หอสมุดกลาง สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER II

LITERATURE REVIEW

2.1 Definition and Standard of Milk

The definition of milk by United States Public Health Service is lacteal secretion, partially free from colostrum, obtained by the complete milking of one or more healthy cows, which contains not less than $8\frac{1}{4}$ % of milk solid not fat and not less than $3\frac{1}{4}$ % of milk fat (12, 13)

The standards of raw milk in Thailand as defined by the notification of Ministry of Public Health number 26, B.E.2522 (14) are

1. Should be free from pathogens.
2. Should contain no colostrum.
3. Should contain no toxic substances such as antibiotics, insecticides in the level that will be harmful to men.
4. Should contain milk solid not fat not less than 8.5% and not less than 3.2% of milk fat.
5. Should be past the pasteurization, sterilization or UHT process before distributing to consumers.

The Standards of pasteurized milk in Thailand by the notification of Ministry of Public Health no.26, B.E.2522 (14) are

1. Should have a specific flavor belong to the specific type of milk.
2. Should be fluid, not in granular or clot form.
3. Should contain no preventive additives.

4. Should be free from pathogens.

5. Should contain no toxic substances from microorganisms in the harmful level.

6. Should have no more than 50,000 colony forming unit of bacteria per one milliliter of milk.

In this notification, the definition of pasteurization is also given as heat treatment process at temperature not lower than 63 °C for not less than 30 minutes or at temperature not lower than 72°C for not less than 16 seconds, then, cool immediately to temperature of 4°C or lower. It may be passed the homogenization process or not (14)

2.2 Composition of milk (12, 13, 15, 16, 17)

Milk contains a large number of substances which can act either as weak acid or weak base. As a result, the pH of milk is very stable. It takes quite a large doses of acid or base to produce any appreciable change in the pH. A solution with this characteristic is called a buffer solution (15). It is liquid food secreted from the mammary glands of mammals(16). It consists of an emulsion of fat and a colloidal dispersion of proteins, together with the milk sugar (lactose) in true solution. These major components are accompanied by various minerals (notably calcium and phosphorus), vitamins, enzymes and various minor organic compounds such as citric acid, some of them nitrogenous in nature. The characteristic opaque color of milk is due mainly to dispersion of milk proteins and calcium salts (12). The quantities of the various main constituents of milk can vary considerably between cows of different breeds and between individual cows of the same breed, so only limited values can be stated for the variations (10). The figure in Table 2.1 are simply examples.

Table 2.1 Quantitative composition of milk (15)

Main constituent	Limits of variation (%)	Mean value (%)
Water	85.5 - 89.5	87.0
Total solids	10.5 - 14.5	13.0
Fat	2.5 - 6.0	4.0
Proteins	2.9 - 5.0	3.4
Lactose	3.6 - 5.5	4.8
Minerals	0.6 - 0.9	0.8

2.2.1 Water The average percentage of water in milk is 87% (12, 15, 16). The water in milk is not different from ordinary water and serve to hold in solution, colloidal and crystal (12)

2.2.2 Fat The average percentage of fat in milk is 4.0% (15). Milk consists of a large quantity of spheres of varying sizes floating freely in the milk, Each sphere is surrounded by a thin skin. These tiny spheres are fat globules, and the skin consists of protein (mucous membrane) and phospholipids. This skin protects the fat from being broken down by enzymes present in the milk (15). Milk fat is the most valuable constituent of milk. It helps the flavor of milk smoothness and palatability (12). The primary component is the triglyceride fraction which is about 98-99% of total milk fat (12). It comprises only traces of monoglycerides and diglycerides which is less than 0.5% of total milk fat (17). These compounds tend to concentrate at the fat globule surface because of free hydroxyl groups which tend to be associated with water. They help stabilize fat emulsions. Milk fat contains at least 140 fatty acids (17). Their kinds, their number, and the manner in which they are associated with glycerol determine the properties of milk fat.

The minor quantity of milk fat are 0.2-1.0% phospholipids which exist in complex with protein in milk and help stabilize fat emulsions(17), 0.35-0.4% sterol which cholesterol is the principal, and fat-soluble vitamins A, D, E and K (17).

Fat is not affected by temperature below 100°C. Some coalescence of fat globules occurs at higher temperatures. Cream separation is, however, impaired somewhat if the milk is heated to 75°C or above (15).

2.2.3 <u>Lactose</u> Lactose is the carbohydrate of milk and is usually the most abundant of the three major solid components of milk. Milk contains on the average about 4.8 percent of lactose (15). Lactose differs from sucrose in molecular configuration, relative sweetness, solubility ahd chemical reactivity (8). It is one-half to one-fifth as sweet as sucrose depending on concentration. In heat-treated milk, in the presence of proteins and certain mineral salts, brown color development occurs quite readily and gives rise to the browning of sterilized milk and of certain condensed-and dried-milk products during storage. The greater chemical reactivity of lactose, as compared with sucrose, is due to the presence of a potentially free aldehyde group in the glucose portion of the molecule.

Lactose undergoes changes at temperature above 100°C. The reaction is between lactose and protein, and give rise to a brownish color (15).

2.2.4 Proteins The average percentage of protein in milk is 3.4% (15). It includes two major proteins and small quantities of others. Casein constitutes about 80% of the total and whey or milk serum proteins 20% of the total (12). This casein is precipitated by acidifying skim milk to a pH value near 4.6 (7, 12).

Whey or milk serum proteins compose of β-lactoglobulin, α-lactalbumin, immunoglobulin, blood serum albumin, proteose and peptone which β-lactoglobulin and α-lactalbumin are the two principal components (16). α-lactalbumin is precipitated by heat, but not by rennet (13). β-lactoglobulin contains free sulfhydryl (-SH) groups in the form of cysteine residues. These have been indicated in the development of cooked flavor when milk is heated (12). Immunoglobulins are antibodies of milk which are especially abundant in colostrum. Blood serum albumin binds and inactivates some antibiotics. Concentrations of blood serum albumin increase when udder tissues become inflamed (17).

Casein does not undergo any detectable change at tempeartures below 100°C, but the rate of coagulation of milk by rennet diminishes with increasing pasteurization temperature. The reason for this is that casein loses its calcium when heated. β-lactoglobulin and albumin begin to precipitate at temperature as low as 70°C (15).

2.2.5 Minerals The percentage of minerals in milk is about 0.8 (15). They are not only important in nutritive but also strongly affect the physical state and stability of milk proteins. They affect flavor, and sometimes catalyze chemical reactions that occur in milk (17). The major minerals in milk and their approximate percentages are calcium, 0.112% ; phosphorus, 0.095% ; potassium, 0.138% ; magnesium, 0.013%; sodium, 0.059% ; chloride, 0.109% ; and sulphur, 0.01% , and minor amounts of iron, 3.0 ppm; silicon, 2 ppm; copper, 0.3 ppm; fluorine, 0.15 ppm. Milk also contains some trace elements such as aluminium, manganese, iodine, boron, titanium, vanadium, rubidium, lithium and strongtium.

The minerals in milk are not affected to any significant degree by heat treatment (15).

2.2.6 Vitamins Vitamins in milk compose of fat-saluble and water-soluble vitamins. Fat soluble vitamins in milk are vitamin A, D, E and K and water-soluble vitamins are vitamin B complex, thiamine, riboflavin, niacin, pantothenic acid, pyroxine, biotin, choline, inositol, folic acid, β-aminobenzoic acid, biotin and vitamin C (13).

Vitamin C is the most sensitive to heat, especially in the presenee of air and certain metals. High temperature short time pasteurization in a plate heat exchanger can, however, be accomplished with virtually no loss of vitamin C. The other vitamins in milk suffer little or no harm from moderate heating (15).

2.2.7 Enzymes Enzymes in milk are important; they may modify the chemical make up of the product so it tastes, smells, appears, or reacts differently and may be used as evidence of quality or of treatment given the product (17).

Raw fresh milk contains enzymes that gain entrance from the cow. Other enzymes are produced in milk or milk products by microorganisms, some inside and some outside the mammary gland (17). The enzymes in raw milk must be considered in handling the product, as they may influence undesirable changes in flavor (16). Enzymes in milk include amylase, catalase, lipase, protease, phosphatase, lactoperoxidase, xanthine oxidase, etc. (12, 17).

2.3 The microbiology of milk

The bacterial flora in milk can vary considerable in numbers and species depending on how the milk is soiled. Thus, milk contains few bacteria in cow's udder, but is later subjected to contamination by man and his habits, i.e. by his animal husbandry methods, and by the practices he uses in collecting milk from the cows. However, both the numbers and kinds of contaminants to be found in milk also depend on the health of the animals. Besides, some finished goods contain sugar, spices, flavors, salt, stabilisers, etc., and each condiment in itself may contribute additional varieties of microorganisms. Initially, therefore, one can consider milk as a product with contaminating micro-organisms in two broad categories; those organisms that are found in raw milk, and those bacteria to be found in pasteurised milk (18, 19).

2.3 .1 The initial microflora of raw milk

a) Total bacterial content

The initial standard plate count of milk may range from <1000 cfu/ml (colony forming units/ml milk), where contamination during production is minimal to > $1x10^6$ cfu/ml (18, 19). The microorganisms present will be derived from one or any combination of the three main sources of contamination, namely the interior of the udder, its exterior and milking equipments. High initial standard plate count in milk e.g.>100,000 cfu/ml, are evidence of serious faults in production hygiene, whereas the production of milk having standard plate count consistently <10,000 cfu/ml reflect good hygienic practices

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(18, 19).

Supaporn Chuaprasert (1984) reported that total bacterial counts of bulk milk from road tankers at arrival in Saraburi (DPO Dairy) are in the range from 3.0×10^5 to 5.3×10^6 cfu/ml, and at arrival in Bangkok (Foremost Dairies) are in the range 2.1×10^5 to 2.1×10^6 cfu/ml (18).

Total bacterial count does not indicate the sources of bacterial contamination in milk, or the identity of production faults leading to high counts. Counts of psychrotrophs, thermoduric organisms, spores, streptococci and coliforms may assist in the diagnosis of faults.

The main groups of microorganisms found in fresh raw milk are shown in Table 2.2

Table 2.2 Types of aerobic mesophilic micro-organism in fresh raw milk (18, 19)

Micrococci	Streptococci	Asporogenous Gram -ve rods	Spore-formers	Gram -ve rods	Miscellaneous
Micrococcus	Enterococcus	Microbacterium	Bacillus	Pseudomonas	Streptomycetes
Staphylococcus	Group N	Corynebacterium	(spores or	Acinetobacter	Yeasts
	Mastitis Streptococci	Arthrobacter	vegetative	Flavobacterium	Molds
	Str.agalactiae	Kurthia	cells)	Enterobacter	
	Str.dysgalactiae			Klebsiella	
	Str.uberis			Aerobacter	
				Escherichia	
				Serratia	
				Alcaligenes	

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b) The thermoduric microflora

The genera surviving laboratory pasteurization are thermoduric microflora as shown in Table 2.3 (18, 19)

Table 2.3 Thermoduric and psychrotrophic micro-organisms in fresh raw milk.

Thermoduric genera[a]	Psychrotrophic genera[b]
Microbacterium	Pseudomonas
Micrococcus	Acinetobacter
Bacillus spores	Flavobacterium
Clostridium spores	Aerobacter
Alcaligenes	Alcaligenes
	Bacillus
	Arthrobacter

a. Survive heating at 63 °C for 30 min.

b. Visible growth at 5-7°C in 7-10 days.

Microbacterium lacticum and bacterial spores normally show 100% survival; some Micrococcus spp. are slightly less heat resistant, and only 1-10% of strains of Alcaligenes tolerans may survive; and probably < 1% of species of streptococci (e.g.Str. faecalis), lactobacilli and some coryneforms normally survive at 63 °C for 30 min. Most reports of coliforms and strains of Escherichia coli surviving pasteurization are probably attributable to the fact that they have been detected in commercially pasteurised milk. Survival of E.coli after heating at 63 °C for 30 min. has seldom been substantiated in laboratory tests (19). The bacillus spore content of raw milk rarely exceeds 5000 cfu/ml and it is generally higher in winter than in summer (19), because these organisms are largely derived from surfaces of teats which have been in contact with bedding materials used for housing cows (19).

In contrast to spore, micrococci and Mycobacterium spp; are almost contaminated from the milking equipments. For this reason, the thermoduric or laboratory pasteurized count has been proposed and used for hygienic quality control of raw milk. Its correlation with total count is poor (19). It is found that milk delivered in cans had higher thermoduric counts than milk collected from refrigerated bulk tanks, but this may have been due to lower levels of thermoduric organisms in the milking machines of the bulk tank producers, rather than to contamination from the milk cans (19).

Spores of Clostridium spp. are thermoduric, its counts are higher in winter, because they are mainly derived from silage used for winter feeding and from bedding materials. Clostridia do not multiply in raw milk.

d) The psychrotrophic microflora

Most raw milk psychrotrophs are heat sensitive gram negative rods, some heat resistant gram positive species have also been isolated as shown in Table 2.3 (19, 20). These latter organisms may cause spoilage of stored heat-treated milk or milk products by their growth, but it seldom exceeds 10/ml (19) and the involvement of specific enzymes has not been reported. The psychrotrophic gram negative bacteria in raw milk are most present in heat treated milk or milk products and do not themselves cause spoilage (other than as post-pasteurization contaminants) but many species produce heat resistant extracellular enzymes capable of degrading important milk constituents. The two groups of enzymes of major economic importance are the proteinases and lipases which, because they are produced extracellularly, can act directly on micelle casein and on the fat globules in milk (19, 20). On average the psychrotrophs is about 10-50% of the initial total count (14).

d) Coliform organisms

The incidence of coliforms and *Escherichia coli* in raw milk implies their association with contamination of faecal origin (19, 20).

It is now well recognised that the presence of coliforms in raw milk is not evidence of direct faecal contamination, and cannot be relied on to detect failure to clean dirty udders before milking. Coliforms can rapidly-build-up in moist, milkly residues in milking equipment, which then becomes the major source of contamination of the milk produced. However, relatively low coliform counts in milk do not necessarily indicate effectively cleaned and disinfectod equipment. Coliform counts regularly in excess of 100 cfu/ml are considered as, evidence of insatisfactory production hygiene (14).

2.3 .2 Udder disease and bacterial content of raw milk

Mastitis is an inflammation of the udder. It is the reaction of milk-producing udder tissue to damages. The damages are not often caused by microorganisms which caused the total bacterial content of raw milk increase (19, 21). Mastitis is responsible for economic loss to the dairy industry because milk yeild is reduced (19, 23). The disease may be present in a clinical or sub-clinical condition. Effect of mastitis will decrease milk yield and most of the composition of milk (22, 23), but increase the numbers of somatic cells (22).

2.3.3 Pathogens for man in raw milk

Raw milk may contain micro-organisms pathogenic for man; and their source may be placed into two groups; 1) Those eliminated in the milk by a diseased cow; 2) Those that may contaminate the milk through some human agency after it has left the cow's udder (19, 24). The most important and serious human diseases disseminated by the consumption of contaminated raw milk are tuberculosis and brucellosis (19, 24). It can also be occurred from infected animals(19). Pasteurization can destroy these dangerous pathogens and render the milk safe for consumption.

Other pathogenic bacteria may also be present in raw milk as a direct consequence of udder disease. Among the organisms commonly producing mastitis Strp. agalactiae, Staph. aureus and E. coli are pathogenic for man.

Infrequently micro-organisms of greater pathogenicity for man produce bovine mastitis and may be present in raw milk. They include Leptospira spp., Listeria monocytogenes, Bacillus cereus, Pasteurella multocida, Clostridium perfringens, Nocardia spp., Cryptococcus neoformans and Actinomyces spp. Additionally, Coxiella burnetti, the causative agent for Q fever (19, 24).

Further hazards stem from the adverstitious contamination of raw milk by pathogenic bacteria from sources external to the udder. Samonallae and thermoduric Campylobacter strains fall into this category. The majority of these stem either directly or indirectly from faecal contamination of the milk.

All of these pathogens, with the exception of Cl. perfringens and B.cereus are destroyed by pasteurization; these two organisms can survive the pasteurization process because of their ability to sporulate. It is from that B.cereus spores and its growth in pasteurized milk leads to the development of off-flavors, and an appearance discouraging consumption (19).

2.3 .4 The microbiology of pasteurized milk

a) Total bacterial contents

The limitation of total bacterial contents of grade A pasteurized milk is 20,000 cfu/ml (17, 19).

The standard plate count on pasteurized milk and related products is something of an index of good manufacturing practices. If the raw product was of satisfactory microbiological quality, the processing was carried out properly,protection from contamination was satisfactory, holding temperatures and times were such that growth of bacteria did not occur to any significant extent, and other less important factors were kept under proper control, the count will be low. However, a low count does not ensure that the product will be free from potential disease-producing organisms (19). A low count does not assure freedom from contamination with bacteria which will cause poor keeping quality (19). Besides; It is reported that bacterial types are more important than numbers in determining shelf-life of milk (25). It is also reported that the organoleptic change in pasteurized milk can be detected when the bacterial populations is excess of 10^6 cfu/ml and frequently 10^7 cfu/ml. Occasionally, even populations of 10^8 cfu/ml or more will not cause an organoleptically detectable change, because of the relative low level of relevant biochemical activity. (19). Most of the reports said that the spoilage of pasteurized milk occured when the counts reach 10^7 cfu/ml (19, 26).

b) Thermoduric bacteria

Thermoduric bacteria are the organisms that survive pasteurization, but do not grow at pasteurization temperatures. Thermoduric organisms are predominantly mesophilic, but a few types are included among the psychrotrophic organisms which can grow slowly at refrigeration temperatures.

The commonly encountered thermoduric bacteria are found in the genera Arthrobacter, Bacillus, Microbacterium, Micrococcus and Streptococcus (18, 19, 27). Micrococcus are thermoduric that frequently associated with poor sanitation of equipment used for handling milk, they are present in insignificant members in milk drawn aseptically. They commonly constitute the major fraction of the thermoduric population of milk. It is found that HTST pasteurization is less effective than holder-type pasteurization in reducing population of thermoduric microorganisms (18, 19).

c) Thermophilic bacteria (19)

Thermophilic bacteria are commonly defined as those which will grow rapidly at 55°C. Some facultative thermophilic bacteria will grow at 37°C or lower.

The thermophilic bacteria found in milk are primarily aerobic or facultatively anaerobic spore-forming rods. They become a problem in pasteurized milk when portions of the milk are held for some time within the temperature range of 50-70°C. Repeated use of the vats for batch pasteurization without thorough cleaning between uses, has been found to permit a build up of thermophilic bacteria in the milk and milk-film residues: When an interruption in normal scheduling occurs, delayed cooling may permit growth and, because the pasteurization temperature of 62.8°C is so suitable for growth of thermophilic bacteria, any prolonged holding in this temperature range of milk, or the equipment with which it comes in contact, must be avoided (19).

The extensive use of HTST pasteurization has markedly reduced problems with thermophilic bacteria. Thus, in a typical operation, less than 2 min. is required for the milk to pass from being cold, raw milk through to being cold (4.4°C) pasteurized milk, and the holding temperature (71.7°C) and holding time (16 s) are a combination not conductive to growth of these bacteria.

d) Psychrotrophic bacteria

Nearly all pasteurised milk and related products held under refrigeration will eventually develop one or more defects due to psychrotrophic bacteria. The rate at which a defect develops will depend upon the initial number of these organisms present, the rate at which the organisms grow at the holding temperature used, and the ability of the organisms to cause an organoleptically detectable change in product. Theoretically, only one cell of a type capable of causing a defect needs to be present in a given container.

When the thermoduric psychrotrophic bacteria of the genera Bacillus, Clostridium and Streptococcus are responsible for a problem, the remedy is either to reduce the numbers of these bacteria in the milk supply, or to increase the intensify of the heat treatment to a level which will kill the responsible organisms. Refrigeration in the upper range of acceptability will increase the probability that this group will develop and shorten the shelf-life of pasteurized milk(19, 27, 28). But organisms of this group do tend to be overgrown by other types of psychrotrophic bacteria.

The psychrotrophic bacteria of the gram-negative group are killed, in most instances by a considerable margin, by normal pasteurization, and their presence in the pasteurized product is the result of post-pasteurization contamination (19, 26, 29, 30). Organisms of this group tend to out grow the thermoduric psychrotrophic types, particularly when the product is held at the lower range of temperatures. Their control depends upon preventing post-Pasteurzation contamination, or at least keeping the level extremely low. Since these organisms are quite easily killed by either heat or chemical microbiocidal treatments, the basic problem usually is one of being sure that no little details are overlooked. Contamination levels so low that they are not detected by the usual microbiological tests can still contribute enough organisms for keeping quality to be a problem.

The temperature at which product is held can have a great effect on the growth of psychrotrophic bacteria. At 10°C and above, non-psychrotrophic thermoduric bacteria are kept to outgrow the psychrotrophic types, but at 7.2°C and below, dominance of the psychrotrophic types is found. As temperature decrease toward 0°C, some of the psychrotrophic organisms will be kept from growing, and those that do grow, will do so at progressively lower rates. The rate of growth of psychrotrophs increased markly from temperature 2°C to 15°C. At temperature between 10 and 15°C, the proportion of the total population that was made up to psychrotrophs would reduce the holding time of milk greatly. The optimum control for psychrotrophic bacteria is to have the least possible post-pasteurization contamination, followed by holding the product at the lowest practical temperature, and certainly not above 5°C. Even relatively short periods of warm-up may so accelerate growth that keeping quality is reduced significantly (19).

Griffiths, Phillips and Muir (1984) reported that the detection of organloeptic changes in most dairy products occurred when the psychrotrophic bacterial number reach log 7.5 cfu/ml (30).

e) Coliform bacteria (19)

This group of bacteria comprises the aerobic and facultative anaerobic, gram-negative, non-spore-forming rods which ferment lactose with the production of acid and gas at 32°C with in 48 hr. The genera Escherichia, Enterobacter and Klebsiella are the typical organisms of this groups, although a few lactose-fermenting species of other genera msy also be included. In dairy products, coliform bacteria are not usually found in properly pasteurised milk immediately following heat treatment. Therefore, test for coliform bacteria are used to detect significant post-pasteurization contamination. Heat-resistant coliform bacteria have been isolated, but experience indicates they are relatively unimportant in pasteurised milk. Excessive numbers of coliform bacteria in raw milk might, under unusual circumstances, permit a few cells to survive pasteurization. Such a situation would be quite unlikely with a properly controlled milk supply.

The common standard is that coliform bacteria shall not exceed 10cfu/ml in pasteurised milk. For a well run plant, the absence of coliform bacteria in 1 ml sample of product would be a desirable objective, and splitting a 10 ml sample between three plates permit a more stringent standard to be employed. Positive results on a 1 ml sample should call for correction of an undesirable situation, but it must be emphasised that the absence of coliform bacteria in 1 ml quantities of product will not assure long-term keeping quality.

Coliform bacteria allowed to grow to numbers of a million or more per ml may cause defects. These defects may include ropiness, grassiness, unclean and medicinal odours, and bitterness. Extensive growth in raw milk may make it unsuitable for processing. In pasteurised milk, keeping quality may be affected, particularly if the milk is held above 7.2°C. Ropiness in refrigerated pasteurised milk may be due to post-pasteurization contamination with strains of Enterobacter aerogenes. The presence of enteropathogenic E.coli has caused illness from other industry products, but pasteurised milk has not been implicated.

Plant with poor cleaning and inadequate microbiocidal treatment of equipment with which the pasteurised milk comes in contact are the most important sources of contamination with coliform bacteria.

Unsanitary practices by personnel, such as assembling equipment without proper washing and microbiocidal treatment of the hands, holding gaskets and miscellaneous small parts in the mouth or in pockets, the carelessness in placing equipment either on the floor or in other areas where it may be splashed upon, are additional sources of contamination.

2.4 Enzymes in milk

Enzymes are proteins which can be denatured and inactivated by high temperatures. Most of milk enzymes are inactivated by pasteurization but some microbial enzymes produced by psychrotroph are heat-resistant (16). The two groups of enzymes of major economic importance are the proteinases and lipases which are produced extracellularly and can act directly on micelle casein and on the fat globules in milk. They can act in stored raw milk which has high population of psychrotrophic bacteria and continue to act in stored heat treated liquid milk and other dairy products (17, 31).

2.4.1 Proteinases of psychrotrophic bacteria

Some proteinase producing thermoduric bacteria are well known for their ability to produce extracellular proteinases and psychrotrophic thermoduric sporeformers have been isolated from milk. However, there have been no definitive reports linking proteases produced by these organisms with changes in milk or milk products.

Proteinase producing psychrotrophic gram negative bacteria are often isolated from milk. They are Ps.species, Ps.fluorescens, Ps.putrefaciens, Ps.fragi, Ps.aeruginosa, Ps.aureofaceins, Ps.putida, Acinobacter spp., Achromobacter sp., Aeromonas sp., Enterobacter liquefaciens, Escherichia fruendii, Flavobacterium spp. The best represented genus is Ps., with Ps. fluorescens as the most common species (19).

Psychrotrophic bacteria are difficult to exclude from raw milk and may grow and produce proteolytic enzymes during refrigerated storage. Many of these protease enzymes are heat stable and survive pasteurization temperatures. Hence, if sufficient heat-stable enzymes are in raw milk prior to pasteurization, defect may become more pronounced after pasteurization during refrigerated storage. Psychrotrophs isolated from raw milk produced proteases that survived 149°C for 10 sec (26). 70 to 90% of raw milks contained psychrotrophs capable of producing heat resistance protease (31, 32, 33). Most of the reports suggested that counts of at least 5×10^6 cfu/ml are necessary before proteolysis is detectable in milk (20). It is found that proteolytic strains of Ps.fluorescens, Ps.fragi and Ps.putrefaciens degraded casein and whey proteins when grown as pure cultures in pasteurized milk at low temperatures. The milk was spoiled by coagulation and bitter off-flavors when the bacterial numbers reach 10^{7-8} cfu/ml (20).

2.4.2 Proteolysis in milk and dairy products.

Cow's milk contains approximately 3.5% (w/w) protein, about 80% of which is classified as casein, a mixture of 4 phosphoproteins, αs_1, αs_2, β and κ-caseins, in the approximate proportions 4:1 : 4:1. All the caseins are insoluble at pH 4.6.

The αs_1-, αs_2- and β-caseins are insoluble at Ca^{2+} concentrations greater than 6 mM, but are stabilized by k-casein in the form of coarse colloidal particles, micelles. Proteolysis of k-casein results in destabilization of the caseinate system and because the caseins, especially β-casein, are strongly hydrophobic, casein hydrolysates tend to be bitter (34).

The protein soluble at pH 4.6 are the whey proteins which are very heterogeneous. The principal proteins, β-lactoglobulin and α-lactalbumin, are remarkable resistant to proteolysis, even by very active, broad specificity proteinases. So, proteolysis in milk is concerned with the caseins only.

The obvious change that occur when milk is treated with proteinases is the formation of a visible clot. Chemical analyses reveal no gross change in composition. The clot is formed by the caseinate fraction of milk, and the presence in the curd of lactose, whey proteins, and fat is due to mechanical occlusion.

Pasteurized milk appears to be more resistant to proteolysis than UHT milk (35). The use of high temperatures during processing may lead to the exposure of new enzyme substrate sites on protein molecules. Hence, much higher proteolysis was required for off flavor development in pasteurized than in UHT milk . Higher enzyme concentrations resulted in clotting of pasteurized milk but gelation in UHT milk (34, 35).

2.4.3 <u>Milk lipases and lipases of psychrotrophic bacteria</u> (20,36)

It is reported that increasing lipolytic flora in stored milk and area dealt with the identification of lipolytic enzymes, the most common sources of lipases are psychrotrophic bacteria. The most strongly active species belonged to genus Pseudomonas. The numbers of these in milk increase during storage and may produce significant amounts of lipase after about 3 days. When the counts of these lipolytic bacteria exceed 10^7 cfu/ml, they can cause rancid flavors. The low incidence of bacterially induce rancidity in milk is very probably due to the inaccessibility of the triglycerides to the lipases, unless the fat globule membrane is damaged by excessive agitation, in these circumstances, the natural lipase in raw milk is very active and would be expected to have a much greater effect than the relatively small amounts of lipase which would be produced by normally-encounter of numbers of psychrotrophs in storred raw milk ($<10^7$ cfu/ml).

Many bacterial lipases differ from milk lipase in that they are not inactivated by pasteurization even though organisms which produce them are destroyed. They can therefore be carried through in an action form into manufactured products and cause fat break down during storage of these products.

Milk lipase is relatively unstable and can be inactivated by salt, acid, light, oxidation and heat. Normal pasteurization of market milk, which inactivates the enzymes prevent further breakdown of the fat due to lipase but not destroy any taint which is already present at pasteurization.

Lipases are more heat resistant in milk than they are in aqueous buffer solution. The fat phase of the milk may offer some protection to denaturation.

2.4.4 Lipolysis in milk

The breakdown of fat is called lipolysis and is caused by the enzyme lipase. Lipolyzed fat has a rancid taste and smell, caused by the presence of low-molecular free fatty acids (butyric and caproic acid) (15). Lipase is present in all raw milk, but can attack the milk-fat only under certain conditions.

The fat in milk exist as small globules surrounded by a natural protective membrane made up of complex fats and proteins. Under normal conditions this membrane is sufficient to prevent the lipase, which is mainly in the skim milk, from attacking the fat. However, if the milk is subjected to certain physical or chemical treatments which disrupt the membrane, the enzyme can gain access to the fat and cause lipolysis which leads to the flavor defect known as hydrolytic rancidity such lipolysis (known as induced lipolysis) does not happen instantaneously but develops during storage (36).

The milk from some cows, although never rancid when it leaves the udder, undergoes lipolysis during storage without being subjected to physical maltreatment. Lipolysis in these milks is initiated simply by cooling the milk soon after it is drawn from the cow and is known as spontaneous lipolysis (36).

When the count of lipolytic bacteria exceed one million/ ml, they can cause rancid flavor (36). This is called microbial lopolysis. Unlike the milk enzymes, many of these lipases can attack the intact fat globule in milk. They can cause lipolysis in milk without any prior activation.

It is generally accepted that a trained grader will detect rancidity in milk with an acid degree value (ADV) greater than 1.5, while most people find milk with an ADV over 2.0 unpalatable (27). At low levels, hydrolytic rancidity in milk is often perceived as old, unclean or cowy flavors. Increasing ADV give bitter, butyric and finally definite rancid flavor. The presence of other off-flavor (Staleness, oxidized flavors) may make low levels of rancidity (36).

2.5 Shelf-life of pasteurized milk (19, 32, 33, 37)

The quality of market milk is very important to the consumer. The time interval between processing and consumption may vary considerably and is often referred to as the "expected shelf-life" of the product (37). Many studies have been conducted on the relationship of shelf-life of the processed product of bacterial quality of the raw milk supply. Production practice at the farm, temperatures of storage, and age of raw milk at processing are factors that can, and invariable do, influence the quality of finished product (19, 32, 33, 37)

It is reported that bacterial types are more important than numbers in determining shelf-life of milk, and the causative spoilage organisms are frequently present to the extent of less than one per milliliter (37). The relationship between the increasing of bacteria and flavor changes in milk stored at low temperatures are positive, but the use of the ratio between gram negative and gram positive bacteria is of more valuable than the standard plate count.

Janzen et al (1982) found that increasing storage time of raw milk at 4.5 °C prior to pasteurization may affect product shelf-life stored at 4.5 °C, but no correlations were significant between raw or pasteurized milk and total bacteria or coliform counts. Related were flavor score and days held raw, shelf-life of the pasteurized milk, and interaction of days held raw and shelf-life of the pasteurized milk. Psychrotrophic counts and age of the raw milk were correlated (32).

The temperature of storage of the processed and packaged milk plays a major role in determining its shelf-life. Both the temperature of the milk following packaging and the ultimate storage temperature are extremely important in determining the shelf-life of the product (37).

Temperature is the principal factor governing the rate of growth of the microorganisms which are responsible for the spoilage of the product (37). The growth of microorganisms followed the first order autocatalytic chemical reaction (38) as

rate of increasing of bacteria = μ (mumber of bacteria)

The constant of proportionality, μ, is an index of the rate of growth and is called the growth rate constant. Since we assume growth to be balanced, μ also relates the rate of increase of any given cellular component to the amount of the cellular component, or in mathematical terms (38),

$$\frac{dN}{dt} = \mu N$$

where N is the number of cells/ml, t is time and μ is the growth rate constant. Other forms of this equation are more useful in practice. up on integration, the equation is

$$\ln N - \ln N_0 = \mu (t-t_0)$$

and on converting natural logarithms to logarithms to the base ten

$$\log N - \log N_0 = \frac{\mu}{2.303}(t-t_0)$$

where the values of N and N_0 correspond to the amount of any bacterial component of the culture at time t and t_0 respectively (38).

In 1981, Janzen et al reported that no correlation was significant ($P>0.1$)for the effect of container types (fiberboard and plastic jug) on ADV (acid degree value), SPC (standard plate count), oxidase-positive bacterial count and flavor score (using eight panelists). However, a closer look at the data for flavor score and ADV found that the mean flavor scores for the milk in fiberboard and plastic jugs are about the same, with plastic jugs exhibiting a slight edge over the carton at 7 and 14 days of storage for both storage temperatures at 4.5 and 7°C (37).

A comparison of the mean ADV with shelf-life of the homogenized products reveals a similar trend. It is found that milk packaged in plastic jugs consistently exhibited a lower ADV compared to the fiberboard carton, for both temperature of storage and storage time as shown in **Table** 2.4 (37).

Table 2.4 Mean flavor scores and days of commercially pasteurized milk (37)

Storage Days	Storage temp (°C)	Package C=carton P=plastic	Flavor score	ADV
0	4.5	C	37.7	1.14
		P	37.7	1.14
7	4.5	C	36.6	1.46
		P	36.7	1.39
14	4.5	C	32.4	2.25
		P	34.1	1.69
0	7	C	37.7	1.14
		P	37.7	1.14
7	7	C	34.1	1.58
		P	34.7	1.45
14	7	C	31.0	2.39
		P	31.7	1.97

* Flavor score, using the 40 (45)-point flavor score. (The current ADSA scoring guide uses 10-point flavor score for Colligiate Compettition use which equates to the 31-40 range normally employed).