CHAPTER II

LITERATURE REVIEW

Microbiological quality

Milk from healthy cows has very low in bacterial numbers. Raw milk can be contaminated with microorganism due to poor milking methods, long duration of transportation, inadequate cleaning of milk equipment, poor cooling and in some cases, as a result of mastitis (Bramley, 1982; Hogan, *et al*,1989; Murphy, 1997; Zehner, *et al*, 1986). Several studies indicated that milking-time hygiene is very important factor in ensuring lower total bacterial counts in milk and integral component of a good milking practice (Natzke, 1977, Pankey, 1989a, b; Bartlett, *et al*., 1992a). Pre-milking sanitation procedure can effectively reduce the microbial contamination immediately before the attachment of milking units and decrease the incidence of udder infections caused by several major environmental pathogens (Galton, *et al*., 1982; Pankey, 1989a; Ruegg and Dohoo, 1997). The legal limit for total bacteria in farm raw milk in United States of America (USA) is 100,000 colony forming unit/milliliter (cfu/ml), milk with counts of 10,000 cfu/ml or less is considered to be of optimum quality ("Grade A" Pasteurized Milk Ordinance (PMO), 1995). Moreover, bacteria produce enzymes that degrade milk proteins, fats and other components, resulting in reduced product yields as well as in product off-flavors. Though pasteurization most often kills a majority of bacteria in milk, some strains produce enzymes that survive the pasteurization treatment. These heat stable enzymes have the capability of further degrading the processed product, especially long-life

milk products (Patel and Blankenagel, 1972). Following is a list of testing methods commonly used to evaluate raw milk bacteriological quality, including the required SPC, as well as auxiliary test:

Standard Plate Count (SPC)

This procedure counts the total viable bacteria in a milliliter (ml) of milk. The SPC is an indicator of sanitation in milking cows, milking system cleanup, and certain types of mastitis. In the United States of America, the legal maximum for producer raw milk is 100,000 cfu/ml (PMO, 1995), whereas it is 600,000 cfu/ml in Thailand (Department of Livestock Development, 1999). Extremely high counts in raw milk (i.e. due to poor cooling) can cause defects in raw milk that result in its rejection, due to off-odors and flavors (i.e. sour, malty). Other microbial defects can carry over into the products (Jenness and Patton, 1959).

Major factors affecting SPC are the level of herd mastitis and high numbers of late lactating cows. Other factors are milker employee hygiene; cow and milking system cleanliness, milking equipment rubber parts condition, and faulty milk pump seal (Blowey and Edmonson, 2000).

Coliform Count (CC)

This procedure counts fecal bacteria in milk and coli organisms shed by cows into milk. In New York State of USA, a count over 750 cfu/ml reduces milk price, and counts below 50 cfu/ml are attainable (Dairy Science Facts, 2000). In Thailand, CC should be less than 10,000 cfu/ml (Department of Livestock Development, 1999).

The main factors affecting CC are milking wet and dirty cows. Coliform mastitic cows rarely contribute to CC but can elevate the count. Occasional problems

are from defective milking system cleaning and dirty condition of equipment parts not touched by hot wash water (Dairy Science Facts, 2000).

Laboratory Pasteurization Count (LPC)

This test counts bacteria that survive milk pasteurization (thermoduric bacteria). These bacteria affect flavor and product shelf life. In New York State of USA, a count over 750 cfu/ml reduces milk price, and counts below 50 cfu/ml are attainable (Dairy Science Facts, 2000), but it should be less than 1,000 cfu/ml in Thailand (Department of Livestock Development, 1999).

Factors affecting LPC are mainly poor equipment cleanup and milk residues in the pipeline traps, and tanks. This can be due to using wash water under 120°F, insufficient agitation, faulty air injectors, not enough or low quality soap and chemicals, and incorrect order of usage. Other factors are poor equipment rubber parts condition and dirt or feed residues on udders and in teat lesion. High LPC cannot result from mastitis (Dairy Science Facts, 2000).

The methylene blue reduction test (MB)

Reduction test for the determination of the bacteriological quality of milk are based on the ability of certain enzymes (dehydrogenases, mainly flavine enzymes) of the bacterial cell to transfer hydrogen from a substrate to biological acceptors. Suitable chemical substances such as methylene blue or resazurin, can also act as acceptors (Figure 1)

During this reaction the chemical substance (dye) is reduced. The rate of reduction depends on the enzyme activity or enzyme concentration and this has been used as an index of the number of bacteria present.

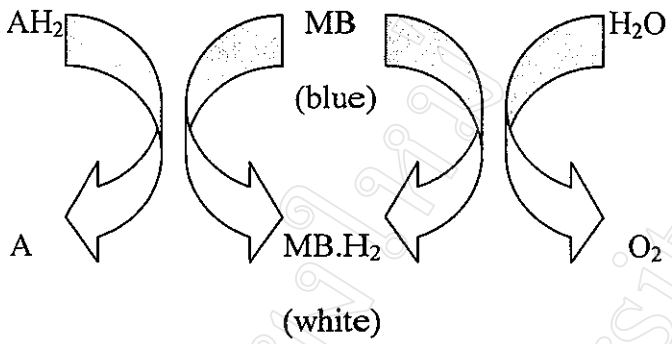

Figure 1 Chemical reaction of the methylene blue reduction test (Luck, 1991)

The principle of these tests is to add the dye to the milk and to measure the color change or to decolorize the dye, often called reductase activity, which is an index of the bacteriological load of the milk (Luck, 1991). In Thailand, this method is a reference method for grading the raw milk quality. Chanlun, *et al* (2003) reported that the milk grading by this method correspond to the total bacterial counts (TBC) in raw milk.

Milk composition

Mammals secrete milk to supply nutrition to their young. For centuries, human has taken advantage of this by taking milk from cows, water buffaloes, goats and sheep (450-500 million metric tons per year worldwide) and using it to make a significant contribution to our diet.

Cows produce more milk than their calves require and man has taken advantage of this since the dawn of time. Breeding programs have successfully increased the amount and quality of milk cows produce. Cow's milk contains about 87.5% water and about 12.5% milk solids (total solids) the latter comprising about 4.0% fat, 3.0% protein, 4.5% lactose (anhydrous) and 1.0% 'other solids', i.e.

minerals, vitamins, etc. (Figure2). In Thailand, milk fat, protein, solid not fat and total solids should not be less than 3.5%, 3.5%, 8.4% and 12.5%, respectively (Department of Livestock Development, 1999). Non-water constituents are present in different physical forms; dissolved (lactose), colloidally dispersed (protein) and emulsified in water (lipids or fats). These physical characteristics are used to facilitate the commercial and analytical separation of the major constituents of milk.

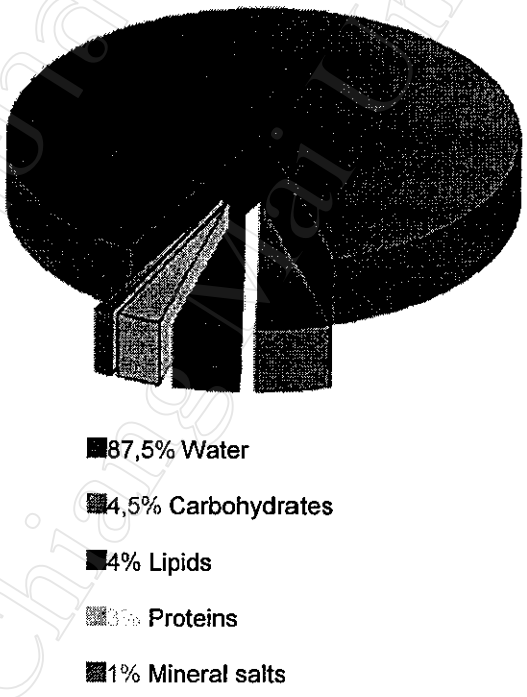

Figure 2 Gross composition of milk (2003 [Online]. Available: http://www.japy.com/htmlgb/lait/lait.htm)

Measurement of total solids in milk

The total solid (TS) of milk are determined simply by evaporating the water and weighing the residue. For the reference method, conditions for the measurement are tightly controlled and rely on oven drying 5 g of milk at 102±1°C for 2 hours until constant mass is achieved. These methods are detailed in International Dairy Federation (IDF), and International Organization for Standardization (ISO) standards (Harding, 1999). Care has to be taken in keeping the solids matter in a dry atmosphere (desiccator) whilst it is being cooled prior to weighing, since lactose is extremely hygroscopic and will readily pick up water, thus giving a falsely high total solids figure. The weight of the added neutralizer is taken into account in the final weighing process.

Moisture balances have been used for rapid determination of total solids. These consist of a combined balance and radiant infrared drying lamp. Whilst these may be used for measuring total solids of milk they tend to be used more for measuring moisture (water) in milk products.

Similarly, microwave ovens have been used for the rapid determination of total solids in milk. These routine during methods have to be checked regularly against the reference method in order to establish a calibration control (Harding, 1999).

Mechanized pipetting followed by oven drying in a flow-through tunnel leading to a balance have in the past been used for mass testing for the total solids where it has been used for quality payment of farmers. However, milk total solid is now considered to be a very crude measure of quality and most payment systems are based on fat and solids-not-fat or fat and protein.

Milk total solids comprise fat (3.9%) and solids-not-fat (SNF) 8.7%, sometimes referred to as non-fat milk solids (NFMS) (Harding, 1999).

Measurement of the solids-not-fat (SNF) of milk

SNF by definition are the total solids other than butter fat. The reference SNF therefore is obtained by taking the reference oven-drying total solids and subtracting the reference (solvent extraction) butterfat.

$$SNF = TS - FAT$$

Many retail products such as creams, butter, etc. are manufactured from fat separated from milk, hence creating a different commercial value for fat than for SNF. Mechanical oven-drying total solids and mechanized Gerber fat determination systems have been used to provide TS and fat—hence SNF.

Measurement of the density of milk using a hydrometer or lactometer (Figure 3) together with determination of fat by Gerber has also been used for routine measurement of fat and SNF. This method gives a simple approximation to the gravimetric SNF. The method is based on the different specific gravities of fat (0.93) water (1.0) and SNF (1.6) of milk. It is based on the observation that the density of milk at a standard temperature (20°C) is related to the fat and SNF content. Hence, measurement of fat (Gerber) and density enable the SNF to be calculated (Harding, 1999).

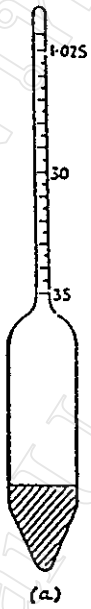

Figure 3 Lactometer (Harding, 1999)

The following formula was arrived by measuring gravimetric SNF, density and fat for thousands of herd milk samples

$$TS = 0.25D + 1.22fat + 0.72SNF$$

$$SNF = TS - fat$$

where TS is total solids and D is density (at 20°C). This derived formula for SNF gives an approximate SNF for individual milk supplies (Harding, 1999).

It is important that errors in density determination are minimized by use of calibrated hydrometers and thermometers. However, the density method of measuring SNF is at best an approximation to the more accurate reference gravimetric method.

Infrared instruments measuring fat, protein and lactose can also provide an SNF or total solids figure when suitable allowances are made for 'other constituents' or 'mineral bias' of milk since the SNF (8.7%) in turn is composed of protein (3.2%), and lactose or milk sugar (4.6%) with vitamins and minerals adding a further 0.9%. The accuracy of such an SNF estimate will depend upon accurate calibration of the infrared instrument for protein and lactose, and regular checks of the constant to be applied for 'other constituents'. This is obtained from measurement of gravimetric SNF, from which reference protein and lactose is subtracted. The 0.92% 'value' proposed for 'other solids' is an average and will vary slightly from herd to herd (Harding, 1999).

The composition of cow's milk varies from cow to cow within breeds and from breed to breed. It also varies during lactation, seasonally and regionally and there are many factors which cause these variations. Milk composition is important since the yield of products made from milk depend on the quantity of particular constituents present in the raw milk. Since the cost of raw material, milk, accounts for about 70% of the cost of products, the financial value of each liter of milk is directly related to its compositional quality; hence it would be illogical to pay farmers only on the volume they sell. In most countries therefore compositional quality is one of factors used to calculate the payment of farmers (Harding, 1999).

Major constituents

Fat

Lipids or fats of milk cumulatively are referred to as butterfat. Biologically, due to the high percentage of carbon in fats, they are 'stored' nutrients with the highest energy or calorific value of all food constituents. The basic structure involves esterification of fatty acids onto a glycerol molecule (Figure 4).

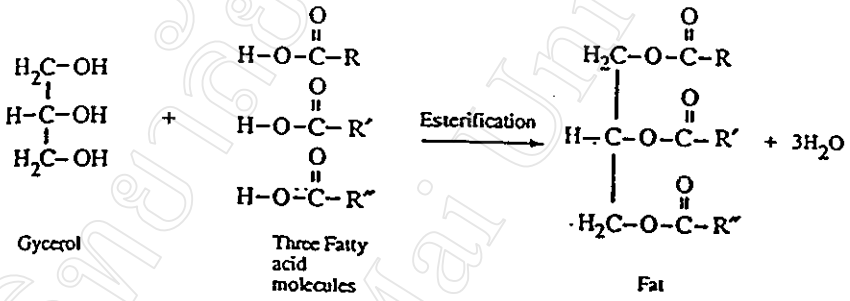

Figure 4 Structure of fat molecule (Harding, 1999)

R, R′ and R″ represent fatty acids which may be the same, or different. Fatty acids are organic acids composed of hydrocarbon chains with a carboxyl group (-COOH) on one end. These can be short-chain, long-chain, saturated or unsaturated.

Saturated fatty acids have no double bond. Monounsaturated fatty acids have one double bond and polyunsaturated fatty acids have two or more double bonds. In oils from fish or plants, fatty acids tend to be polyunsaturated (PUFA) whereas animal fats tend to be saturated. However, fatty acids of butterfat vary in chain length and in levels of monounsaturated fatty acid and this variation can affect both the nutritional value of fat and the quality and characteristics of products made from it. Milk fat contains more short-chain fatty acids (C_4 and C_6) than most vegetable oils.

Proteins

Proteins are the most valuable components of milk in terms of their importance in human nutrition and their influence on properties of dairy products containing them. This, together with the availability of rapid instrumental methods of measurement, has led to increased use of protein as a quality parameter in payment of farmers.

Proteins are large-molecular-weight complex organic compounds which contain carbon, hydrogen, oxygen and nitrogen; sulphur phosphorus and other elements may also be present. Protein molecules are made up of amino acids. These amino acids link together via peptide bonds to form long chains (Figure 5).

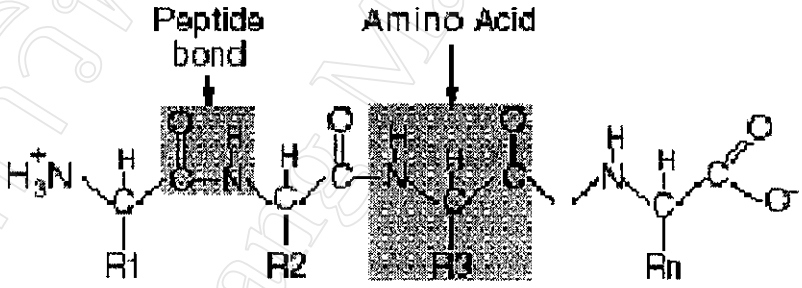

Figure 5 Structure of protein molecule: R1, R2, etc., are radicals specific to each amino acid. The number of amino acids in the caseins of milk varies from 199 to 209 (Wattiaux, 2003 [Online]. Available: http;//babcock.cals.wisc.edu/de/html/ch19/reproduction_eng_ch19.htm)

The total protein component of milk is composed of numerous specific proteins. The primary groups of milk proteins are the caseins. Caseins are fairly easily digestible in the intestine compared with many other food proteins available. The high quality, easily digestible protein in cow milk is one of the key reasons why milk is such an important human food.

Casein composes of several similar proteins which form a multi-molecular, granular structure called a casein micelle. In addition to casein molecules, the casein micelle contains water and salts (mainly calcium and phosphorus). Some enzymes are associated with casein micelles, too. Individual molecule of casein alone is not very soluble in the aqueous environment of milk. However, casein micelle granules are maintained as a colloidal suspension in milk. If the micelle structure is disturbed, micelles may come apart and the casein may come out of solution, forming the gelatinous material called the curd. This is part of the basis for formation of all non-fluid milk products. Because the casein micelle is in suspension, it can be separated from the rest of milk by centrifugation at a very high speed (Rowland, 1938). Generally the milk is first defatted (the cream is removed) from whole milk by low speed centrifugation (at about 5,000 to 10,000 x g), resulting in the cream layer at the top, the aqueous supernatant in the middle layer, and a small pellet of leukocytes and other debris at the bottom of the centrifuge tube. The aqueous supernatant is the skim milk (sometimes called the plasma phase of milk). Centrifugation of the skim milk in an ultracentrifuge (usually about 50,000 x g or greater) results in pelleting of the casein micelles and in a supernatant called whey (also sometimes called the serum phase of milk) which contains the water, lactose and soluble non-casein proteins.

Once casein has been removed, then all other proteins left in the milk preparation are considered to be whey proteins.

Lactose

Lactose, with the exception of water, is about 4.6%. Although the lactose is the principle component of milk, it is the least important of the solids both nutritionally and commercially. Lactose—milk sugar—is the major carbohydrate in the milk of most mammals. Hence mammalian milk is the major source of lactose which is one of the most common natural disaccharide. Lactose is a disaccharide composed of the monosaccharides D-glucose and D-galactose, joined by a β-1,4-glycosidic linkage (Figure 6) and is digested or broken down into these constituent parts by the enzyme lactase.

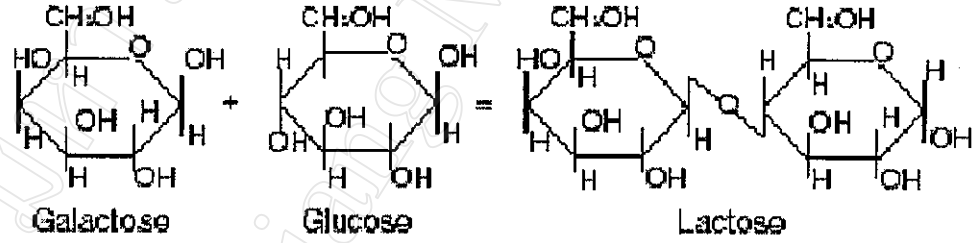

Figure 6 Structure of lactose molecule: Lactose is synthesized in the udder from glucose and galactose (Wattiaux, 2003 [Online]. Available: http;//babcock.cals.wisc.edu/de/html/ch19/reproduction_eng_ch19.htm)

Minerals

The major components of minerals found in milk are calcium and phosphorus. These minerals are required in large quantities by the rapidly growing neonate for the

bone growth and development of soft tissues. They are both mostly associated with the casein micelle structure. Consequently, whey has relatively little calcium and phosphorus compared with whole milk. Milk also contains most other minerals found in the body.

Other Components in Milk

Milk contains all the major vitamins. The fat soluble vitamins A, D, E, and K, are found primarily in the milk fat; milk has only limited amounts of vitamin K. The B vitamins are found in the aqueous phase of milk.

Assessing compositional quality using infrared; Milkoscan

The principle of using infrared absorption to measure the quality of milk was first proposed by Goulden (Goulden, 1964) working at the National Institute for Research in Dairying (Reading, Berkshire, UK).

Fat

For fat content, the principle of the infrared analysis of milk involves counting of ester linkages by measuring their infrared absorption (Figure 7). The fat molecule consists of a glycerol 'backbone' to which about three fatty acid chains are bound.

Two different wavelengths, 5.7 μm and 3.5 μm, can be used to determine the fat in milk. The 5.7 μm filter is referred to as Fat A and the 3.5 μm as Fat B.

Fat A

The absorption at 5.7 μm is due to stretching vibrations in the C=O bonds of the carboxyl group in fat (Figure 7). Since there is only one carboxyl group per fatty acid, this measurement 'counts' the number of fat molecules regardless of the carbon chain length and molecular weight of individual fatty acids.

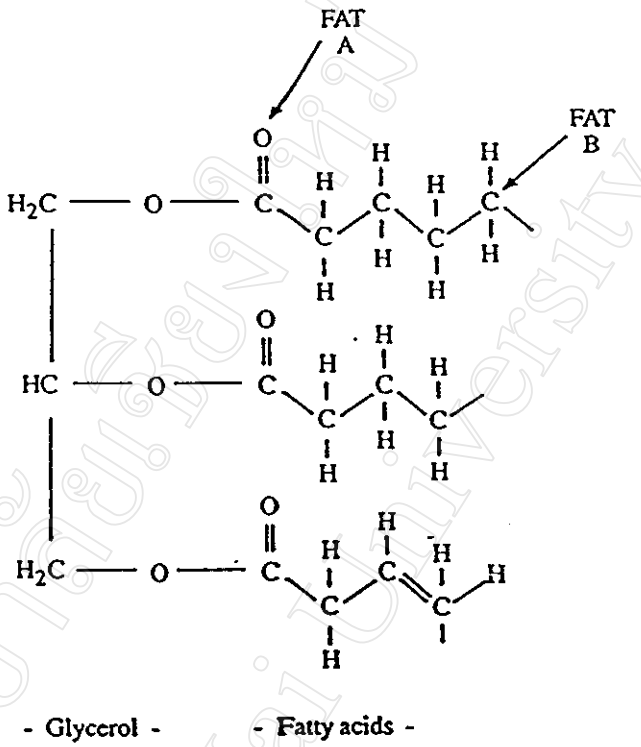

Figure 7 Infrared measurement of fat. A, measuring carbonyl; B, measuring ester linkages (Harding, 1999)

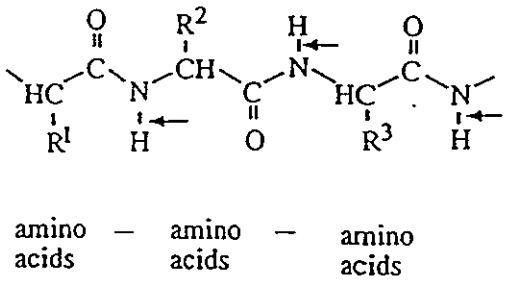

Figure 8 Infrared measurement of protein (Harding, 1999)

If the average chain length (mean molecular weight) of fatty acids is changed, the number of triglyceride molecules per unit weight will change too, and an error will occur in the result unless the change is compensated by recalibrating the instrument.

Comparisons between infrared and reference gravimetric methods had identified errors associated with band A filters and these resulted in the production of B filters in 1981. B type filters use the CH_2 and CH_3 absorption centers of the fatty acids which represent 73% of the fat by weight, and they are less sensitive to variations in fatty acid chain length and degree of unsaturation.

Fat B

The absorption at 3.5 μm is due to stretching vibrations in the saturated C-H bonds of the fatty acid chains (Figure 7). This measurement is, therefore, related to both the size and the number of fat molecules in the sample, as the number of carbon-hydrogen bonds increase substantially in proportion to the molecular size.

Measurement at 3.5 μm also includes free fatty acids that may have formed during storage; these are not measured at 5.7 μm.

Protein

The protein molecule consists primarily of amino acid units joined together in a long chain by peptide bonds (Figure 8).

The wavelength for protein determination is 6.5 μm, and it relates to the nitrogen-hydrogen bonds within peptide bonds that are responsible for the infrared absorption. Thus, the measurement represents the number of amino acids rather than their weight, but the composition of protein in milk is fairly constant, this causes no problems. In contrast to the reference (Kjeldahl) method the infrared measurement

does not measure all non-protein nitrogen. In fact, this technique measures only proteinaceous nitrogen, except urea.

Lactose

Lactose consists of one glucose molecule and one galactose molecule joined together (Figure 9). The hydroxyl group (OH) is characteristic of carbohydrates. It is the bond between the hydroxyl group and the carbon atom which absorbs infrared energy at the lactose wave length, 9.5 μm.

The Milkoscan's 'lactose' determination is not actually specific for lactose, but will measure other carbohydrates containing the OH group which may be present in the sample.

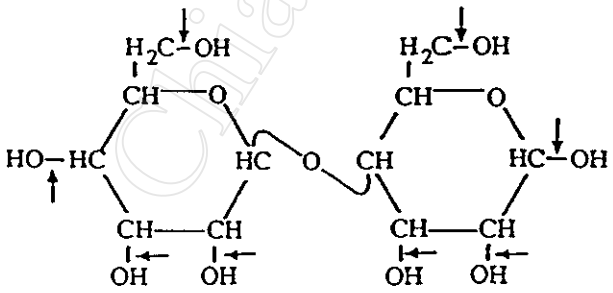

Figure 9 Infrared measurement of lactose (Harding, 1999)

Somatic Cell Count (SCC)

Somatic cells are simply animal body cells which present at low levels in normal milk. High levels of these cells in milk indicate abnormal, reduced-quality milk that is caused by an intramammary bacterial infection (mastitis).

The majority of the cells in a somatic cell count are leukocytes (white blood cells), and some are cells from the udder secretory tissue (epithelial cells). Epithelial cells are part of the normal body function and are shed and renewed in normal body processes. White blood cells serve as a defense mechanism to fight disease (infection), and assist in repairing damaged tissue.

Somatic cell count has become the "gold standard" measure of milk quality. The herd SCC level is dependent on the number and duration of infections present plus rate of new infections.

A low BTSCC (bulk tank somatic cell count) not only consistently correlates with low level mammary gland inflammation but also with other important milk quality factors such as microbiological quality (Plate counts, PI counts, Coliform counts) (Bennett, 1987; Schukken, *et al*, 1992). Food safety and the relative risk of antibiotic residue can be positively correlated to rising BTSCC (Ruegg and Tabone, 2000)

Numerous studies have shown a correlation between established mastitis control practices and SCC (Barlett, *et al*, 1992b; Crist, *et al*, 1982; Erskine, *et al*, 1987; Goodger, *et al*, 1993; Moxley, *et al*, 1978; Neave, et al, 1969; Wilson and Sears, 1995). The National Mastitis Council (NMC) five-point control plan: post milking teat dipping, dry cow therapy, pre-milking hygiene, and proper function and operation of milking equipment, as well as appropriate treatment of clinical cases, all

have been proven effective in lowering SCC (Nicolai and Philpot, 1974). Most of these studies were done comparing BTSCC of less than 400,000 with BTSCC greater than 400,000 to 1,000,000. The key milk quality element being regulated is SCC. High SCC levels are not known to pose a direct public health risk, yet they reflect mammary infection and overall quality of management. Moreover, lower SCC levels have been shown to be related to higher milk yield and better dairy product quality, and are, therefore, of economic value (Ma et al., 2000). SCC level of 200,000 cells/ml or less is considered physiologically typical (Laevens, *et al.,* 1997). The EU requires that milk used for dairy products which are sold in its territory have SCC levels below 400,000 cells/ml. New Zealand and Australia require similar levels, and Canada requires milk to gave below 500,000 cells/ml (Sargeant *et al.,* 1998; Norman *et al.*, 2000). In the United States, the current national penalty level is 750,000 cells/ml and over. Many US (organic) dairy cooperatives also require SCC less than 400,000 cells/ml. In Thailand, milk collecting centers also want SCC less than 500,000 cells/ml (Department of Livestock Development, 1999). While this raises the question of whether there are additional practices associated with reaching lower BTSCC expectations of today. During the past dozen years, environmental factors such as free stall and bedding management have also been recognized as important factors effecting BTSCC (Hogan, *et al*, 1988). Barkema *et al.* (1998) differentiate management practices between "low" BTSCC (<150,000), "mid" BTSCC (150,000-250,000) and "high" BTSCC (250,000-400,000). They found that those management practices known to be important for managing "high" BTSCC (>250,000) herds, such as post milking teat dipping, dry cow therapy, milking technique and antibiotic treatment of clinical cases, were also important in differentiating the "mid" and "low"

category BTSCC herds. In the "low" (<150,000) category herds, significantly more attention was paid to general hygiene ($p<0.05$) than the higher BTSCC herds. For example, factors which involve BTSCC <150,000 are cleanliness of cows and drinking cups, removal udder hair, cleanliness of free stalls, bedding quality, surveillance of clinical mastitis in dry cows, cleanliness of calving pens and milking parlors, post milking teat dipping, dry cows treatment, apt nutrient supplementation for spring heifers, dry and lactating cows

Hutton *et al.* (1990) found similar subtle differences in management detail and consistency between low BTSCC herds and higher BTSCC herds. These findings are not surprising, only vindication for those who have been emphasizing these points for many years.

Antibiotic residues

Antibiotics are used on dairy cattle to help control disease. The most usual treatment is intramammary infusion into an inflamed quarter of mastitis. The drug is infused by using a syringe-like application. The length of time of the drug is retained in the udder is controlled by the base in which it is dispersed. Antibiotics dispersed in oil-based solvents are retained in the udder longer than those in water-based solvents and drug companies set the 'retention' time for their preparations by trials involving milking cows. Once the upper limit of the antibiotic acceptability—the MRL—is set, the drug manufacturer normally has to satisfy licensing authorities that, providing farmers follow the withholding time on the preparation, they will not fail to meet the MRL for the bulk supply.

Antibiotic residues are undesirable in milk. It is argued that regular ingestion of antibiotics may encourage the development of antibiotic-resistant strains of

organisms in human and may lead to failure to respond to therapeutic doses of antibiotic at the later stage. Some people are also highly sensitive to antibiotics. For example, penicillin allergy could be triggered by trace levels in food. Antibiotics not only inhibit or kill pathogenic organisms in human but also inhibit the bacteria used to produce cheese and yogurt (Jurdi and Asmar, 1981), therefore antibiotic residues pose not only problems in human health but also a threat to the quality of manufactured products.

Antibiotic residue test method

Test methods must have a sensitivity which meets the MRLs for consumer protection as well as those required to protect starter cultures. The most widely used methods are microbial inhibitor tests. These methods, as the name describes, involve growth of specific test organisms such as *Bacillus stearothermophilus* being inhibited if an antibiotic is present in milk. The test organism is cultured in a small well in the presence of nutrients and an indicator dye. Under normal conditions the culture grows and the dye color is changed from purple to yellow. If an antibiotic is present the culture is killed and the dye remains purple. Such test methods are generally used as screening tests and positive samples should be retested. Whilst sensitive to a range of antibiotics, the test organism most widely used is generally more sensitive to penicillin than to other antibiotics. Modification of the pH of the test organism can however change the test sensitivity to different antibiotics.

Until recently an MRL has been set for penicillin without specified limits being given for other antibiotics. This is now changing with MRLs now having been set for a wide range of antibiotics (Table 1) for which appropriate test methods are being developed.

In northern Thailand, the microbial inhibition test kit that is commonly used is the test kit of the Department of Medical Science, Ministry of Public Health. This test kit has 90.5% of specificity and 100% of sensitivity (Rungrodejanarak, 1998). The detection limit of specific drugs that this test kit can be detected is shown in Table 1.

Milk quality in Thailand

There is only a few of Thailand's literatures about milk quality. Such as the research by Tangjaipatana *et al* (1995a), they had surveyed the raw milk quality in Saraburi Province, Ratchaburi Province and Bangkok. They found that the quality of cleanliness was fair; the nutrition value implied that the percentages of milk fat of most samples were in the range of 3.6-4.5; 18.92% of the samples were found positive for drug residues; and the somatic cell count was fair.

Additionally, Chayaratanasin and Kiatsoonthorn (2000) reported the raw milk quality at the cooperative level from 12 dairy plants, 105 milk collecting centers of 8 regions (33 provinces) in Thailand. The milk samples were collected from January 1998 to January 1999. The information was adapted from the total 1,235 items and presented in Table 2.

Table 1 Antibiotics maximum residue limits (MRL) in food (EC Directives 2377/90, 675/92 and 3093/92) and the detection limit of the antibiotic residue test kit for milk, Department of Medical Science, Ministry of Public Health (Rungrodejanarak, 1998)

Antibiotic	MRL (ppb)	Detection limit of test kit (IU/ml or μg/ml)
Benzyl penicillin	4.0	0.004
Ampicillin	4	0.004
Amoxicillin	4	0.004
Oxacillin	30	-
Cloxacillin	30	-
Dicloxacillin	30	-
Sulphonamides	100	6.0
Trimethoprim	50	-
Dapsone	25	-
Tetracyclines	100	0.10
Spiramycin	150	7.5
Levamisol	10	-
Tylosin	50	0.0625

Table2 Mean value of raw milk quality taking from the dairy cooperatives' bulk tanks (January 1998 to January 1999) (Chayaratanasin and Kiatsoonthorn, 2000)

Test	Number	Mean (min-max)	Note
Total solid percentage	973	12.44 (9.33-13.95)	-
Solid not fat percentage	1,193	8.27 (6.44-9.01)	-
Fat percentage	1,043	4.23 (3.05-5.52)	-
Temperature (°C)	562	5.72 (2.44-8)	-
Methylene blue reduction test (hours)	581	4.05 (2.30-6.20)	Hours that milk turns to white color
Standard Plate Count (cfu/ml)	895	739,000 (11,000 – 8,200,000)	-
Coliform Count (cfu/ml)	107	7,600 (80-180,000)	-
Laboratory Pasteurization Count (cfu/ml)	23	16,000 (410-51,000)	-
Somatic Cell Count (cells/ml)	567	998,000 (20,000-11,276,000)	-
Antimicrobial Residue	429	No. of +,- and +/- No. of test was between 0/89 – 1/121 and reported in percentage	The result was not shown because of many variables of the result.

Another research about milk quality in Thailand is the research of Aiumlamai *et al* (2003). They had surveyed the quality and composition of tank milk from dairy cooperatives in Thailand. They reported that the average SCC and total direct bacterial microscopic count were 1,027,880 ± 141,418 cell/ml and 924,476 ± 346,690 cell/ml, respectively. The average percentages of total solid (%TS), fat (%F) and solid not fat (%SNF) in milk composition were 12.58 ± 0.34, 4.14 ± 0.27 and 8.44 ± 0.15, respectively. Moreover, there are some researches about the antibiotic residue in raw milk. For example, the research of Tangjaipatana and Vecharungsun (1995b) showed that antibiotic residues in raw milk in Central region of Thailand during March 1993 – February 1994 which equivalent to 0.003 – 0.004 I. U. per milliliter of milk were found 14.28 – 35.49% of milk samples in each month. The residues equivalent to 0.003 – 0.004 I. U. of penicillin per milliliter of milk were evident in 0-10.61% of milk samples in each month.

In Northern Thailand, there are only a few researches about dairy farming and milk quality. Rojanasthien *et al* (2003) reported that the number of the dairy farm which has BTSCC higher than 500,000 cell/ml in Chaiprakarn District, Chiang Mai Province is in the range of 0-16% of the total sample. This result showed that farmers in Chaiprakarn could produce good quality milk with low SCC. About the dairy farming in Chiang Mai and Lamphun Province, Rojanasthien *et al* (2002) had studied about the results of herd health and production management in small holder farms in this area. This research indicated that the farmers who always pay attention to have good farm practice, got good milk quality and milk quantity. In addition, Phongphaew and Simasatitkul (1996) had surveyed on the mastitis control techniques in small-scale dairy farmers (n = 51) in Chiang Mai. They found that most farmers had

mismanaged as follows: 88.3% did not wash udders with chlorine solution, 88.2% used a towel for every cow, 56.9% dripped foremilk on the floor instead of using strip cups, 52.94% did not dip teats after milking, 82.4% dried cows with no medication, 60.8% used to have mastitis cows in herds and 3.9% had 1-2 cows with clinical mastitis while surveying. About subclinical mastitis knowledge, 41.2% knew nothing, 58.8% slightly had but never realized its harm.

Because there have been more developments and differences in the dairy farming in Northern Thailand in every year, the results of many researches in the past should have been changed. Moreover, there have never had any research to survey the milk quality of raw milk at the milk collecting center level in this area; therefore, this area still lack of basic knowledge for researchers to produce a milk quality research.

The Milk Collecting Centers in Thailand (MCC)

The milk collecting centers is the place to buy and collect the raw milk from the farmers before transport it to the dairy plant. In Thailand, the milk collecting centers can be divided into 2 types. First type of MCC in Thailand is simple MCC. These MCCs are only for collecting the raw milk. Another type of MCC in Thailand is combination of MCC and the dairy plant. These MCCs buy the raw milk from the farmers and have other parts to produce the processed milk products (Khaotian, 1996).

Nowadays, Food and Drug Administration of Thailand tries to set the Good Manufacturing Practice (GMP) of the MCC before 2003, 24 July (followed the government), so the MCC have to develop their management according to the GMP. The basic knowledge of milk quality and factors affecting raw milk quality at this level becomes necessary information of every MCC in Thailand.