or its products as a reliable indication of faulty methods of production and handling. Further these organisms are considered to be the frequent cause of spoilage in dairy products (Sadek and Eissa, 1957).

Many food poisoning outbreaks have been reported incriminating *E.coli* from many countries due to the consumption of contaminated food including dairy products. The enteropathogenic strains of the organisms have also been responsible for idiopathic, acute and infantile diarrhoea in Bangladesh, India, and United Kingdom (Kahlon and Joshi, 1983).
**Bacillus cereus**

Among the organisms responsible for causing food borne infections *Bacillus cereus* has received much attention of researchers during the last few decades (Soolton and Norris, 1987; Stephen et al. 1993).

The *B. cereus* is ubiquitous in nature and occurs widely in soil, cereals, spices, vegetables, dairy products, food and environment (Giffel et al. 1995). Starch rich foods like rice are considered to be the chief source of *B. cereus* (Blakey and Priest, 1980). Occurrence of *B. cereus* has been reported from foods like meat, snack and lunch foods (Bachhil and Jaiswal, 1988; Agarwal et al. 1997; Varadaraj et al. 1992). *Bacillus cereus* is a gram- positive, spore forming, motile, aerobic rod that also grown well anaerobically. It has been recognized as an opportunistic pathogen of increasing importance. Two types of illness have been attributed to the consumption of food contaminated with *B. cereus*. The diarrhoeal syndrome and the emetic syndrome (Granum, 1994). For both types of food poisoning, the food involved has usually been heat treated, and surviving spores germinate to produce somatic cells and toxins (Anon, 1972).

Optimal temperature for the growth of *B. cereus* is 30°C. The minimum and maximum temperatures for growth are 10°C and 49°C. The pH range for growth is 4.9 to 9.3. The incidence of *B. cereus* in milk samples was around 8.0 percent which may be attributed to the poor hygienic condition (Meena et al. 2000). The importance of *B. cereus* in milk and milk products have been emphasized by many workers (Ahmed et al. 1983; Kulshreshtha et al. 1984; Hin-chung et al. 1988). The organism is reported to produce several extracellular products such as hemolysin, phospholipase and a lethal factor responsible for food poisoning outbreaks (Johnson and Bonventre, 1967).
Staphylococcus aureus

Staphylococcus aureus is the commonly occurring food poisoning organism that produces enterotoxin in food during its growth. The toxin is termed as an enterotoxin because it causes gastroenteritis or inflammation of the lining of the intestinal tract.

The bacterium usually produces the enzyme which ferments mannitol and various sugars to form acid but no gas; Growth occurs over a wide temperature range from 6.5 to 50°C with the optimum between 30°C and 40°C depending on other growth conditions. Similarly Staphylococcus aureus can grow over a wide pH range of 4.2 to 9.3 with optimal growth between 7 and 7.5 (Baird Parker, 1965). This organism can usually occur in the presence of 15 percent sodium chloride and are relatively resistant to drying and heat.

Other important cultural characteristics of Staphylococcus aureus are colour pigmentation, generally a golden yellow and beta hemolysis on blood agar, although both of these characteristics are variable and also may be associated with strains of Staphylococcus epidermis. Several selective and differential media have been used for the isolation of Staphylococcus aureus from various sources. Staphylococcus aureus have been identified by the black coloured colonies on tellurite-based media. They reduce the tellurite salt to elemental tellurium (Varadaraj and Ranganathan, 1985).

In addition to extracellular coagulase, alpha-hemolysin and lipase, strains of Staphylococcus aureus produce enterotoxin that can cause food poisoning in human beings. Staphylococcal food poisoning is characterised by severe cramping, abdominal pain, nausea, diarrhoea, and vomiting that occur 2 to 6 hours after ingestion of food in which Staphylococcus aureus grew and produced
enterotoxin. The short incubation time is indicative of a true food intoxication. Duration of acute symptoms is usually less than 24 hours and the disease is rarely fatal, although fluid replacement may be necessary to compensate for the fluid lost through diarrhoea and vomiting.

Presence of *Staphylococcus aureus* in frozen dairy dessert also have been established (Foley and Sheuring, 1965; Tamminga *et al.* 1980). Foods can become contaminated with *Staphylococcus aureus* by a cough, or sneeze, but usually contamination is from an individual with an infection on the hands or with a cold or sore throat (Gourma *et al.* 1991).

**PROTEOLYTIC BACTERIA**

This is a heterogeneous group of bacteria which produces extracellular proteinases, so termed because the enzymes diffuse outside the cells. All bacteria have proteinases inside the cell, but only a limited number of kinds have extracellular proteinases. The proteolytic bacteria may be divided into those which are aerobic or facultative and may be spore-forming or not and those which are anaerobic and spore forming. *Bacillus cereus* is an aerobic, spore forming, proteolytic bacterium. Many of the species of *Clostridium*, *Bacillus*, *Psuedomonas*, and *Proteus* are proteolytic. Some bacteria termed "acid-proteolytic", carry on an acid fermentation and proteolysis simultaneously (Frazier, 1967). Some bacteria decompose proteins anaerobically (putrefactive) to produce foul smelling compounds such as hydrogen sulphide, mercaptans, amines, indole and fatty acids. Putrefication of split products of proteins can also take place.

Protein hydrolysis by microorganisms in food may produce a variety of odor and flavour defects. Some of the common psychrotropic spoilage bacteria are strongly proteolytic and cause
undesirable changes in dairy, meat, poultry and sea food products particularly when high populations are reached after extended refrigerated storage. On the other hand, microbial proteolytic activity may be desirable in certain foods, such as in the ripening of cheese where it contributes to the development of flavour, body, and texture (Jay, 1972).

**LIPOLYTIC BACTERIA**

This is a heterogenous group of bacteria which catalyses the hydrolysis of fats to fatty acids and glycerol. Many of the aerobic, actively proteolytic bacteria are also lipolytic.

\[
\text{Fatty foods} + \text{lipolytic microorganisms} \rightarrow \text{Fatty acids} + \text{Glycerol}
\]

The source of these organisms are mainly from soil, water, utensils.

Many foods contain significant amounts of fat and these fats are susceptible to hydrolysis and oxidation which lead to changes in flavour. Although many of the problems of fat breakdown are non-microbial in origin, numerous bacteria, yeasts and molds are also capable of causing both hydrolytic and oxidative deterioration. Only fatty acids of low molecular weight which are sufficiently volatile contribute directly to flavour changes. There are free fatty acids (FFA) released by hydrolysis is more susceptible to oxidation than is a fatty acid esterified in a triglyceride. Thus measurement of fat hydrolysis gives some indication about the oxidative changes taking place in the sample under investigation (Alford and Pierce, 1961; Alford et al. 1964).

The foods most often involved in problems of lipolysis are cream, butter, margarine, dressings, and other high fat products. Desirable flavors in many cheese and some other fermented foods also are associated with changes in the fat (Alford and Steinle, 1967).
The changes that microbes cause in foods are not limited to the results of degradation. They may also be caused by products of microbial synthesis. Some microorganisms discolour foods as a result of pigment production. Slimes may be developed in or on foods by microorganisms capable of synthesizing certain polysaccharides (Pelczar and Reid, 1985).

The genera *Pseudomonas*, *Achromobacter* and *Staphylococcus* amongst the bacteria, *Rhizopus*, *Geotrichum*, *Aspergillus* and *Penicillium* amongst the molds, and the yeast genera *Candida*, *Rhodotorula* and *Hansenula* contain many lipolytic species (Lawrence, 1967).

**PSYCHROTROPHS**

Since widespread use of refrigerated storage of milk products is in vogue, organisms capable of growth at these low temperature have assumed increasing importance. These organisms that grow at refrigeration temperature although their optimum growth temperature may be considerably higher, have been termed psychrotrophs (Eddy, 1960).

These bacteria do not constitute a taxonomic group but are found scattered in several unrelated genera such as *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Micrococcus*, *Serratia*, *Aeromonas*, *Acinetobacter*, *Enterobacter*, *Escherichia*, *Lactobacillus*, *Bacillus*, *Streptococcus*, *Alcaligenes*, *Proteus*, *Staphylococcus*, *Corynebacterium*, *Arthrobacter*, *Clostridium* and yeast and molds.

Psychrotrophs are distributed in diversified habitats as water, soil, utensils and vegetation. Most psychrotrophic microorganisms in milk and dairy products are come from soil, water and vegetation. The importance of psychrotrophic bacteria has greatly increased with extended storage of raw and pasteurized milk and other dairy products at cold temperatures. These bacteria are
generally non-pathogenic, but in dairy products they can cause a variety of off-flavours, including fruity, stale, bitter, putrid and rancid flavours, as well as physical defects. They are also involved in loss of flavour in cultured dairy products (Parker, 1953).

Most of these bacteria are gram-negative rods, although some are gram-positive, spore-forming and thermoduric rods belonging to the genera *Bacillus* and *Clostridium* (Overcase and Atmaram, 1974; Shedata and Collins, 1971). The numbers of psychrotrophic bacteria in raw milk depend upon sanitary conditions prevailing during production and upon the time and temperature of milk storage before processing.

The influence of psychrotrophic bacteria on the shelf-life of pasteurized milk will depend mainly upon the number present after packaging, the rate of growth, the storage period and the biochemical activity of the organisms. Even if no detectable changes occur, an increase in numbers of psychrotrophs may cause problems in meeting bacterial standards.

The presence of these organisms in milk and milk products indicate not only insanitary conditions but also the keeping quality of the products. It is well known that the condition under which ice cream is produced, stored, transported and distributed are far from satisfactory in India. Such a situation warrants a strict vigilance and exposes the need for suitable sanitary standards for the manufacture and sale of milk products preserved at low temperature.

At present in India there are no legal standards for psychrotrophs in milk products. Lack of legal standards and the ignorance of the producer in scientific methods of manufacture and handling has resulted in the poor quality of milk products. Entry of microorganisms in ice cream occur through ice cream mix, air and dispenser (Foster *et al.* 1957). Laxminarayana (1961) suggested that various types of equipments through which ice cream passed during
manufacture and handling might act as sources of contamination. Witter (1961) was of the view that the initial source of psychrotrophs in the dairy industry was water and the secondary sources were improperly cleaned equipments and utensils. Thomas et al. (1966) stated that most psychrotrophic organisms in milk and milk products usually come from soil, water and vegetation.

**YEAST AND MOLD**

**Yeast**

Yeast may be defined as microorganisms in which the unicellular form is conspicuous and which belong to the fungi (Lodder, 1970). The yeasts are ovoid, elliptical or rod shaped organisms varying in size between 10-15μ length. They are gram positive and non-motile with growth temperature ranging from 25-40°C. High acidity (pH 3.5) is suitable for their growth. They are strongly fermentative or oxidative in their metabolism of carbohydrates and organic acids (Foster et al. 1957). The different genera of yeasts related to dairy industry are *Candida* sp, *Rhodotorula* sp, *Saccharomyces* sp, *Torulopsis* sp and *Trichosporon* sp. (Lodder, 1970; Harrigan and McCance, 1976).

**Mold**

Mold are complex multicellular fungi which are capable of sexual and asexual multiplication. The growth depends on a wide range of pH, temperature and osmotic pressures. Mold actively dissimilate carbohydrates, fat and proteins from the substrates by intracellular and extracellular enzymatic systems (Foster et al. 1957). The main features of different genera of molds in relation to dairy industry are *Aspergillus* sp, *Cladosporium* sp, *Fusarium* sp, *Penicillium* sp and *Rhizopus* sp. (Harrigan and McCance (1976) and Onions et al. 1981).
Among the spoilage microorganisms yeasts and molds occur frequently in dairy products as contaminants. The contamination of yeast and molds in milk and milk products originates from soil, air, feed, coat of the animal, utensils used for handling of milk, manufacturing and packaging equipments and place of storage. The yeast and molds extend their contamination by utilizing a wide range of substrates and sporulate freely under variety of conditions.

The yeasts and mold generally grow in conditions that are unfavourable for growth of many bacteria. Further, they grow and multiply at low pH, reduced water activity levels and wide range of temperature. The incidence of yeast and molds in milk and milk products have been reported by various workers (Deak and Beuchat, 1987; Khoburger and Fachat, 1975; Fleet and Mian, 1990 and Ghodekar et al. 1980).