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 promotors 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 B₁), riboflavin (vitamin B₂), pyridoxine (vitamin B₆), pantothenic acid, niacin, biotin, folic acid, cyanocobalamine (vitamin B₁₂) 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 Kowski 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 (Veerraju 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 inactivitated 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 *Micrococcic* 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. Balasubramaniam 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.
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 Powseney, 1979). Furthermore, 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
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 out breaks 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.

Fatty foods + lipolytic microorganisms → Fatty acids + 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 dispensor (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).
MATERIALS AND METHODS
MATERIALS AND METHODS

SOURCES OF MILK SAMPLES

Homes
Milk samples were obtained from the individual houses where milk was produced from a few animals reared.

Vendors
Milk samples were obtained from milk vendors who supply milk regularly to houses in the morning and evening.

Local Dairy Farms
Milk produced by herds of dairy animals reared in the individual farms was sold either at the production site or taken to private selling outlet. Samples of such milk were collected.

Milk Producer’s Society
Milk from individual farms located in that area sold as it is to people or sent to far off destinations for the preparation of milk products was also collected.

Packaged Milk
Commercially available popular brands of packaged milk were purchased and subjected to various analysis.
Two hundred and fifty milliliter of each sample was collected for analysis.

SOURCES OF BUTTER AND DAHI SAMPLES

Homes
Butter and dahi samples were obtained from individual houses where butter is prepared.

Vendors
Butter and dahi samples were purchased from the milk maids selling in the street.
Retail Shops

Butter and dahi samples were collected from shops meant for selling dairy products. In the case of dahi, samples were also collected from Restaurants in and around Courtallam.

Sample size: 250 gm

SOURCES OF KHOA SAMPLES

Vendors

Khoa samples were purchased from the vendors selling in the streets.

Retail Shops

Khoa samples were obtained from shops meant for selling dairy products.

Sample size: 250 gm

SOURCES OF ICE CREAM SAMPLES

Seven well known brands of ice cream sold in Courtallam area were taken for this study.

Sample size: 200 gm

SAMPLING OF MILK

Several precautions were taken during sampling of milk. The samples were collected in sterile glass stoppered bottles and care was taken not to wet the stopper or the neck of the bottle. The stopper was removed from the bottle until necessary and replaced immediately after the sample was obtained. The bottle was not filled more than three-quarters so that milk might be shaken before examination. The sample must be the representative one of the milk examined. When samples from bulk milk was taken the contents of the milk tank was thoroughly mixed with a sterile plunger and a
Milk being received in a milk processing unit

Milk is poured into the receptacle for further processing
Sample collection for laboratory analysis from milk producer’s society

Sample collection from street vendor
Pasteurization plant in a milk processing centre

Packaging of milk in the processing centre
Packaged milk taken for the study

Measurement of specific gravity of milk immediately after sample collection
Cream separator in a milk processing centre

Butter Churn in a milk processing centre
quantity proportional to the amount of milk in the tank was removed with a sterile dipper. After collection, the samples were cooled and carried for analysis in an ice-box and brought to the laboratory immediately. Representative milk samples were obtained for evaluating its biochemical and microbiological quality.

The milk samples were thoroughly mixed by shaking the bottle 25 times with an excursion of about 1 foot for a time occupying approximately 12 seconds to obtain a uniform sample for plating.

Six samples were collected from various sources namely Homes, Vendors, Local dairy farms in Tenkasi, Melagaram, Courtallam, Shencottah and Viswanathapuram.

In addition to the above, twelve milk samples were collected from Milk Producer's Society located at Tenkasi and Shencottah. Six samples of seven brands of commercially available packaged standardized milk were collected for both biochemical and microbiological studies. At that time of sampling, temperature and aseptic condition were also properly maintained.

For microbiological examination, one portion of the sample was preserved in a clean sterilized container and kept in the refrigerator at 4 ± 1°C.

**SAMPLING OF BUTTER**

A total of ninety samples of butter were collected from five different places in sterile wide mouthed bottles for biochemical analysis and enumeration of standard plate count, coliforms, proteolytic, lipolytic, psychrotrophic bacteria and yeast and mold counts with due precautions. These six samples of butter were collected from Tenkasi, Melagaram, Courtallam, Shencottah and Viswanathapuram.
While sampling the butter the following principles were observed.

1. True representative samples were collected and examined.
2. The samples were collected without the entering of any extraneous matter.
3. Aseptic condition was maintained during the period between collection and analysis.

With a sterile stainless steel spatula 120 gram of butter was removed from 3 different corners of the container by inserting the trier diagonally through the butter samples and stored suitably at a temperature between 4 ± 1°C. While drawing the sample for bacteriological examination, all the equipments and containers were sterilized at 160°C in a hot air oven for not less than 2 hours.

All the samples were analysed biochemically and microbiologically. The butter samples were stored at 4 ± 1°C for 30 days and the same evaluation tests were conducted.

**SAMPLING OF DAHI**

A total of one hundred and eight samples were collected from five places in and around Courtallam. Six samples from each source namely homes, vendors, retail shops for dairy products and restaurants were collected in a sterile wide mouthed bottles with glass stopper. Minimum time was allowed to elapse between collection and examination of the sample. All biochemical and microbiological evaluations were done as per milk samples.
**SAMPLING OF KHOA**

Sixty samples of market khoa were collected from vendors and shops in Tenkasi, Melagaram, Shencottah, Courtallam and Viswanathapuram. The samples were carefully collected in sterilized containers and brought to the laboratory in an ice-box for biochemical and microbiological analyses.

For the microbiological analysis the samples were well grained and made into a paste using 0.9 percent sodium chloride solution as diluent and then serial dilution were made before the commencement of microbiological studies.

**SAMPLING OF ICE CREAM**

Samples of ice cream were purchased from various sources in and around Courtallam. Seven types of packaged ice cream locally and commercially produced were analysed.

The various brands of ice cream products examined were referred to as A, B, C, D, E, F and G. The representative portion of the samples were transferred aseptically into a sterile container of not less than 100 milliliter capacity. Frozen samples were left at room temperature for a maximum period of one hour until thawed. A portion of the sample was taken and kept in a sterile container for microbiological studies.
BIOCHEMICAL ANALYSIS OF MILK

DETERMINATION OF pH

pH of all the test samples were determined by systronics pH meter model LI – 120.

The pH value, or negative logarithm of hydrogen ion concentration, gives a measure of the true acidity of milk. The relationship between pH and acidity of milk is only approximate. The pH test is mainly used for the detection of abnormal mastitis milk (ISI, 1960).

DETERMINATION OF SPECIFIC GRAVITY OF MILK

The specific gravity of milk was determined according to the method followed by (ISI, 1977).

Principle

The specific gravity of milk is the ratio between the weight of certain volume of milk at the standard temperature of 15.6°C and the weight of the same volume of water at the same temperature. Since temperature influences specific gravity, it should be done at standard temperature.

Procedure

The sample of milk to be treated was thoroughly shaken and brought to a temperature between 10°C to 21.1°C. The milk was taken in the cylinder and lactometer was lowered into the milk without touching the walls of the cylinder. The lactometer reading was noted.

Calculation

\[
\text{Specific gravity} = \frac{\text{C.L.R.}}{1000} + 1
\]
DETERMINATION OF MOISTURE IN MILK

The Moisture content in milk was carried out as per APHA (1975).

Procedure

A previously weighed, heated and cooled crucible with 10 grams of milk was weighed accurately. The crucible was heated in an hot air oven at 70-100°C for 4 hours. Then the crucible was transferred into a desiccator, cooled, and then weighed. The crucible was placed in the oven for 30 minutes, cooled in a desiccator and weighed. The above procedure was repeated till the concordant value was obtained.

\[
\text{Percentage of moisture} = \frac{W_1 - W_2}{W_1 - W} \times 100
\]

where

\[W = \text{Weight of empty crucible}\]
\[W_1 = \text{Weight of crucible + milk before drying}\]
\[W_2 = \text{Weight of crucible + milk after drying}\]

DETERMINATION OF TOTAL SOLIDS

Estimation of total solids was carried out according to the method given in APHA (1975).

Principle

The total solid content of the sample determines the solids present in milk.

Procedure

A clean, dry, empty silica crucible was weighed with lid (w₁). Five milliliter of the representative milk sample was pipetted out into the crucible and was weighed with the lid (w₂). Then the crucible was placed in a water bath. The base of the crucible was
kept horizontal to promote uniform drying and it was protected from direct contact with the metal of the water bath. After 30 minutes the crucible was removed, the bottom was wiped and was transferred to a well ventilated oven maintained at 98 – 100°C. After 3 hours the crucible and the lid was transferred to a desiccator. The crucible was allowed to cool for 30 minutes and reweighed (W₃). The weighing was repeated until the loss of weight between successive weighing does not exceed 0.5 milligram and the lowest reading was noted.

**Calculation**

\[
\text{Percentage of total solids} = \frac{W₃-W₁}{W₂-W₁} \times 100
\]

where

- \(W₁\) = Weight of empty crucible
- \(W₂\) = Weight of crucible + sample
- \(W₃\) = Weight of crucible + residue after drying

**DETERMINATION OF VOLATILE SOLIDS**

Volatile solids of samples were determined by the method described in APHA (1975).

A known quantity of dry matter was taken in a pre-weighed silica crucible and kept in the muffle furnace for 30 minutes, which was set for 550°C. After cooling in a desiccator for 30 minutes, the weight was taken. This process was repeated to get the concordant value. From the weight the percentage of volatile solids was calculated as follows.
Calculation

\[
\frac{(A-B) \times 100}{W}
\]
Percentage of volatile solids = \(--\)

where,

- \(A\) = Weight of crucible + sample before ignition
- \(B\) = Weight of crucible + sample after ignition
- \(W\) = Dry weight of the sample.

ESTIMATION OF FAT (Gerber method)

The estimation of fat was carried out according to the method given in ISI (1977).

Principle

When definite quantities of sulphuric acid and amylalcohol are added to definite volume of the sample, protein will be dissolved and the fat globules will be set free, and remain in liquid state due to heat produced by the acid. On centrifugation, fat being lighter will be separated on the top of the solution.

Reagents

(i) Sulphuric acid (Gerber) - Density 1.807 – 1.812 g/ml at 27°C.

(ii) Amylalcohol - Density 0.803 – 0.805 g/ml at 27°C.

Procedure

Ten milliliter of Gerber sulphuric acid from automatic measure was taken into the butyrometer. Accurately 10.75milliliter of representative milk sample was pipetted out into the butyrometer without allowing the milk to mix with the acid. This was done by
allowing the jet of milk from the pipette to hit the inside wall of the butyrometer, by holding the pipette in a slanting manner and resting the tip on the mouth of the butyrometer. With the help of automatic measure one milliliter of amyl alcohol was added to the butyrometer. The stopper was tightened and the contents were mixed well. Then the butyrometer was centrifuged for five minutes at 1200 rpm. After centrifugation the butyrometer was kept in a water bath at 65°C for five minutes. The reading was taken after adjusting the fat column to be within the scale of butyrometer and recorded.

**ESTIMATION OF TITRABLE ACIDITY**

Estimation of titrable acidity was carried out according to the method described in ISI (1960).

**Principle**

The acidity is determined by neutralization of the acid by the known amount of standard alkali solution. Normally 0.1 N alkali neutralizes 0.009 gram of lactic acid.

**Reagents**

1. 0.1 N Sodium hydroxide solution
2. Phenolphthalein indicator

**Procedure**

Ten milliliter of milk was taken in a clean conical flask and equal quantity of distilled water was added to it, followed by the addition of few drops of phenolphthalein indicator. It was titrated against 0.1 N Sodium hydroxide solution until a faint pink colour persists. The titrations were repeated to get concordant values.
Calculations

Percentage of acidity \( = \frac{9 \times N \times V}{W} \)

where,

\( V \) = Volume of 0.1 N sodium hydroxide required
\( W \) = Weight of sample
\( N \) = Normality of sodium hydroxide solution

DETERMINATION OF LACTOSE (Bock's method)

Lactose estimation was carried out by the method given in Oser (1965)

Principle

Benedict's reagent for the estimation of reducing sugar contains potassium thiocyanate as well as copper sulphate and in the presence of the former a white precipitate of cuprous thiocyanate is formed by reduction instead of the usual red precipitate of cuprous oxide. The small amount of potassium ferrocyanide also aids in keeping cuprous oxide in solution. The appearance of dirty white precipitate with the loss of all blue tint indicates complete reduction of the copper. The alkali used is sodium carbonate which has the advantage over the hydroxides of causing less likely destruction of small amounts of sugar and is recommended for its simplicity and accuracy.
The carbonate, citrate and thiocyanate were dissolved in enough water and filtered. The copper sulphate was dissolved separately in about 10 milliliter of water and poured slowly into the above mixture with constant stirring. Ferrocyanide solution was added and diluted to exactly 1000 milliliter with distilled water.

**Reagents**

1. 2/3N sulphuric acid
   1.86 milliliter of concentrated sulphuric acid was taken and made upto 100 milliliter using distilled water.
2. Sodium tungstate - 10 percent solution

**Procedure**

Twenty milliliter of the sample was taken in 100 milliliter standard flask. Twelve milliliter of 10 percent sodium tungstate and 12 milliliter of 2/3N sulphuric acid were added to it. The contents were mixed well and made upto the mark with distilled water. The contents were filtered and the filtrate was filled in a burette. Twenty five milliliter of benedict’s solution was pipetted out into a conical flask. A few bits of porcelain and a pinch of sodium carbonate were
added to it. The solution was boiled for a few minutes and titrated against solution in the burette. Since, the titration must be done in a hot condition, the benedict solution was heated each time after adding a few drops of titrants. When the colour changed to greenish, the titrant was added in drops. The end point is the appearance of dirty white precipitate.

**Calculation**

\[
\text{Percentage of lactose} = \frac{0.067 \times 100 \times 100}{\text{Titre value x weight of sample}}
\]

**Weight of sample** = Specific gravity of sample x Volume of Sample

(25 milliliter of Benedict's reagent is equivalent to 0.067 milligram of lactose).

**ESTIMATION OF NITROGEN CONTENT**

Nitrogen content of samples were estimated by the method given by Jackson (1958)

**Principle**

Nitrogen present in the samples will be converted in to ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed was subsequently decomposed by a fixed alkali (sodium hydroxide) and the liberated ammonia was trapped in a weak acid (boric acid) of known strength. Finally it was titrated against very dilute sulphuric acid and the nitrogen content was computed.
Reagents

1. 40 percent sodium hydroxide
2. 2 percent boric acid
3. Mau-Zazuga indicator (Double indicator)
   80 milligram of bromocresol green and 16 milligram of methyl red were dissolved and the volume was made up to 100 milliliter with 95 percent ethanol. The pH range was adjusted using a drop of hydrochloric acid.
4. N/70 sulphuric acid:
   This N/70 sulphuric acid was prepared accurately from 0.1 N sulphuric acid.
5. Preparation of 0.1 N Oxalic Acid
   Exactly 630 milligram of oxalic acid was dissolved in distilled water and the volume was made up to 100 milliliter in a volumetric flask.
6. 0.1N Sodium Hydroxide
   Accurately 1.0gram of sodium hydroxide was dissolved in 250 milliliter of distilled water.
7. Standardization of N/70 Sulphuric Acid
   Sodium hydroxide (0.1N) was taken in the burette. 5.0 milliliter of oxalic acid was pipetted out in a conical flask. A drop of phenolphthalein indicator was added. This was titrated against 0.1N sodium hydroxide. The end point was the appearance of pink colour. The titration was repeated to get concordant values.
   The normality of the sodium hydroxide solution was calculated. Five milliliter of 0.1N sulphuric acid was pipetted out and was titrated against the 0.1N sodium hydroxide solution using the phenolphthalein indicator. The end point was the appearance of pink color. The experiment was repeated to get concordant value and the normality of the sulphuric acid solution was calculated.
From this normality, the volume needed for preparing the N/70 sulphuric acid was calculated.

8. Standard for Nitrogen

Accurately 36.39 milligram of Ammonium sulphate was weighed and dissolved in distilled water and made upto 100 milliliter. 5.0 milliliter of this solution contains 1.0 milligram of nitrogen. This was distilled in the microkjeldhal distillation apparatus and the liberated ammonia was collected in 2 per cent boric acid and titrated with N/70 sulphuric acid and milligram of nitrogen equivalent to 1.0 milliliter of N/70 sulphuric acid was calculated.

Procedure

Exactly 100 milligram of oven dried powdered sample was digested with 3.0 milliliter of concentrated sulphuric acid, a drop of perchloric acid and a pinch of copper sulphate till the solution became colourless. The digested sample was made upto 50.0 milliliter with nitrogen-free water. In the case of milk sample, one milliliter was wet digested in the similar manner indicated above. 2.0 milliliter of this solution was pipetted out into kjeldhal distillation flask followed by 4.0 milliliter of 40 per cent sodium hydroxide solution and distilled for about 10 minutes. The liberated ammonia was collected in a receiver containing 5.0 milliliter of 2 per cent boric acid containing the indicator. The liberated ammonia was titrated against N/70 sulphuric acid.

Calculation

\[
\text{Percentage of Nitrogen} = \frac{(A-B)}{\text{Volume of digested sample}} \times \frac{\text{Total volume of sample}}{\text{Quantity of sample taken for digestion}} \times 100 \times \text{Strength of N/70 } H_2SO_4
\]

\[A = \text{Titer value of the sample} \quad \quad B = \text{Blank}\]
ESTIMATION OF PROTEIN

The protein content of the samples were calculated by multiplying the nitrogen content by the factor 6.25 for milk and by 6.38 for milk products.

ESTIMATION OF CALCIUM

Estimation of calcium was carried as per the method described in APHA (1975).

Principle

Ammonium oxalate precipitates calcium quantitatively as calcium oxalate. The precipitated calcium oxalate is dissolved in acid and titrated with permanganate. The amount of permanganate required to oxidize the oxalate is proportional to the amount of calcium.

Reagents

1. 4 percent Ammonium oxalate solution
2. 2 percent Ammonia solution
3. 2N Sulphuric acid
4. 0.1 N Potassium permanganate solution diluted to 0.05N.

Standardisation of Potassium Permanganate

The stock solution was prepared by dissolving 3.2 gram of potassium permanganate in a litre of distilled water by heating to 70 to 80°C. This solution was stored in an amber coloured bottle. 110 milliliter of the stock solution was made up to 1 litre. This diluted potassium permanganate solution was standardized using 25 milliliter of 0.1N oxalic acid mixed with equal volume of dilute sulphuric acid. The titration was done in hot condition. The end
point was the appearance of a pale permanent pink color. From this, the normality of potassium permanganate was calculated.

**Procedure**

Two grams of the sample was taken in a silica crucible and it was ashed up in a muffle furnace at 550°C. This was dissolved in 2 or 3 drops of concentrated hydrochloric acid and made up to the final volume of 25 milliliter with distilled water. Two milliliter of ammonium oxalate was added to 2.0 milliliter of this solution and allowed to stand over night. Then it was centrifuged and supernatant was removed without disturbing the precipitate, was washed with 2 percent ammonia solution till it gave no precipitate with calcium chloride solution. The precipitate was then warmed with 2.0 milliliter of 2 N sulphuric acid and titrated against 0.05 N potassium permanganate to a pale permanent pink color, which persisted for about a minute. Two milliliters of 2 N sulphuric acid served as the blank.

**Calculation**

\[
\text{Calcium (milligram / 1000 gram or liter)} = \frac{(A-B) \times N \times 20040}{2}
\]

where,

- \(A\) = Titre value for the sample
- \(B\) = Titre value for blank
- \(N\) = Normality of potassium permanganate.
ESTIMATION OF MAGNESIUM (Titan yellow method)

The estimation of magnesium was carried out according to the procedure given by Neil and Neely (1956).

Principle

Magnesium is complexed with titan yellow (a dye) in an alkaline medium and the intensity of the red colour is measured colorimetrically.

Reagents

1. Polyvinyl alcohol 0.05 percent
2. Titan yellow 0.05 percent
3. Sodium hydroxide 4 N

Procedure

One milliliter of representative solution was taken in a clean test tube along with 2 milliliter of distilled water and mixed well. One milliliter of 0.05 percent polyvinyl alcohol was added followed by 1 milliliter of 0.05 percent titan yellow. The mixture was shaken thoroughly. Finally 2 milliliters of 4 N NaOH was added and mixed well. Immediately the colour was read in a spectrophotometer (Spectronic 21 – Bauch and Lomb) at 540 nm against the blank treated in the same manner with one milliliter of calcium chloride instead of sample. Simultaneously 1.0 milliliter of working standard solution containing 0.04 milligram of magnesium was taken and treated in the same way as the sample. The quantity of magnesium was calculated as follows.

Calculation

\[
\text{Magnesium (milligram/l)} = \frac{\text{Density of unknown} - \text{Density of blank}}{\text{Density of standard} - \text{Density of blank}} \times 1000
\]
ESTIMATION OF IRON

The estimation of iron was carried out according to the method given by Banerjee (1978).

Reagents

1. Potassium thiocyanate - 3 N
2. Saturated potassium persulphate solution.
3. Concentrated sulphuric acid.

Procedure

Two grams of the sample was ashed up in a muffle furnace at 550°C and dissolved in 2 or 3 drops of concentrated hydrochloric acid and made up to 25 milliliter with distilled water. To one milliliter of this solution, 0.4 milliliter of saturated potassium persulphate solution, 0.3 milliliter of concentrated sulphuric acid and 1.6 milliliter of 3N potassium thicyanate were added. Final volume was made up to 10.0 milliliter with distilled water. The colour developed was read in a spectronic-21 at 540 nm against a reagent blank within 10 minutes. The iron content of the unknown sample was calculated from the standard graph drawn by using ferrous ammonium sulphate.

Calculation

<table>
<thead>
<tr>
<th>Standard Value</th>
<th>Sample OD</th>
<th>Volume of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (µ/100ml)</td>
<td>0.1 OD</td>
<td>Amount of digested sample taken for analysis</td>
</tr>
<tr>
<td></td>
<td>x</td>
<td>Amount of sample taken</td>
</tr>
</tbody>
</table>
ESTIMATION OF SODIUM

The estimation of sodium was carried out according to the method given in APHA (1995). The quantity of sodium in the samples was determined. The solution under analysis is sprayed in a flame photometer using appropriate filter.

The flame photometer was standardized using the known standard sodium solution, 5 milliliter of the solution was fed directly into the flame photometer. The sodium content was read as ppm. For samples containing high concentrations of sodium appropriate dilution with deionised distilled water were made before feeding into the flame photometer.

ESTIMATION OF POTASSIUM

The estimation was also carried out by flame photometry given in APHA (1995)

Principle

The solution under analysis is sprayed as a fine mist into a non-luminous flame which becomes coloured according to the characteristic emission of the metal. The wave length corresponding to the element being analysed is selected by a light filter and allowed to fall on a photodetector whose output is a measure of concentration of the element. The output of the photodetector is connected to an electronic metering unit to provide the read out.

Reagents

1. Ammonia Solution

Forty milliliter of concentrated ammonia solution was mixed with 200 milliliter of distilled water.
2. **Stock Standard**

Accurately 1.907 gram of dried potassium chloride was dissolved in distilled water and made up to 1000 milliliter. The concentration of this solution is 1000 ppm.

3. **Working Standard**

Ten milliliter of stock potassium solution was made up to 100 milliliter. The concentration of this solution now is 100 ppm.

**Procedure**

Samples digested for nitrogen estimation was used for the determination of potassium. Five milliliter of the digested sample was neutralized with equal amount of ammonia solution and fed into the flame photometer. The potassium content was directly read out as ppm. The instrument was standardized using different concentrations of working standard solutions.

**ESTIMATION OF CHLORIDE**

Estimation of chloride was done according to the method described in AOAC (1975)

**Principle**

Silver nitrate reacts with chloride to form very slightly soluble white precipitate of silver chloride. At the end point when all the chlorides get precipitated, free silver ions react with chromate to form silver chromate of redish brown colour.

**Reagents**

1. Silver Nitrate (0.1 N)
2. Potassium dichromate indicator (10 percent)
Procedure

Nine milliliter of milk sample was pipetted out in a clean conical flask. One milliliter of potassium dichromate was added as indicator. The contents were titrated with 0.1 N silver nitrate till the appearance of first perceptible pale red-brown colour lasting for 30 seconds.

Calculation

\[
\text{Percentage of } \text{chloride} = \frac{\text{Volume of silver nitrate} \times \text{Normality of silver nitrate}}{\text{Weight of sample}} \times 3.55
\]

Factor 3.55 = \[
\frac{35.46}{1000}
\]

where 35.46 is the molecular weight of chloride.

Weight of sample = Volume of milk \times \text{specific gravity of milk}

ESTIMATION OF PHOSPHORUS

The phosphorus content of the samples were determined by the method given by Fiske and Subbarow (1925)

Principle

Phosphorus reacts with molybdic acid to form phosphomolybdic acid. This on treatment with 1, 2, 4 amino napthosulphonic acid is relatively reduced to produce a deep blue color (Molybdenum blue) which is probably a mixture of lower acids of molybdenum. This color is measured in a spectrophotometer. Actual quantity of phosphorus was estimated from the standard graph prepared from standard phosphorus solution treated in the same way.
Reagents

1. Molybdate solution – I
   Accurately 2.5 grams of ammonium molybdate was weighed and dissolved in 5.0N sulphuric acid and made up to 100 milliliter.

2. Molybdate solution – II
   Exactly 2.5 grams of ammonium molybdate was weighed and dissolved and made up to 100 milliliter with 3.0 N sulphuric acid.

3. Ansa Reagent (Fiske and Subbarow reagent)
   Accurately 0.5 gram of 1,2,4 amino naphthalosulphonic acid was weighed and dissolved in 100 milliliter of glass distilled water. This was stored in an amber coloured bottle in a cool place.

4. Phosphorous Solution (Stock Standard)
   Precisely 35.1 milligram of potassium dihydrogen phosphate was weighed and dissolved in water with one milliliter of 10N sulphuric acid and made up to 100 milliliter in a volumetric flask.

5. Working Standard
   Ten milliliter of the stock standard solution was pipetted out and made up to 100 milliliter in a volumetric flask with distilled water.

Procedure

Various volumes of working standard solution 1.0, 2.0, 3.0, 4.0 and 5.0 milliliter were pipetted out into a series of test tubes. The concentrations of above solutions were 8, 16, 24, 32 and 40 microgram of phosphorus respectively. One milliliter of the digested sample was pipetted into another test tube. ANSA (0.4 milliliter) and 1.0 milliliter of molybdate I were added to standard and 1.0 milliliter of molybdate II was pipetted into the unknown samples respectively.
The volume was made up to 10 milliliters with distilled water. It was mixed well and the developed color was read in a spectronic – 21 at 660 nm after 20 minutes.

The standard graph was drawn using the standard values.

**MICROBIOLOGICAL ANALYSIS OF MILK**

**CLOT ON BOILING (C.O.B) TEST**

The test was conducted according to the procedure given in ISI (1960).

This is a quick test to determine the developed acidity and the suitability of milk for processing.

Five milliliters of the sample was taken in the test tube placed in a boiling water-bath and held for about 5 minutes. The sample was smelt for any acidic flavour. The tube was removed and rotated in an almost horizontal position and examined the film of milk or side of the test-tube for any precipitated particles. The formation of clot was indicative of a positive test. Milk which gave a positive COB test had an acidity generally above 0.17 percent (as lactic acid) and was considered as not suitable for distribution as liquid milk for processing.

**METHYLANE BLUE REDUCTION TEST**

This test was carried out by ISI (1960). When methylene blue is mixed with contaminated milk, the methylene blue loses its colour (becomes reduced). This is the basis for the reductase test.
Procedure

Ten milliliters of thoroughly well mixed milk was taken in a sterile test tube, one milliliter of methylene blue was added to the above tubes. Then the tube were sealed with a sterile rubber stopper with a sterile forceps and was slowly inverted three times to mix the dye with the milk and immediately the tube was placed in a water bath at 37.5°C. The level of the water in the water bath should be above the level of the milk in the tubes.

Every half an hour the tubes were observed and those which had decolourized were removed from the bath and the time taken for reduction was noted. Those which had not decolourized were inverted once and replaced in the water bath and those which had shown partial decolourization were not inverted and placed in the water bath again. The tube without addition of methylene blue also was kept as a control.

The reduction time of milk is roughly proportional to the bacterial count of milk. Under results the relationship may be summarized as follows:

<table>
<thead>
<tr>
<th>Class of milk</th>
<th>Reduction time</th>
<th>Number of organisms per milliliter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I - good</td>
<td>Over 4½ hours</td>
<td>200,000 or less</td>
</tr>
<tr>
<td>Class II - Average</td>
<td>2½ to 4½ hours</td>
<td>200,000 – 2,000,000</td>
</tr>
<tr>
<td>Class III - poor</td>
<td>Less than 2½ hours</td>
<td>2,000,000 – 10,000,000</td>
</tr>
</tbody>
</table>

Preparation of Methylene Blue Solution

One tablet was dissolved in 200 milliliter of cold sterile glass distilled water in a sterile flask. Then it was made upto 800 milliliter using cold glass distilled sterile water. The flask was closed with sterile rubber bung and stored in cold dark place. The solution should not be kept for more than 14 days.
DETERMINATION OF TOTAL MICROBIAL POPULATION

The total microbial population was carried out according to the method described in Standard methods for the examination of dairy products (APHA, 1978). Nutrient agar medium was used for the enumeration of bacterial population.

**NUTRIENT AGAR MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>5</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.5</td>
</tr>
<tr>
<td>Glucose (Dextrose)</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>6.5</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
</tbody>
</table>

pH was adjusted to 7.0 ± 0.1 after sterilization.

The ingredients were dissolved, sterilized at 15 lb pressure in an autoclave at 121°C for 20 minutes. After cooling, the medium was used for plating. Sterile distilled water or 1.25 percent sodium citrate or 0.9 percent sodium chloride solution was used as diluent.

**Dilution**

Three or more test tubes containing exactly 9 milliliter of 0.9 percent sterile saline solution were arranged in a test tube rack. These are called dilution blanks. One milliliter of thoroughly mixed milk sample was pipetted by means of a sterile pipette and transferred to the first tube of the dilution blank. All the transfers were done under aseptic conditions. Care was taken to see that only the tip of the pipette was inserted into the liquid. In order to rinse the pipette the contents of the pipette was raised and lowered six times.
times and finally allowed about three seconds to drain and the remaining contents were blown out. All these manipulations were carried out under aseptic condition.

**Plating Technique**

One milliliter of thoroughly mixed sample was transferred to the test tube containing 9 milliliter of diluent. From this dilution serial dilutions were made ranging from $10^{-1}$ to $10^{-4}$. One milliliter of $10^{-4}$ dilution was poured into the pre-sterilized petriplates and then 15-20 milliliter, well mixed and previously cooled nutrient agar medium was poured into the petriplates. The plates were rotated for the contents to mix well. All aseptic precautions were taken till inoculations were completed. After setting of the medium, the plates were inverted and kept in the incubator at 37°C for 24-48 hrs. Then the colonies were counted using colony counter.

**Calculation**

$$\text{Number of bacteria / ml} = \text{Number of colonies per plate} \times \text{dilution factor}$$

**DETERMINATION OF COLIFORM COUNT – APHA (1978)**

The methods used for the determination of coliform organisms present in the market samples were

1. Presumptive coliform test
2. Confirmatory test
3. Completed test

1. Presumptive Test

This is the preliminary test for the determination of coliform organisms. This test was done using violet red bile agar media.
**VIOLET RED BILE AGAR MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>3</td>
</tr>
<tr>
<td>Bile salt</td>
<td>1.5</td>
</tr>
<tr>
<td>Peptone</td>
<td>10</td>
</tr>
<tr>
<td>Lactose</td>
<td>10</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.03</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.002</td>
</tr>
<tr>
<td>Agar</td>
<td>20</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.4 after sterilization.

**Procedure**

Dilutions were prepared using 1.25 percent sodium citrate as diluent or 0.9 percent sodium chloride. One milliliter of the dilutions were plated in violet red bile agar media. The same media was used as a cover layer. Then the plates were incubated at 37°C for 24±2 hours and examined for the presence of coliform organisms. Appearance of colonies with dark red center and colourless periphery indicated presence of coliforms.

2. **Confirmatory Test**

A confirmatory test should be made on doubtful colonies of violet red bile agar plates by transferring each of colonies to tubes of 2 percent brilliant green lactose bile broth.
BRILLIANT GREEN LACTOSE BILE BROTH

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10</td>
</tr>
<tr>
<td>Lactose</td>
<td>10</td>
</tr>
<tr>
<td>Bile salt</td>
<td>20</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.0133</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.2 after sterilization.

Procedure

A series of fermentation tubes (Durham's tubes) containing brilliant green lactose bile broth (after sterilization) were inoculated and incubated at 37°C for 48 hours.

Observation

The acid and gas formation was observed in the Durham's tubes. The broth was changed to yellow by the formation of acid produced by the coliform organism and gas bubbles were observed on the surface of the broth.

3. Completed Test

The completed test was used as the next step following the confirmatory test. It was applied to the brilliant green lactose bile broth fermentation tubes showing gas in the confirmatory test.

A completed test was made by streaking the materials from typical colonies on eosine methylene blue agar media.
The pH was adjusted to 7.1 after sterilization.

The plates were incubated at 37°C for 24 hours. Coliform colonies on eosine methylene blue agar media was observed as a green metallic sheen.

A differentiation of the coliform group had been carried out on the basis of the results of four tests, Indole, Methyl red, Voges Proskauer and Sodium citrate, often referred collectively as ‘IMVIC’ test. After the completed test, the organisms were subjected to IMVIC test for further confirmation.

**Indole Test**

A positive result of this test indicates the production of indole due to the partial decomposition of tryptophan.

<table>
<thead>
<tr>
<th>PEPTONE WATER MEDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredient</strong></td>
</tr>
<tr>
<td>Bactopeptone</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.2 after sterilisation.
The tubes were inoculated with 3 or 4 similar colonies taken from plates of solid media and incubated at 37°C for 48 hours. About 0.5 milliliter of freshly prepared Kovac's reagent was added to both the inoculated and control tubes and the tubes were shaken gently. A deep red colour with this reagent indicated the presence of indole. The cultures were removed periodically after 24 hours and tested for indole production.

**KOVAC'S REAGENT**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoamyl alcohol</td>
<td>15 milliliter</td>
</tr>
<tr>
<td>Paradimethyl amino benzaldehyde</td>
<td>1 gram</td>
</tr>
<tr>
<td>Concentrated Hydrochloric acid</td>
<td>5 milliliter</td>
</tr>
</tbody>
</table>

Paradimethylaminobenzaldehyde was dissolved in alcohol and then the acid was added slowly. The presence of indole was indicated by a deep red colour in the reagent.

**Methyl Red Test**

This test depends on the ability of the organisms to produce acid from glucose in amounts sufficient to reduce the pH to 4.2 or less and to maintain this low pH at least for 4 days. Buffered peptone glucose broth was used both for Methyl red and Voges Proskauer tests.

**GLUCOSE PHOSPHATE PEPTONE WATER MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>5</td>
</tr>
</tbody>
</table>
Peptone and dipotassium hydrogen phosphate were dissolved in water by steaming the mixture for sometime. The medium was filtered while hot and after cooling, the pH was adjusted between 7.4 and 7.6. Then 5 grams of glucose was added. The medium was dispensed in 4 milliliter quantities in test tubes and sterilized at 121°C for 25 min. They were inoculated from a plate of solid media. Control tubes were also maintained. All the tubes were incubated at 37°C for 48 hours. A few drops of methyl red solution was added to the 48 hours culture, as well as control tubes. A resultant definite red colour was considered as positive. An yellow colouration was considered as negative.

The indicator was prepared by dissolving 0.1 gram of methyl red in 300 milliliter of 95 percent ethanol and diluting to 500 milliliter with distilled water.

Voges Proskaver Test

This test may be carried out using the medium as described for the methyl red differential test or if desired an alternative salt peptone glucose medium may be used.

Reagents Used

1. Naphthol solution (5 percent)
2. Potassium hydroxide solution (40 percent)

Naphthol Solution

Five grams of purified alpha - naphthol was dissolved in 100 milliliter absolute ethyl alcohol. This solution should be prepared fresh each day.
Procedure

Five milliliter of either culture media were inoculated and incubated at $35 \pm 0.5^\circ C$ for 48 hours. To one milliliter of culture 0.6 milliliter of naphthol solution and 0.2 milliliter of 40 percent potassium hydroxide solution were added. Development of a pink to crimson colour in the mixture from 2 to 4 hours after adding the reagents gives a positive test.

Sodium Citrate Test

Koser's citrate broth was used to the citrate medium. Organisms capable of utilizing citrate as the sole carbon source could grow in this medium.

**KOSER'S CITRATE MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>5</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.29</td>
</tr>
<tr>
<td>Ammonium dihydrogen phosphate</td>
<td>1</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>2</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>1</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>Trace</td>
</tr>
</tbody>
</table>

The salts were dissolved and the pH was adjusted to 7.2 after sterilisation.

The medium was dispensed in test tubes and sterilized at $121^\circ C$ for 15 minutes. Inoculations were made from solid media, and the tubes were incubated at $37^\circ C$ for 24 hours. Utilization of carbon from citrate would change the medium from green to deep blue.
Enterobacter was differentiated from *E. coli* using Malonate broth (APHA, 1978).

### MALONATE BROTH

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>1.0</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>2.0</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
<td>0.6</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>0.4</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium Malonate</td>
<td>3.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.25</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.025</td>
</tr>
</tbody>
</table>

The final pH was adjusted to 6.7 ± 0.1 after sterilisation.

All the ingredients were dissolved dispensed into test tubes and autoclaved at 15 lb pressure for 20 minutes at 121°C.

**ISOLATION OF *Bacillus cereus***

This was carried out by the method given in Standard methods for the examination of dairy products using polymyxin Pyruvate- egg yolk – Mannitol bromothymol blue – Agar (PEMBA) (APHA, 1978).
PEMBA MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>1.0</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.0</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.1</td>
</tr>
<tr>
<td>Disodium Hydrogen Phosphate</td>
<td>2.5</td>
</tr>
<tr>
<td>Potassium Dihydrogen Phosphate</td>
<td>0.25</td>
</tr>
<tr>
<td>Sodium Pyruvate</td>
<td>10.0</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.12</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

The pH was adjusted to $7.2 \pm 0.2$ after sterilization.

The above chemicals were dissolved and the medium was dispensed in 95 milliliter quantities and sterilized. Before pouring the medium, 5 milliliter of sterile egg yolk emulsion and 10 units of membrane filtered polymyxin B - sulphate (Himedia) were added to the molten medium. Then the medium was mixed well and poured into the pre-sterilized petriplates. The plates with medium was surface dried in the refrigerator at 2-8°C but not more than 48 hours. Then the plates were dried in the incubator at 35°C for 2 to 4 hours with lids on and agar surface upward. Then one milliliter of $10^{-4}$ dilution sample was surface streaked using spreader rod and incubated at 37°C for 36 to 48 hours. *Bacillus cereus* colonies were appeared as peacock blue coloured surrounded by a zone of egg yolk precipitation.

**Calculation**

$$\text{Number of bacteria / ml} = \text{Number of colonies per plate} \times \text{dilution factor}$$
**ISOLATION OF *Staphylococcus aureus***

Bromothymol blue lactose agar was used for the isolation of pathogenic *Staphylococcus aureus* (APHA, 1975).

### **BROMOTHYMOL BLUE LACTOSE AGAR MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3.0</td>
</tr>
<tr>
<td>Proteose peptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.17</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

The final pH was adjusted to 8.6 ± 0.2 after sterilization.

All the ingredients were dissolved by slow heating and sterilized at 121°C using 15 lbs pressure for 15 minutes. Then, it was poured into the pre-sterilized petriplates to a thickness of 5 millimetre and kept in the refrigerator at 2-8°C for not more than 48 hours for surface drying. Then the plates were dried in the incubator at 35°C for 2-4 hours with lids on and agar medium upward. The plates were inoculated with one milliliter of $10^{-4}$ dilution sample using a spreader rod. After inoculation, the plates were incubated for about 36-48 hours at 35°C. Appearance of golden yellow colonies were counted using colony counter.

**Calculation**

Number of bacteria / ml = Number of colonies per plate x dilution factor

Egg yolk – tellurite – glycine – pyruvate – agar medium (ETGPA) was also used for the isolation of pathogenic *Staphylococcus aureus* (APHA, 1978).
### ETGPA MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>5.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>12.0</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>10.0</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
</tbody>
</table>

The final pH was adjusted to 7.0 ± 0.2 after sterilization.

The medium was dispensed in 95 milliliter amounts and autoclaved at 121°C using 15 lbs pressure for 15 minutes. Prior to pouring the plates, 5 milliliter of sterile egg yolk (50 percent emulsion) and 0.3 milliliter of 3.5 percent membrane filtered potassium tellurite were added to the molten medium, mixed well, and it was poured into the pre-sterilized petriplates and kept in the refrigerator at 2-8°C for not more than 48 hours for surface drying. Then the plates were dried in the incubator at 35°C for 2-4 hours with lids on and agar medium upward. The plates were inoculated with 0.5 milliliter of 10⁻¹ dilution sample in duplicate using a spreader rod. After surface inoculation, the plates were incubated for about 24-48 hours at 37°C. Colonies of *Staphylococcus aureus* on ETGPA were black, shining, with a surrounding halo and a clearing zone of egg yolk.

**Calculation**

\[
\text{Number of bacteria/ml} = \frac{(\text{Number of colonies in one plate} + \text{Number of colonies in other plate}) \times \text{dilution factor}}{2}
\]
Gram Staining

One loop of 24 hour bacterial culture was placed on a clean glass slide. The culture was fixed by smearing and drying heat. Then the crystal violet (Primary stain) was applied for 1 minute and washed with water and then Grams iodine was applied for 1 minute and washed with water. Iodine acts as mordent. Then the slide was decolourised with 95 percent alcohol for 30 seconds. After this process safaranin was added as counter stain and dried by blotting. Slide was viewed under the microscope. Gram positive and gram negative bacterial colonies would appear as purple and pink coloured colonies respectively.

DETERMINATION OF YEAST AND MOLD

Potato dextrose agar was used for enumeration of yeast and molds. The medium was prepared as per the procedure given in ISI (1980)

POTATO DEXTROSE AGAR MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>20.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Mashed white potatoes*</td>
<td>200.0</td>
</tr>
</tbody>
</table>

* White potatoes were peeled, sliced and boiled until soft and mashed. The diluent used was phosphate buffer.

Procedure

One milliliter of 10⁻¹ dilution was plated in potato dextrose agar. Before pouring the agar the pH was adjusted to 3.5 using the 10 percent tartaric acid solution (3 drops per 10 milliliter). The plates were rotated well for the contents to mix well. The plates
were incubated at 22 ± 2°C for 3 to 5 days. Convex shining star shaped yeast and mold colonies appearing as cottony or wooly were counted.

**Calculation**

Number of yeast = Number of colonies per plate x dilution factor and mold / ml

**Staining**

After counting, the yeast and mold colonies were stained. The edge of a mold colony was taken out using a sterile inoculation needle on to a clean slide. The slide was stained using a drop of cotton blue and examined directly under a microscope. Similarly a yeast colony was stained. Cotton blue stain was used for staining.

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**BIOCHEMICAL ANALYSIS OF BUTTER**

**ESTIMATION OF FAT**

Fat estimation of butter was carried out by Gerber method followed by ISI (1966).

Five grams of the butter sample was weighed in the butter butyrometer without any air bubble. About 15 milliliter of 1 : 1 sulphuric acid was transferred into the butter butyrometer followed by the addition of 1milliliter of amyl alcohol. After shaking well, the butyrometer was placed in a water bath maintained at 65°C. After 30 minutes it was centrifuged for 5 minutes. The clear supernatent reading was read from the butyrometer.
ESTIMATION OF TITRABLE ACIDITY

The estimation of titrable acidity of butter was carried out according to the method described by ISI (1966).

Accurately 20 grams of butter sample was weighed in a dry 250 milliliter conical flask. Ninety milliliter of boiled water was added and the contents were mixed well. While still hot, it was titrated with 0.02 N sodium hydroxide, using two or three drops of the phenolphthalein indicator.

Titrable acidity (as lactic acid) percent by weight = \[
\frac{9 \times NV}{W}
\]

where,
- \(N\) - Normality of sodium hydroxide solution
- \(V\) - Volume of sodium hydroxide solution used
- \(W\) - Weight of butter sample taken in grams

ESTIMATION OF MOISTURE (Kohnan's method)

The estimation of moisture was carried out according to the method followed by ISI (1966). Five grams of the butter sample was taken to determine the moisture percent and the procedure followed was similar to that of milk sample.

ESTIMATION OF SALT

The estimation of salt was carried out according to the method described by ISI (1966).

Principle

The butter is melted in hot water and chloride present in the mixture was titrated with a solution of silver nitrate using potassium chromate as indicator.
Reagents
1. Potassium chromate 5 percent (W/V) solution.
2. Calcium carbonate – analytical grade free from chloride
3. Standard silver nitrate solution (0.1N).

Five grams of the butter sample was weighed in a 250 milliliter conical flask. One hundred milliliter of boiling distilled water was added with occasional swirling for 5 to 10 minutes. After cooling to 50-55°C, two milliliter of potassium chromate, to which 0.25 gram of calcium carbonate was added and again mixed by swirling. The contents were titrated at 50-55°C with standard silver nitrate solution until the brown colour persists for half a minute. A blank test was done with all the reagents in the same quantity except the sample material.

Calculation

\[
\text{Percentage of Sodium chloride} = \frac{5.85N (V_1 - V_2)}{W}
\]

where,

- \(N\) = Normality of silver nitrate solution
- \(V_1\) = volume of silver nitrate used in the sample titration
- \(V_2\) = volume of silver nitrate used in the blank titration
- \(W\) = weight of the sample in gram
MICROBIOLOGICAL PARAMETERS STUDIED IN BUTTER

BACTERIAL POPULATION

Enumeration of bacterial population was carried out as per the procedure described in APHA (1978). The samples collected for examination were transported in jars packed with ice and stored in the refrigerator until examination. They were tested within 36 hours, after collection. All the samples were properly preserved till the time of analysis. Butter samples were kept at 4 ± 1°C for 30 days to study the keeping quality of butter.

Diluent

Sterile sodium citrate solution (1.25 percent) was used as a diluent for butter because it facilitates easy and uniform dispersion of butter granules.

Procedure

The samples were kept in the water bath maintained at 40°C until the butter becomes milky white liquid. (The time required for melting the butter should not exceed 15 minutes). Dilutions were prepared using 1.25 percent sodium citrate. One milliliter of the dilutions were plated by using nutrient agar media. Then the plates were incubated at 37°C for 48 hours. The typical colonies were observed and counted.

Procedure for Dilution

The samples of butter in the sterile wide mouthed sample bottle as well as sterile Ringer's diluent (1.25 percent sodium citrate) were warmed in a water-bath maintained at 40 to 45°C. Then the sample was agitated thoroughly so as to obtain uniform mixing of the serum, water and fat. Butter solidifies at ordinary temperature since there is a tendency for the fat globules to clump
together. Therefore, the plating of butter is not as easy as milk. It is necessary to keep all the water blanks and pipettes warm by keeping the blanks in a water bath maintained at 40-45°C. The portion of butter was weighed and melted at 45°C. One milliliter of the melted butter was poured in petriplates using sodium citrate as diluent.

With a previously warmed sterile pipette one milliliter of butter was transferred to a 9 milliliter sterile Ringer’s solution to give a dilution of 1:10 which is at 40 to 45°C.

For preparing 1:100 dilution, one milliliter of 1:10 dilution was drawn using previously warmed sterile pipette to another 9 milliliter sterile Ringer’s diluent warmed at 40 to 45°C. Likewise a series of 1:1000 and 1:10000 dilutions were also prepared.

**Calculation**

Number of bacteria / ml = Number of colonies per plate x dilution factor

**COLIFORM COUNT**

The procedure followed was similar to that of milk.

**ISOLATION OF PROTEOLYTIC BACTERIA**

Skim milk agar medium was used to enumerate the proteolytic bacteria in butter (APHA, 1975).
SKIM MILK AGAR MEDIUM

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight in grams per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone or trypticase</td>
<td>5.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Reconstituted skim milk (10 percent solids)</td>
<td>100 milliliters</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.1 after sterilization.

The melted medium was poured into the pre-sterilized petriplates. The sample was streaked on the surface of plates and kept at 21°C for 72 hours. Then the plates were flooded with one percent hydrochloric acid or ten percent acetic acid solution for one minute. Clear zones were found around the colonies indicated the presence of proteolytic organisms in the given sample.

Calculation

\[
\text{Proteolytic count/ml} = \text{Number of clear zone around colony} \times \text{dilution factor}
\]

ISOLATION OF LIPOLYTIC ORGANISMS

Isolation and counting of lipolytic organisms were carried out as per the procedure described by Kannan (1996). Lipolytic organisms produce an enzyme lipase which catalyses the hydrolysis of fats as fatty acids and glycerol. Many of the aerobic active proteolytic bacteria and a few molds are capable of producing lipase and are lipolytic.
Procedure

The samples of butter, as well as the sterile Ringer's diluent (quarter strength) were warmed in a water bath maintained at 40° to 45° C. The pipette was wetted with warm diluting solution. Immediately 25 milliliter of thoroughly well mixed, melted sample was transferred into 225 milliliter of warm diluting solution. The solution was well mixed and one milliliter of diluted sample was transferred to butter fat agar plates. The plates were incubated at 22°C for 5 days, then the plates were flooded with copper sulphate solution for 10-15 minutes. Then the copper sulphate solution was drained and the plates were washed with running water for one hour. Appearance of greenish blue zone around the colonies indicated the presence of lipolytic bacteria.

Calculation
Lipolytic count/ml = Number of colonies per plate x dilution factor

<table>
<thead>
<tr>
<th>BUTTER FAT AGAR MEDIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Butter fat</td>
</tr>
<tr>
<td>Yeast extract</td>
</tr>
<tr>
<td>Peptone</td>
</tr>
<tr>
<td>Agar</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.8 after sterilization.
RINGER SOLUTION (Full strength)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight in grams per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>9</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.42</td>
</tr>
<tr>
<td>Calcium chloride (anhydrous)</td>
<td>0.48</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.20</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.0 after sterilization.

For quarter strength one part of the full strength was mixed with 3 parts of distilled water and then sterilized.

PSYCHROTROPHIC COUNT

Psychrotrophic count in butter was carried out by Standard methods for the examinations of dairy products APHA (1978).

The procedure was same as that of total microbial population for milk but, the incubation temperature was 7°C for 10 days.

Calculation

\[
\text{Psychrotrophic count} = \text{Number of colonies} \times \text{dilution factor in butter/ml or gram}
\]

YEAST AND MOLD

The procedure was same as that of milk.
BIOCHEMICAL ANALYSIS OF DAHI

ESTIMATION OF pH

pH of dahi was carried out as done with milk samples.

ESTIMATION OF FAT

In case of dahi 15 grams of sample was weighed accurately in a conical flask. To this 0.75 milliliter of liquor ammonia was added and mixed well and used for the estimation of fat similar to the procedure followed for the milk.

ESTIMATION OF ACIDITY

Estimation of acidity in dahi was carried out in a slightly modified manner. To 10 grams of accurately weighed dahi sample, 12.5 milliliter of distilled water was added and heated near to boiling. Two or three drops of phenolphthalein was added and titrated against 0.1N sodium hydroxide as in the case of milk.

ESTIMATION OF PROTEIN

The procedure followed was similar as that of milk.

MICROBIOLOGICAL PARAMETERS STUDIED IN DAHI SAMPLES

Total microbial population, Coliform, Bacillus cereus, Staphylococcus aureus and yeast and mold counts were carried out according to the method given for milk samples using respective media.
PREPARATION OF KHOA

Typically buffalo milk in 4-6 litre lots is simmered with vigorous scraping in a shallow pan over direct fire till a semi-solid mass is formed. Considerable amount of free fat is released towards finishing - stage, and the mass turns relatively non-sticky on the heating surface. Each lot takes 5-10 minutes to finish. Product made from buffalo milk is whiter while that made from cow milk yellowish, both with slight tinch of brown.

BIOCHEMICAL ANALYSIS OF KHOA

ESTIMATION OF MOISTURE

In case of Khoa five grams of accurately weighed sample was transferred into a dish. The sample was spread at the bottom of the dish with flattened glass rod and kept in an oven maintained at 70 to 100°C for 4 hours. Other manipulations and calculations followed were similar to that of milk sample.

ESTIMATION OF ACIDITY

Two grams of Khoa was weighed accurately in a porcelain dish. Three milliliter of hot water was added and made into a fine paste using a pestle and mortar. Then 17 milliliter of hot water was added and diluted to 20 milliliter. One milliliter of phenolphthalein indicator solution was added and titrated against standard sodium hydroxide (0.1 N) solution. The persistence of a slight pinkish tinge for 30 seconds was the end point. The calculations followed were similar to that of milk sample.
Process of Khoa preparation

Packaging of Khoa
ESTIMATION OF LACTOSE

Five grams of Khoa sample was accurately weighed and made into a paste using hot distilled water. The procedure followed was similar to that of milk sample.

DETERMINATION OF FAT

Three grams of finely divided Khoa sample was taken into the cheese butyrometer. Ten milliliter of Gerber sulphuric acid and 10 milliliter of hot distilled water were added followed by one milliliter of isoamyl alcohol. The butyrometer was closed with the stopper and it was shaken well till all contents were well mixed. The butyrometer was placed in a water bath at 65 ± 2°C and shaken periodically until the solution of the sample was mixed well. Other procedure followed and calculations were similar to that of milk sample.

ESTIMATION OF NITROGEN

In order to estimate the nitrogen content of Khoa, two hundred milligrams of the sample is taken for digestion. The reagent used, distillation and titration carried out were similar to that of milk sample. The nitrogen content determined must be multiplied by a factor 6.38 to obtain the value of protein in case of khoa.

MICROBIOLOGICAL PARAMETERS STUDIED IN KHOA

Microbial population, coliform, Bacillus cereus, Staphylococcus aureus and yeast and mold were carried as per the technique described in milk using appropriate media.
BIOCHEMICAL ANALYSIS OF ICE REAM

DETERMINATION OF ACIDITY

Determination of acidity was performed as per the method described in ISI (1964).

Ten grams of ice cream was weighed in a clean conical flask to which 25 milliliters of recently boiled, cooled water and 1 milliliter of phenolphthalein were added. Then the contents were titrated against 0.1 N sodium hydroxide solution as in the case of milk.

\[
\text{Percentage of acidity in ice cream} = \frac{9 \text{ } NV}{W}
\]

where,

\( V \) = Volume of sodium hydroxide required for titration
\( N \) = Normality of sodium hydroxide
\( W \) = Weight of the ice cream taken

ESTIMATION OF FAT

The moisture, acidity, fat and protein contents of ice cream were carried out similar to that of milk after thawing the ice cream samples.

MICROBIOLOGICAL PARAMETERS STUDIED IN ICE CREAM

Microbial population, coliform, \textit{Bacillus cereus}, \textit{Staphylococcus aureus}, yeast and mold counts were cultured and enumerated in appropriate medium similar to that of milk samples. However, the procedure for psychrotrophics was the same as that of microbial population for milk, but the incubation temperature was 7°C for 10 days.
BIOCHEMICAL TESTS FOR BACTERIA

The biochemical tests were carried out to identify the bacteria present in the sample. This was done according to the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The tests conducted were as follows:

1. Hydrogen sulphide production
2. Casein hydrolysis
3. Catalase test
4. Indole formation
5. Methyl red test
6. Voges-Proskauer reaction
7. Citrate utilization
8. High and Leifson test (or) oxidation / fermentation test
9. Carbohydrate fermentation test or utilization test
10. Gelatin liquification
11. Haemolysis
12. Nitrate reduction test
13. Salt tolerance test
14. Urease test
15. Malonate utilization
16. Potassium cyanide utilization
17. Phenylalanine deaminase production
18. Ammonia test
19. Arginine hydrolysis
20. Decarboxylase test
21. Gluconate oxidation
22. Starch hydrolysis
23. Mannitol fermentation
24. Coagulase test
1. Hydrogen sulphide production

The filter paper strips impregnated with 10 percent lead acetate solution were placed inside the medium (without touching). Then the tubes were incubated for 12 hours at 37°C. The paper strips would be changed to black in positive reactions.

2. Casein Hydrolysis

Skim milk agar medium was poured into the pre-sterilized petriplates. The test culture was streaked on the surface of the medium as a single line and incubated at 37°C for 24 to 48 hours. After the incubation period a clear zone seen around the colonies indicated positive reaction and presence of casein hydrolyzing organisms.

**SKIM MILK AGAR MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk powder</td>
<td>100</td>
</tr>
<tr>
<td>Peptone</td>
<td>5</td>
</tr>
<tr>
<td>Agar</td>
<td>15</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.2 after sterilisation.

3. Catalase test

One milliliter of one percent Tween 80 was taken in a 5 milliliter screw caped bottle. A colony picked up from agar plates was transferred to this solution and vertexed. Half a milliliter of hydrogen peroxide solution was added to the above contents and capped. Appearance of small bubbles in 30 seconds was the indication of catalase positive reaction.

4. Indole formation

A positive result of this test was due to the production of indole by the partial decomposition of tryptophan. The procedure is already given under microbiological test for coliforms.
The procedures of methyl red (5), Voges-Proskaver reaction (6) and citrate utilization (7) were already given under the methods adopted for coliforms in milk.

8. Hugh and Leifson test or Oxidation/Fermentation test

**MEDIA COMPOSITION**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.3</td>
</tr>
<tr>
<td>Agar</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.0 after sterilization.

To the prepared medium 15 milliliter of 0.2 percent sterile bromothymol blue and 10 grams of pasteurized dextrose were added. Then the medium was dispensed in 15 millimeter aliquots into the test tubes under aseptic conditions. They were heated in boiling water-bath for 10 minutes to remove oxygen in the medium, then the culture was added after cooling.

One set of test tubes were sealed with 2 percent agar for a height of 1 to 2 centimeter to maintain anaerobic condition. Another set of tubes were kept as it is to maintain aerobic condition. All the tubes were incubated for 24 hours at 37°C. The bromothymol blue would be changed to yellow in colour showing the acid production. Acid production in both aerobic and anaerobic tubes indicated the fermentative metabolism.

9. Carbohydrate fermentation test / utilization test

Glucose, fructose, lactose, sucrose, arabinose, raffinose, xylose, mannose, sorbitol, glycerol were dissolved (5 percent W/V) in nutrient broth. A few drops of bromothymol blue were added.
Durham's tube filled with respective medium were placed into the test tubes containing 10 milliliter of the medium upside down. The culture was inoculated and incubated for 24 hours at 37°C. Displacement of medium with bubble's in Durham's tubes indicated the production of gas. The change of colour from blue to yellow indicated the acid production. Active fermentation might produce a trapped bubble which would measure at least 2-3 millimeter.

10. Gelatine liquefaction

Gelatin plates were made by incorporating 2 percent (W/V) gelatin in nutrient agar. These plates were incubated for 24 hours at 37°C after adding the culture. Later the plates were flooded with saturated ammonium sulphate solution or 20 percent trichloroacetic acid. Halo appearance around colonies indicated liquefaction of gelatin.

11. Haemolysis

Blood agar medium was prepared by adding one percent of human or rabbit blood to nutrient agar. Wells in the agar plates were prepared using a 5.0 millimetre diameter cork borer. Bacterial culture was inoculated into the wells and incubated for 48 hours at 37°C. Formation of clearing zone around the well was considered as the index of haemolytic activity.

12. Nitrate reduction test

The organism was grown in nutrient broth and a few drops of 10 percent aqueous ferric chloride solution was added. Then it was incubated for 4 hours at 37°C and acidified with few drops of 1N hydrochloric acid. Half milliliter of freshly prepared 0.2 percent solution of sulphanilamide hydrochloride was added. Absence of pink colour was considered as positive reaction.

In nutrient broth containing 0.1 percent potassium nitrate, the culture was inoculated and incubated at 30°C. After 3 days a pinch of zinc dust was added to the broth. Failure of colour
development indicated that the nitrate was completely reduced and the appearance of pink colour showed the absence of nitrate reductase activity.

13. Salt tolerance test

Nutrient broth containing various concentration of sodium chloride (2-6.5 percent) was inoculated with culture and incubated for 48 hours at 37°C. The growth was estimated in terms of absorbance at 550 nm.

14. Urease test

<table>
<thead>
<tr>
<th>COMPOSITION OF UREASE MEDIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
</tr>
<tr>
<td>Peptone</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
</tr>
</tbody>
</table>

To this 0.1 milliliter of 0.1 percent phenol red and 2 percent filtered and sterilized urea were added.

Urease production was studied by growing one millilitre of overnight culture in 5 milliliter of urease medium. The inoculated tubes were incubated at 37°C for 24 to 48 hours. The colour change from yellow to pink was the indication of urease production.

15. Malonate utilization

The procedure followed is similar to that of the test for coliforms in milk.

16. Potassium cyanide utilization

Three loopful of culture was transferred to potassium cyanide broth and incubated for 48 hours at 35°C. The turbidity of the broth indicated the growth of organism and positive reaction. If there was no turbidity the reaction as well as growth was negative.
POTASSIUM CYANIDE BROTH

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease peptone</td>
<td>3.0</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>5.64</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>0.225</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.6 after sterilization.

The above ingredients were dissolved in distilled water with stirring. Aliquots of 100 milliliter were autoclaved for 15 minutes at 121°C. Then 1.5 milliliter of 0.5 percent potassium cyanide solution was added to the above basal medium (This solution should not be pipetted out by mouth). The medium was distributed in sterile test tubes and inoculated. After incubation for 24 hours, the turbidity was observed.

17. Phenylalanine deaminase production

The slants of phenylalanine agar was prepared and inoculated with a fairly heavy inoculam of an agar slant culture. The inoculated slants were incubated at 37°C for 24 hours. Later 4 or 5 drops of 10 percent ferric chloride reagent was allowed to run down over the growth of the slant. If phenyl pyruvic acid has been formed a green colour would develop in the syneresis fluid and medium.

18. Ammonia test

The culture was inoculated in nutrient broth and incubated at 30°C for 5 days. A strip of filter paper wetted with Nessler’s reagent was placed in the upper part of the culture tube. The tube was warmed in a water bath at 55-60°C. If filter paper turned to brown, it would indicate the presence of ammonium ion.
19. Arginine hydrolysis

The culture was inoculated with sterile arginine broth.

**COMPOSITION OF ARGinine BROTh**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.0</td>
</tr>
<tr>
<td>Potassium hydrogen phosphate</td>
<td>2.0</td>
</tr>
<tr>
<td>L-Arginine mono hydrochloride</td>
<td>3.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.0 after sterilization.

The medium was prepared and dispensed in test tubes in 15 milliliter aliquots and autoclaved. The culture was inoculated and incubated for 24-48 hours at 37°C. Then a few drops of neutral red was added to the tube. Appearance of brown colour indicated the hydrolysis of arginine.

20. Decarboxylase test

**Falkow's Decarboxylase Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The pH was adjusted to 6.72 after sterilization.

Ten milliliter of 2 percent of bromocresol purple was added to the medium. Then 0.5 gram of lysine or ornithine or arginine was added. The medium was distributed in one milliliter aliquots in small test tubes containing sterile liquid paraffin to provide a layer about 5 millimeter thickness above the medium. The
test tubes were autoclaved at 121°C for 15 minutes. Through the paraffin layer the culture was inoculated with a straight wire. The tubes were incubated and observed daily for 4 days. Decarboxylation would be indicated by the colour change to purple or violet in positive decarboxylation reaction. The control tubes would remain yellow showing negative reactions.

21. Gluconate oxidation

**COMPOSITION OF GLUCONATE MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>1.0</td>
</tr>
<tr>
<td>Peptone</td>
<td>1.5</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Potassium gluconate</td>
<td>40.0</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.0 after sterilization.

The medium was dispensed into the test tubes in one milliliter quantities. After inoculation, the tubes were incubated for 48 hours at 37°C. To this equal volume of Benedict's qualitative reagent was added and boiled for 10 minutes in a water bath. The positive result would show orange brown precipitate of cuprous oxide.

22. Starch hydrolysis

The organism was plated in starch agar medium (10 percent starch in nutrient agar). Then plates were incubated for 5 days and flooded with iodine solution. Clear zone around the colonies in a blue background indicated positive reaction (hydrolysis of starch).

23. Mannitol Fermentation

Anaerobic fermentation of mannitol was a reliable test for detecting coagulase positive *Staphylococci* in milk.
24. Coagulase Test

This test is the most accurate and taxonomically correct test for identification of catalase positive colonies suspected to be *Staphylococcus aureus*.

a) Plasma (0.5ml) in a tube, 0.5ml of an 18-24 hrs nutrient broth culture of the test organism was added. Tubes were examined for clotting every 30 minutes upto 4 hours.

b) Three or four colonies were inoculated in 0.3 ml of brain heart infusion broth (BHI) and emulsified thoroughly. The culture suspension was incubated for 18 to 24 hours at 35 to 37°C. Then 0.5ml of coagulase plasma with EDTA was added to the above culture and mixed well. It was incubated at 35 to 37°C and examined periodically over a six hour interval for clot formation 3+ or 4+ of well clot formation would indicate the positive reaction for coagulase. And the formation of 1+ or 2+ would indicate the negative reaction.

**COMPOSITION OF BRAIN HEART INFUSION BROTH**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in grams per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf brain, infusion</td>
<td>200.0</td>
</tr>
<tr>
<td>Beef heart, infusion</td>
<td>250.0</td>
</tr>
<tr>
<td>Protease peptone or polypeptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The pH was adjusted to $7.4 \pm 0.2$ after sterilization.

The salts were dissolved and dispensed in test tubes and sterilized for 121°C for 15 minutes.
Hydrogen sulphide production

Oxidation / Fermentation test
Indole production - A deep red colour seen

Methyl red - Positive by the appearance of red colour.
Vogesproskauer test - Appearance of pink colour

Citrate utilization: Development of blue colour
Nitrate reduction - Appearance of brown colour

Starch hydrolysis - Halo space around the colony
STATISTICAL METHODS

All the procedures followed were according to the methods described in Snedecor and Cochran (1997).

ANALYSIS OF VARIANCE

It is the most efficient way of detection and estimation of the different components of variation in a set of data obtained under statistical planning. In analysis of variance two or more treatments are compared simultaneously. A significant variation among the treatments gives an overall picture and then further tests with the critical difference are made to see which treatment differs from which.

CORRELATION

The correlation provides an excellent tool for the prediction of parameter values within reasonable degree of accuracy. The term correlation indicates the relationship between two such variables. We can use the correlation to find out the relationship between all possible milk quality parameters. Correlation coefficients among the milk and milk quality parameters of different sources were calculated. The range of numerical values of correlation ‘r’ ranged from -1 to +1.