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**THE INVESTIGATION OF THE QUALITY OF
MILK PRE-CONCENTRATED BY AN
ON-FARM EVAPORATION SYSTEM**

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE DEGREE OF MASTER OF
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ABSTRACT

Pre-concentrating milk on-farm is likely to become an economical operation in the milk processing as the volume of milk processed in a dairy plant is steadily increasing, which means that the milk collection area is becoming larger and larger. A major concern of introducing the on-farm concentration system is the quality of pre-concentrated milk.

The objectives of this study were to find out the most suitable evaporating temperature and the optimum concentration of milk to be done in an on-farm evaporator system.

A Centritherm evaporator, which will be similar to the evaporator used in the on-farm evaporator system, was mainly employed to concentrate milk and a single tube falling film evaporator was also used for comparison in the experimental work.

The effect of evaporation conditions on the changes in quality of pre-concentrated milk during the storage on farm, transportation from farm to processing factory, and the storage at factory were investigated. This consisted of two aspects: microbiological and chemical/physical.

The microbiological study concentrated on the microbial growth rate and microbial numbers in the concentrated milk, including total bacteria, psychrotrophic bacteria, coliform bacteria, thermophilic bacteria.

It was found that in general the numbers of total bacteria were reduced as both the evaporating temperature and the concentration of milk were increased. When the evaporating temperature was 50°C, the total bacteria was decreased gradually as the concentration of milk rose. But when the evaporating temperature was above 60°C, the numbers of total bacteria were decreased significantly with the increase of the concentration of milk.

When storage time on farm (at 5°C) was increased, the standard plate counts of raw milk and the samples of milk evaporated at 50°C increased. However, the standard plate counts of the samples of milk evaporated at 60°C and 70°C were nearly kept constant.

An important finding is that there were no psychrotrophic bacteria detected in the samples of milk evaporated at 60°C and 70°C. Whereas a large numbers of psychrotrophic bacteria were found in the raw milk and the samples of milk evaporated at 50°C.

It was also found that the numbers of coliform bacteria were greatly reduced in the milks evaporated at 50°C and the population was totally killed when the milk was concentrated at evaporating temperature over 60°C.

During the transportation to and storage at the processing factory, the concentrated milk is likely to be recontaminated. An experiment to imitate the transportation process was designed to check the growth of bacteria in the raw milk and the 40% and 50% total solids (TS) milks concentrated at 60°C without contamination or with contamination, by adding 5% raw milk into the concentrated milk. It was found that the numbers of total bacteria in the concentrated milk without contamination did not change during the simulated transportation to and storage at the factory for two days, whereas the numbers of total bacteria in the concentrated milk with contamination were increased during the simulated transportation and storage. The increased bacteria were mainly the psychrotrophic bacteria, but the higher the concentration of milk, the lower the rate of bacterial growth.

The investigation of the chemical and physical properties of the pre-concentrated milk was to examine the apparent viscosity, fat globule size distribution, the content of free fatty acids and denatured whey proteins.

The apparent viscosity of concentrated milk increased gradually as the concentration was raised up to 50% TS, but only slightly increased with the storage time. However, a

great increase in the viscosity with concentration and storage time occurred in the samples of about 54% TS concentration.

The fat globule size distribution in the samples of concentrated milk was examined and it was found that as the milk concentration increases, the volume (%) of the large size globules (around 4 μm) was reduced rapidly before the concentration reached about 30% TS, but for the greater increase in concentration the distributions of fat globules size were nearly kept constant.

The contents of free fatty acids in the raw milk and milks concentrated at 60°C and 70°C were nearly the same (less than 2 m.Mols/l milk), whereas the content of free fatty acids in the milk concentrated at 50°C was higher and increasing with storage time (4.5-7.0 m.Mols/l milk). This was ascribed to both the damage of natural fat protection due to the fracture of the globule membrane during evaporation, and the increase of lipases from the high population of psychrotrophic bacteria in the samples of milk evaporated at 50°C.

No obvious denaturation of whey protein was found in the samples of milk (30-40% TS) concentrated at 60°C for 40 to 90 minutes. However when the evaporation temperature was 70°C, about 50% the bovine serum albumin (BSA) was denatured as concentration of milk was increased beyond 40% TS.

Based on these experimental results, suitable conditions were determined to be an evaporating temperature of 60°C, and the concentration of milk could be 40% to 50% TS. The storage time of three days at farm and two days at factory could be acceptable if the temperature of concentrated milk is kept at 5°C, and concentrated milk can be stored at a dairy factory longer than raw milk when they are under the same transportation and storage conditions.

The results obtained from the falling film evaporator showed that the reduction of the numbers of total bacteria and the numbers of psychrotrophic bacteria with the increasing of concentration and evaporating temperature was quicker than that in the Centritherm

evaporator. The fat globule size distribution is nearly the same in both evaporators under the similar experimental conditions.

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CHAPTER 1

INTRODUCTION

Milk transportation from a dairy farm to the processing factory is an expensive component of milk processing in the dairy industry. If milk can be concentrated on farm and the pre-concentrated milk is then transported to the factory, there will be many benefits, including:

- (1) a great reduction in the milk transportation cost;
- (2) a reduction in factory evaporation and effluent disposal costs;
- (3) a reduction of factory effluent;
- (4) a reduction in refrigeration loads on the farm.

The idea of on-farm concentration has attracted world-wide interest for many years and trials have been undertaken for application of membrane technology (Hobman, 1982; Anon, 1992).

A major concern of introducing the on-farm concentration system is the quality of pre-concentrated milk. However, few papers are found in the literature in which the details of changes in chemical and microbiological qualities for pre-concentrated milk have been given.

Evaporation is, and no doubt will remain, a major technique used for removal of water in the dairy industry (Fergusson, 1989). If a suitable design is used, taking into account the whole energy consumption on the farm, an evaporation system would be an effective method of on-farm concentration.

An on-farm evaporation system is being developed in the Department of Food Technology at Massey University. The proposed on-farm evaporation system is shown in Figure 1.1. This system will be operated as follows: The fresh whole milk is first collected in the buffer tank and then pumped to the evaporator. The concentrated milk

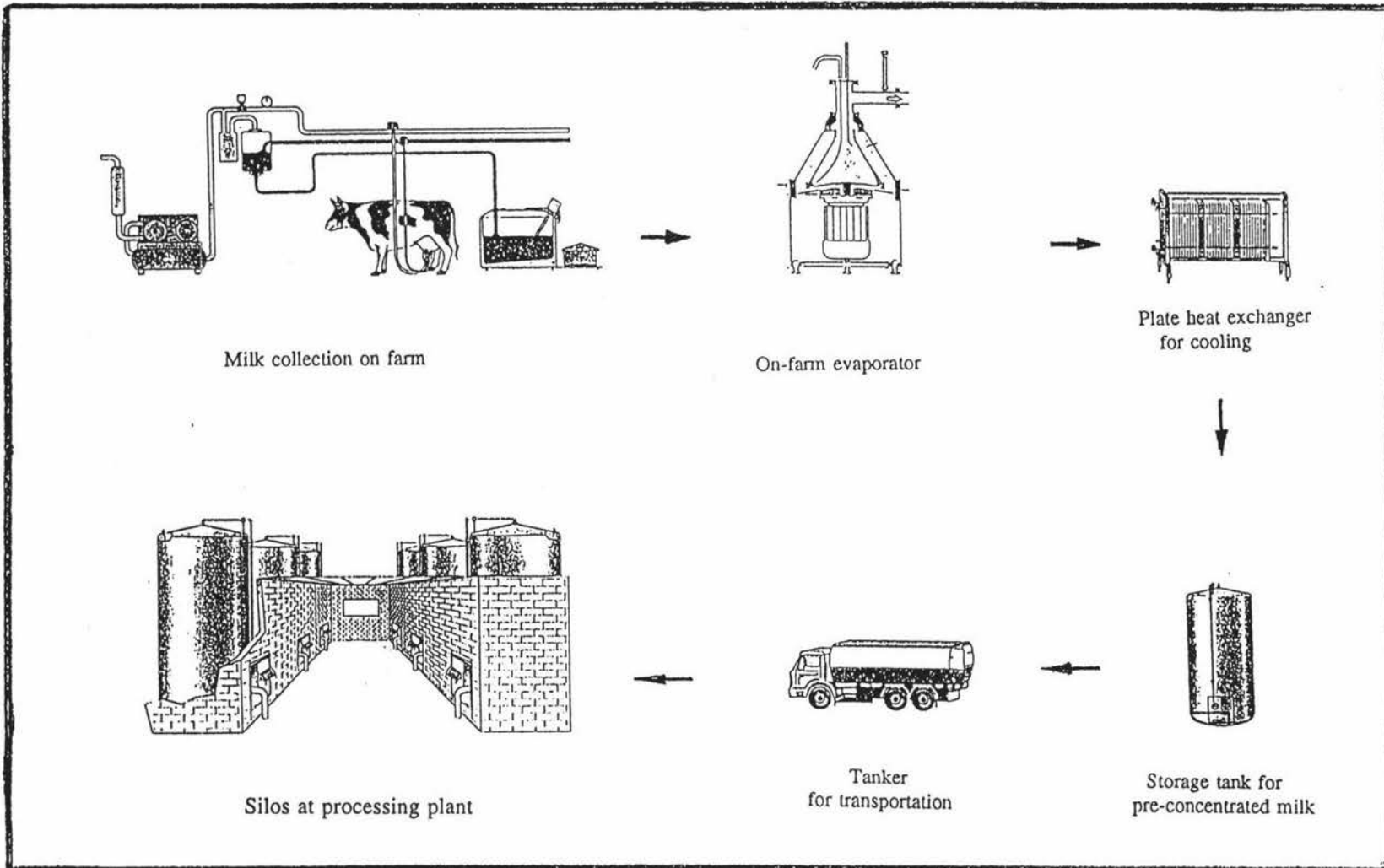


Fig. 1.1 The proposed on-farm evaporation system

from the evaporator is chilled in a plate heat exchanger and then passed to the storage tank. The storage temperature is about 5°C and the maximum storage time is about 3 days. Finally the concentrated milk is transported by tanker to the dairy factory for further processing and may be stored in the dairy factory for a while (maximum two days) before processing.

The evaporator proposed for the on-farm evaporation system is similar to the Centritherm evaporator. Therefore, the Centritherm evaporator was used mainly for the experimental work. The Centritherm evaporator has a heated rotating surface, which results in an extremely high heat transfer coefficient and a very short residence time of concentrated liquid in the evaporator. It is the most suitable evaporator for concentrating heat sensitive materials in a small or medium capacity.

Nowadays, the dominant evaporator used in the dairy industry is the falling film evaporator, which has satisfied the demands of the modern dairy industry, i.e. the possibility of large capacities, economical running, reliable operation, careful treatment of the products, etc.. So, some experimental work was carried out on the falling film evaporator to compare the results from both evaporators.

In an evaporator, heat must be supplied to the liquid through a heat exchange surface, and the vapour and liquid separated. Evaporation is therefore both a heat exchange operation and a vapour-liquid separation.

Basically an evaporation system consists of:

- (1) a heat exchanger to supply heat to raise the liquid to its boiling point and provide the latent heat of vaporisation;
- (2) a separator in which the vapour is separated from the concentrated liquid phase;
- (3) a condenser to effect condensation of the vapour and its removal from the system;
- (4) a vacuum device to withdraw the non-condensables and maintain the consistent evaporating temperature.

If milk is evaporated on the farm, then there may be changes in milk components as a result of heating time, temperature, concentration, etc.. There could also be changes in microbial numbers and types. Therefore, it is necessary to study the microbiological and chemical/physical qualities of pre-concentrated milk in this on-farm evaporation system.

The aims of this project were to investigate the effect of different evaporation conditions on the quality of milk, to find out the most suitable evaporating temperature and the optimum concentration of milk to be done in a farm evaporator to ensure that the pre-concentrated milk quality at factory reception is no worse than that of the milk from the present system of milking and collection.

The microbiological study concentrated on the microbial growth rate and microbial numbers in the concentrated milk. The microbial changes of the milk during the evaporation and storage on farm were studied. During the transportation and storage at factory, the temperature of concentrated milk may increase and the milk may be contaminated by microorganisms in the transportation and storage equipment. So the microbial changes of concentrated milk during simulated transportation and storage in the factory were also investigated.

The contents and changes of total bacteria, coliform, thermophilic and psychrotrophic bacteria in the milk samples were estimated. The reasons for examining these bacteria in this study are:

- (1) The contents of total bacteria and coliform bacteria in milk are normally used to evaluate milk quality and contamination.

The Standard Plate Count (SPC) is suitable for estimating bacterial population in most types of dairy products. This procedure is also recommended for industry application in detecting source of contamination by testing line samples taken at successive stages of processing (Messer, *et al.*, 1985).

The coliform group of bacteria comprises all aerobic and anaerobic gram-negative, nonspore-forming rods able to ferment lactose with production of acid

and gas at 32°C within 48 hours. They are found in intestines, in manure, in soil, in contaminated water and on plants. Typically, these organisms are classified in the genera *Escherichia*, *Enterobacter*, and *Klebsiella*. A few lactose-fermenting species of other genera are included in the coliform group (Hartman & LaGrange, 1985; Bylund, 1995).

Coliform bacteria are killed by high temperature short time (HTST) pasteurisation. Application of the test for coliforms is intended neither to detect faecal pollution specifically nor to identify *Escherichia coli* in dairy products, but rather to be used as test organisms for routine bacteriological quality control in dairies. Such tests are conducted following pasteurization primarily to detect significant bacterial recontamination of processed dairy products. In proportion to the numbers present, existence of any of these types in dairy products is suggestive of unsanitary conditions or practices during production, processing, or storage. Results of tests on raw samples are to be interpreted differently from those obtained by testing pasteurized milk. Coliforms in small numbers may enter raw milk and cream under normal condition of production and handling (Hartman & LaGrange, 1985; Bylund, 1995).

(2) For on farm pre-concentrated milk, the raw milk should be concentrated at lower temperature to minimise changes in chemical composition. However, the lower evaporation temperature will be very suitable for the growth of the thermophilic bacteria, although some other types of organism will be destroyed. According to Bramley and McKinnon (1989) and Nelson (1989), operation over an extended period provides an opportunity for development of a considerable thermophilic population. Therefore, it is necessary to investigate the development of thermophilic population during the pre-concentration processing.

(3) Psychrotrophic bacteria can multiply at or below 7°C, irrespective of their optimum growth temperature, and the increase in total counts observed during prolonged storage of raw milk < 7°C is caused by multiplication of its psychrotrophic flora. The psychrotrophs Gram-negative bacteria in raw milk are

not present in heat-treated milk or milk products and do not themselves cause spoilage (other than as post-pasteurization contamination) but many species produce heat-resistant extracellular enzymes capable of degrading important milk constituents (Law, 1979; Frank, *et al.*, 1985). Therefore, the psychrotrophs have to be explored in this evaporation system to investigate the numbers and growth rate of psychrotrophic bacteria and the relationship between the psychrotrophs number and free fatty acids content in the samples.

The chemical and physical properties of the pre-concentrated milk chosen to be checked in this study were:

- (1) Fat globule size distribution: There may be some changes in fat globule size distribution as a result of the evaporation. This can affect the milkfat properties and free fatty acids level.
- (2) Free fatty acids: The content of free fatty acids in pre-concentrated milk evaporated at different evaporation conditions was determined. The free fatty acids level can be influenced by the enzymes in the raw milk and/or be produced by microorganisms in milk and the changes in the fat globules.
- (3) Apparent viscosities: The apparent viscosities of the milk at different concentration levels were checked before and after holding at 5°C, and the effects of evaporating temperature, concentration level and holding time on age thickening of pre-concentrated milk were investigated. The viscosities of pre-concentrated milk will be an important property for the further processing in the factory.
- (4) Denatured whey protein: The denatured whey proteins in the concentrated milk evaporated at 60°C and 70°C with different concentrations were detected. The content of denatured whey protein in the concentrated milk will affect the properties of the final products.

CHAPTER 2

LITERATURE REVIEW

As heat processing is involved in the evaporation, this review will mainly focus on the effects of heat treatment on milk components, milk concentrate qualities, and microflora in milk.

2.1 Effects of heat treatments on milk components

Heat processing is generally considered to be one of the most important single factor influencing milk components and the properties of milk and concentrated milk. However, much of the effect of heat treatment depends on the degree of the treatment and on media conditions (pH, presence of ions such as Ca^{2+}) (Robin, *et al.*, 1993).

2.1.1 Effects on caseins

Casein may be defined as the protein precipitated by acidifying skim milk to a pH value near 4.6. The casein content of milk is about 26 g/kg, representing about 80% of milk protein. The principal casein fractions are: α_{s1} -casein (10 g/kg), α_{s2} -casein (2.6 g/kg), β -casein (9.3 g/kg), κ -casein (3.3 g/kg), and γ -caseins (0.8 g/kg) (Goff & Hill, 1993).

Compositionally, the hallmark of the caseins is ester-bound phosphate. All of the casein polypeptide chains have at least one such group per molecule (Walstra & Jenness, 1984). Phosphate groups are important to casein association and the structure of the casein micelle. Calcium binding by individual caseins is proportional to phosphate content (Goff & Hill, 1993). None of the four kinds of casein has a highly organized secondary structure. None of the various methods of analysis suggests anything more than short lengths of α -helix or β -sheet structure in them (Walstra & Jenness, 1984).

Caseins in micellar form are exceptionally thermostable; typically, milk withstands heating at 140°C at pH 6.7 for 20 minutes before coagulation occurs. The remarkable stability of caseins at high temperatures is principally due to the low levels of secondary and tertiary structures (Goff & Hill, 1993; Singh & Fox, 1989).

2.1.1.1 Low temperature (0-80°C)

From a physical-chemical point of view, heating or cooling milk above or below physiological temperature causes a shift in the calcium phosphate equilibrium which affects some properties of milk, especially rennet coagulability. On cooling, colloidal calcium phosphate (CCP) dissolves (Singh & Fox, 1989), and some casein, especially β -casein, dissociates from the micelles (Fox & Guiney, 1973), contributing to the increase in the rennet coagulation time (RCT) of milk observed during cold storage (Robin, *et al.*, 1993). This cold ageing effect is readily reversed by heating at 40°C for 10 minutes or by the high temperature short time (HTST) pasteurization (Singh & Fox, 1989). Conversely, on heating up to 70°C, CCP precipitates, the soluble β -casein reassociates with the micelles and the RCT is reduced. But more severe heating prolongs RCT due to denaturation of whey proteins and their interaction with κ -casein via sulphhydryl-disulphide interchange (Robin, *et al.*, 1993; Singh & Fox, 1989).

The individual caseins undergo temperature-dependent association as the temperature is increased from 0°C, indicating the strongly hydrophobic characteristics of the caseins. β -casein is the most hydrophobic casein and its association is also the most strongly temperature dependent (Singh & Fox, 1989). All caseins self-associate and intermolecular aggregation occurs both in the presence and absence of calcium. The order of decreasing sensitivity to calcium precipitation is α_{s1} -, α_{s2} -, β -, and, finally, κ -casein, which stabilizes the casein system against calcium precipitation. Caseins do not form aggregates with whey proteins except during heat treatment (Robin, *et al.*, 1993; Walstra & Jenness, 1984).

The α_{s1} - and α_{s2} -caseins are precipitated by $\text{Ca}^{2+} > 6 \text{ mM}$ at all temperatures. When temperature is $< 20^\circ\text{C}$, β -casein is quite soluble in the presence of Ca^{2+} , but it is very insoluble above this temperature (Singh & Fox, 1989).

2.1.1.2 High temperature (80-150°C)

Heat treatments in the range of 80-150°C, such as preheating of milk, in-container sterilization, and ultra-high temperature (UHT) processes, induce changes in caseins such as dephosphorylation, proteolysis, covalent bond formation, changes in casein micellar structure, maillard reaction and so on, but the heat-induced changes throughout this temperature range appear to differ only in rate and not in nature (Singh & Fox, 1989).

2.1.2 Effects on whey proteins

The proteins remaining after casein has been removed from skim milk are known as whey proteins or milk serum proteins. The total concentration of whey proteins in milk is 5 to 7 g/L, not including about 1.1 g/L of proteose peptone (Goff & Hill, 1993). The native whey proteins differ both in structure and properties from the caseins. The whey proteins are, in contrast to the caseins, not associated into micelles but are molecularly dissolved and susceptible to heat denaturation (de Wit, 1981).

Whey proteins are composed of α -lactalbumin, β -lactoglobulin, bovine serum albumin and immunoglobulin.

According to de Wit (1981), in contrast to caseins, whey proteins are relatively heat labile, being denatured completely in 60 minutes at 77.5°C, 30 minutes at 80°C, or 5 minutes at 90°C. This thermolability of the whey proteins is a consequence of the marginal stability of their compact three-dimensional structure. Near the denaturation temperature, the slim margin of the stabilizing forces is counter-balanced by the destabilizing forces, resulting in an unfolding of the protein molecule. Heating globular proteins causes them to unfold and this unfolding is accompanied by an endothermal

heat effect (heat uptake) (Robin, *et al.*, 1993). Although under appropriate conditions proteins may refold again to their original three-dimensional structure, in many practical cases the unfolded molecules polymerize to aggregates of irreversibly denatured proteins (de Wit, 1981).

Denatured whey proteins do not precipitate from milk but coprecipitate with casein on acidification, salting out, or ultra-centrifugation. The protection of denatured whey proteins against precipitation appears to be nonspecific and possessed by all casein fractions; sequestering of Ca^{2+} by caseins may be responsible (Fox, 1981).

2.1.2.1 β -lactoglobulin

β -lactoglobulin is the most important whey protein, both quantitatively and qualitatively (de Wit, 1981). β -lactoglobulin contains five cysteine residues, four of which are involved in internal disulphide bridges leaving one free, but normally inaccessible, thiol group (Pearce, 1979). The existence of this thiol group is of great importance for changes occurring in milk during heating, as it is involved in reactions with other proteins, notably κ -casein and α -lactalbumin (Walstra & Jenness, 1984). It has considerable α -helix and β -structure, and exists naturally as a noncovalently linked dimer. The dimers are further associated to octamers in the isoelectric region (pH 3.5 to 5.2) but are dissociated to monomers at pH below 3.4 (Goff & Hill, 1993).

β -lactoglobulin, with a denaturation temperature of 78°C, is the most stable of the serum proteins. A second thermal change appears near 140°C caused by the breakdown of disulphide bonds and additional unfolding of the molecule. The thermal behaviour of the whey proteins is mainly governed by the properties of β -lactoglobulin (de Wit, 1981). The heat denaturation of β -lactoglobulin is pH dependent (Robin, *et al.*, 1993).

The denaturation of β -lactoglobulin by heat has shown to occur in two stages, an initial reversible denaturation followed by irreversible steps. The latter have received the most attention because of their commercial consequences (Pearce, 1979). It was observed by fluorescence spectroscopy that at pH 6.5, irreversible conformational changes

commenced above 70°C, whereas below 70°C the changes were reversible by cooling (de Wit, 1981). β -lactoglobulin undergoes irreversible thermal denaturation due to aggregation involving sulphuryl groups followed by less specific aggregation (Sawyer, 1968). de Wit (1981) observed that at 80°C, and pH 6.8 to 7.5, β -lactoglobulin is partially denatured without aggregation and loss of solubility. It seems that thiol groups unmasked and activated at pH > 6.8 initiate intramolecular disulphide rearrangements that stabilize the molecule.

It is accepted generally that β -lactoglobulin and κ -casein interact by sulphuryl-disulphide interchange, and this interaction markedly affects heat stability and rennet coagulability of milk (Fox, 1981).

2.1.2.2 α -lactalbumin

α -lactalbumin is generally regarded as the second most important whey protein (Pearce, 1979). α -lactalbumin contains eight cysteine groups, all of which are involved in disulphide bridges, and four tryptophan residues. α -lactalbumin has a highly ordered secondary structure, and hydrodynamic data indicate a compact, spherical tertiary structure (Goff & Hill, 1993). In recent years it has been identified as a calcium-metalloprotein binding one mole of calcium per mole of protein, essential to the maintenance of the native conformation of the protein (Pearce, 1979).

The denaturation temperature of α -lactalbumin is 62°C (Robin, *et al.*, 1993). It has long been assumed that α -lactalbumin was the most stable serum protein due to the reversibility of the heat denaturation at pH 6.0. Since 1977, it has been recognised that α -lactalbumin is, in fact, one of the least heat-stable proteins in milk. Recent studies have clearly shown that the reversible denaturation of α -lactalbumin is due to calcium ion dissociation and reassociation from the protein, which is a calcium metalloprotein (Goff & Hill, 1993; Pearce, 1979). α -lactalbumin has no free -SH groups and therefore there is little opportunity for SH-SS exchange reactions which might promote coagulation in whey or complex form with casein micelles via the -SH groups of κ -casein (Pearce, 1979).

2.1.2.3 Bovine serum albumin and immunoglobulins

Bovine serum albumin (BSA) is a major component of blood serum. It is synthesized in the liver and gains entrance to milk through the secretory cells, but it comprises only about 5% of total whey protein in milk and contains 17 disulphide bonds and one free thiol group per molecule. This holds the molecule in a multiloop structure. It appears to function as a carrier of small molecules (e.g., fatty acids) that bind to it. Little is known of its behaviour in milk and milk products and its possible influence on their properties (Walstra & Jenness, 1984; Pearce, 1989). Jelen and Rattray (1995) reviewed that thermal analysis of BSA revealed complex thermal behaviour; this is possibly due to the high molecular weight and presence of numerous disulphide bonds. Defatted BSA was denatured at thermization temperature. However, the binding of fatty acids to BSA markedly increased thermal stability. Denaturation temperature for BSA are reported as 71.9°C at pH 6.5, 74°C at pH 4.5 (Bernal & Jelen, 1985). The increase of thermal stability, perhaps, is because that fatty acids stabilize the tertiary structure in manner analogous to the stabilization of α -lactalbumin by calcium (Prarce, 1989; Jelen & Rattray, 1995).

Immunoglobulins are antibodies synthesized in response to stimulation by macromolecular antigens foreign to the animal. IgA, IgG and IgM have been identified, IgG being predominant in mid- and late-lactation milk but with larger quantities of IgA and IgM in colostrum and early lactation milk. The Ig proteins of milk appear to be identical to their blood counterparts, being composed of two heavy chains and two light chains joined by disulphide bonds. Although Ig makes up about 10% of total whey protein in milk, their contribution to the functional properties of milk and dairy products has been largely ignored (Pearce, 1989; Jelen & Rattray, 1995). Very little public literature can be found regarding the thermal denaturation of the immunoglobulins. Larsen and Roller (1955) found that the immunoglobulins fraction was the most resistant of the whey proteins to heat precipitation. de Wit and Klarenbeek (1984) reported that the denaturation temperature for IgG was 72°C and at acidic pH thermal stability was reduced.

2.1.3 Effects on fat and fat globules

Milk fat is secreted in the form of fat globules. The fat globules in the bovine milk can vary somewhat in their size distribution but average 2-4 μm , in diameter (Christie, 1983). Each fat globule of milk is surrounded by a thin protective layer, 8 to 10 nm thickness (Goff, *et al.*, 1993), usually called a membrane. Its composition and properties are completely different from either milk fat or plasma. The main components are proteins (almost half of the membrane material) and phospholipids (roughly one-third) (Driessen, 1989). All interactions between fat and plasma must take place through the membrane. Its total area is considerable, and it contains substances and enzymes. It functions to prevent the fat globules from flocculation and coalescence, and it protects the fat against enzyme action. Properties of the membrane are also of great practical importance, partly for the same reasons. Consequently, the membrane largely determines the amount of deteriorative reactions, such as lipolysis and autoxidation (Walstra & Jenness, 1984, Goff, *et al.*, 1993).

Milk fat contains various lipids, but more than 98% consist of triglycerides. The other lipids include mono- and diglycerides, phospholipids, free fatty acids and sterols (Siezen, *et al.*, 1994).

Heating may cause physical and chemical changes in milkfat globules. Physical changes include those in the stability of milkfat globules towards creaming, flocculation, coalescence and disruption. Chemical changes are due mainly to reactions of fatty acids residues (van Boekel & Walstra, 1995). Some changes in milkfat globule are reviewed below.

When it comes to describing the effects of heating, two aspects must be considered. First, heating is always accompanied by some agitation. This is of importance for physical stability as it may lead to changes in globule size as a result of coalescence or disruption (Mulder & Walstra, 1974). Second, changes in surface layers may take place: desorption of membrane material in the case of coalescence; and adsorption of surface-active material from the milk plasma in the case of disruption. When foaming

occurs, fat globules may come into contact with bubbles yet uncovered by plasma proteins; hence, spreading of membrane material over an (air or vapour) bubble may occur and when such a bubble disappears, this material is released into the plasma; meanwhile, plasma proteins adsorb on to the partly denuded fat globules (Mulder & Walstra, 1974). These changes are thus not the result of heating, but of the agitation during heating, although a high temperature may promote coalescence as well as disruption. It depends on the type of apparatus used whether coalescence or disruption occurs (Mulder & Walstra, 1974; van Boekel & Walstra, 1995).

According to van Boekel & Walstra (1989), membrane proteins will denature above about 70°C and reactive groups may be exposed. A pronounced effect is the release of sulphhydryl compounds, notably H₂S. The cysteine residues in membrane proteins seem to be very reactive. Therefore, disulphide interchange reactions may occur and whey proteins are also reactive in this respect. Whey proteins may thus become associated with membrane proteins. Houlihan, *et al.* (1992) demonstrated that serum proteins, especially β-lactoglobulin, became associated with the membranes of natural milkfat globules at temperatures > 70°C and κ-casein also was increasingly incorporated into the membrane with heating time at 80°C. van Boekel & Walstra (1995) claimed that heating (at about 80-90°C) did not have a profound effect on membrane composition, but that the presence of serum proteins in combination with heat may initiate some changes. When the temperature is above 100°C, membrane proteins and lactose may participate in the Maillard reaction. Also possible is the formation of dehydroalanine from cysteine residues.

Heating may induce some changes in phospholipids. To some extent, phospholipids may migrate from the fat globules to the aqueous phase during heating (Houlihan *et al.*, 1992), perhaps together with other compounds (Mulder & Walstra, 1974). However, it is not certain whether this occurs as a result of heat or as a result of agitation during heating when membrane material desorbs because of coalescence of fat globules (van Boekel & Walstra, 1989).

Changes in membrane composition and structure could result in improved as well as in impaired stability. An indirect consequence of heating is the disappearance of the phenomenon of cold agglutination. In low-pasteurized milk some cold agglutination may occur, but in high-pasteurized milk the effect has disappeared (Mulder & Walstra, 1974; Walstra, 1984). According to van Boekel & Walstra (1989), if the 'agglutinin' is inactivated, creaming (albeit slowly) results in a cream layer in which the fat globules are closely packed (in contrast with a cream layer of agglutinated fat globules with loose floccules). In such a closely packed cream layer, fat globules are pressed more or less together, and this may lead to partial coalescence if part of the fat is or becomes solid (formation of a cream plug). This partial coalescence is also a consequence of impaired coalescence stability caused by heating. The coalescence stability may be related to the milkfat globule size distribution. As described above, the agitation during heating may cause coalescence as well as disruption. If fat globules have increased in size during the heating process, this alone causes a decreased stability against coalescence. If fat globule size has decreased during heating, the resulting globules will, after heating, be more stable against coalescence (Walstra & Jenness, 1983).

2.2 Factors that influence storage stability of concentrated milk

2.2.1 Heat treatment

Bloore & Boag (1981) pointed out that the heat load during sterilization appeared to be the rate-determining step of age-thickening. Intense heat treatment delays gelation. Preheating the milk before concentrating it in the manufacture of evaporated milk has no effect, but heating the concentrate to temperature above 110°C for several minutes improves the stability to gelation (Walstra & Jenness, 1984). Hostettler, *et al.* (1968) suggested that casein-whey protein complexes, formed on heating, dissociate from the micelles during storage resulting in the destabilization of casein. If the heat treatment is severe, complex formation is irreversible and protects the micelles from further changes during storage. Bloore & Boag (1981) found a high temperature, short time preheat treatment have a lower viscosity of concentrate than a low temperature, long time treatment giving a similar Whey Protein Nitrogen Index (WPNI). It also gave a lower rate of viscosity increase with time. The periods of gelation was found to depend

on the time and temperature of the heat treatment either before or during sterilization. Preheating treatment at a higher temperature and for longer period delay gelation but increases sedimentation defects and flavour deterioration. Sterilization (HTST) of concentrated milk at higher temperature and holding times also retards gelation. However, at equivalent sterilizing effectiveness, higher sterilization temperatures with shorter exposure times result in reduced resistance to gelation (Zadow & Chituta, 1975; Harwalkar, 1982). Manji & Kakuda (1988) suggested that if free amino groups are required for gel formation, with higher heat treatment, loss of lysine groups by the browning reaction may be the reason of retarding the gelation process.

2.2.2 Cold storage

Cold storage of concentrate before sterilization markedly influences the relative viscosity during storage, also if no bacterial proteinases are formed (Walstra & Jenness, 1984; Harwalkar, *et al.*, 1983). Harwalkar, *et al.* (1983) demonstrated that as the time of cold storage of concentrates before sterilization increased, viscosity increased and the tendency to thicken or gel during subsequent storage of evaporated milk increased. de Koning, *et al.* (1992) reported that from the electron micrograph of gelled evaporated milk, it appeared that aggregated micelles were connected by thread-like structures to a three-dimensional network. Thus, they suggested that the effects of age-thickening and gelation are stimulated by cold storage of the concentrate before sterilization and have to be attributed to a phasic-chemical transformation of the casein micelles.

2.2.3 Milk composition and season

The viscosity of skim-milk concentrate is influenced by the composition of the raw milk (Buckingham, 1978). Snoeren, *et al.* (1982) suggested that the viscosity of skim-milk concentrate is a function of the volume fraction of the dispersed particles. The volume fraction depends on the hydration of the protein, the protein composition and the protein content, which varies during lactation. At the end of the lactation period the viscosity of the concentrate, at a given dry matter content, is much higher than in early lactation. Bloore & Boag (1981) also found that the marked increase in concentrate viscosity

towards the end of each dairying season is largely attributable to the protein content of the milk, which rises at this time. Harwalkar (1982) reported the increasing of whey protein content hastens the onset of gelation, but increasing the casein content does not affect the gelation period of UHT sterilized concentrated milk. Buckingham (1978) suggested that the heat denaturation of the milk proteins could result in molecular aggregation of the protein and an increase in the amount of associated bound water. This, in turn, would cause an increase in viscosity and eventual gelation of concentrate.

2.2.4 Quality of milk

Bacteriological quality of raw milk is also important in gelation; sterilized milk from poor quality milk is very susceptible to gelation during storage. The type of microorganisms or spores present in poor milk is important, organisms that produce heat-stable enzymes cause most serious gelation problems (Snoeren *et al.*, 1979; Zadow & Chituta, 1975). Driessen & van der Waals (1978) found a higher enzymatic activity in UHT evaporated milk is accompanied by increased casein degradation and an enhanced tendency to gel. The protease activity is important in age gelation, but proteolysis cannot be the only cause of age gelation. Snoeren, *et al.* (1979) observed that UHT-treatment concentrated milk gelled sooner than unconcentrated milk even though the rate and extent of proteolysis was considerably lower in the former. Kocak & Zadow (1985) showed that gelation time is not directly related to the microbiological quality of raw milk or to the degree of proteolysis in either the fresh or prepared UHT milk, or the gelled samples.

2.2.5 Other factors

Gelation of concentrated milk is hastened by increasing total solids (Harwalkar, 1982). Bloore & Boag (1981) found that the viscosity of skim-milk concentrate rose exponentially with increasing concentrate total solids.

Increasing the temperature generally accelerates gelation (Walstra & Jenness, 1984; Manji & Kakuda, 1988; Harwalkar, 1982).

Additions of sodium phosphate and citrate hasten the gelation process. Additions of polyphosphates and polyhydric compounds like lactose, sucrose, dextrose and sorbitol delay gelation. MnSO_4 , ZnSO_4 or FeSO_4 usually also delay gelation. The addition of CaCl_2 or the alteration of pH usually has little and often non-consistent effects (Walstra & Jenness, 1984; Buckingham, 1978; Harwalkar, 1982).

2.3 Microflora in raw milk

2.3.1 Total bacterial content and contamination sources

The initial microbiological quality of milk depends on the milking and storage conditions and varies enormously. Cow's udder, and milking and storage equipment, are the main contamination sources (Bramley & McKinnon, 1989; Flückiger, *et al.*, 1980).

Total bacterial content (TBC) may range from $< 1,000/\text{ml}$ where contamination during production is minimal, to $> 1 \times 10^6/\text{ml}$ of milk (Bramley & McKinnon, 1989). The TBC in freshly milked milk obtained by milking machine under hygienically satisfactory conditions, is normally 1,000 to 10,000 /ml (Kessler, 1981). According to the IDF Monograph on the 'Bacteriological quality of cooled bulk milk', the total viable count of ex-farm milk produced under good hygienic conditions should not exceed 10,000 bacteria/ml (Flückiger, *et al.*, 1980).

2.3.1.1 Contamination of cow's udder and milking

The contamination from the cow's udder comes from both interior and exterior of udder. Tolle (1980) reported that in the absence of disease the numbers of non-pathogenic bacteria present in the gland or teat duct will not significantly increase the bacterial numbers in milk. But mastitis may result in about 1×10^6 bacteria/ml in the bulk milk.

Under normal farm conditions of machine milking, dirty teats may contribute up to 1×10^6 bacteria/ml milk. Even if the teats appear to be visually clean the bacteria from teat surface may result in high counts particularly if the cows are housed on fine sand

bedding or other bedding which harbours large numbers of bacteria. If the teats are washed and dried carefully, the numbers of bacteria getting into milk from the exterior of the udder may be less than 5,000/ml (Joergensen, 1980).

According to Palmer (1980), milking equipment is frequently the major contamination source of bacteria in ex-farm milk. Because some inner surfaces, e.g. of milking machines and pipelines, cannot be checked visually, and if they are not effectively washed, large numbers of bacteria accumulate at these sites and may contribute more than 10,000 bacteria/ml to the milk supply. Detailed attention to equipment design and effective washing should reduce the numbers of bacteria in the milk derived from equipment to less than 5,000/ml.

The microorganisms present in raw milk will be derived from one or any combination of the several main sources of contamination described. Consequently high initial TBC values in milk, e.g. > 100,000/ml, are evidence of serious faults in production hygiene, whereas the production of milk having TBC values < 20,000/ml reflects good hygienic practices (Bramley & McKinnon, 1989).

2.3.1.2 The contamination during storage and transport

Raw milk is an ideal medium for the growth of most microorganisms present, and cooling of the milk is an effective way to lengthen its keeping quality, because lowering the temperature of milk decreases the rate of bacteria growth.

Milk must be cooled at the farm on collection, and temperatures in the range 3-5°C are readily achieved. Such cooling inhibits general growth of bacteria. Where milk is cooled to and stored at < 4°C, the low temperature will normally prevent most of bacterial multiplication for at least 24 hours, many organisms with optimum growth temperatures around 20°C will grow, but more slowly at refrigeration temperatures (Bramley & McKinnon, 1989; Muir, 1989). However, some organisms are capable of growth at temperature 5°C or less (Langeveld & Cuperus, 1980). The most important group of these organisms consists of the Gram-negative rods such as *Pseudomonas*

(Cousin, 1982). Thus storage of milk at the farm at say 5°C not only decreases the overall bacterial growth rate but also changes the microbial balance in favour of those organisms, the psychrotrophs, which multiply at this temperature. At 5°C the population of these bacteria is doubled very approximately every 8 hours so that for milk produced under good hygienic conditions (e.g. TBC 10,000/ml and psychrotrophs 1,000/ml) the number of those organisms will become dominant on storage by multiplying (Gehrigler, 1980).

Unfortunately, the transport of milk from the farm and subsequent storage at the dairy factory results inevitably in further contamination and also the temperature may rise (Flückiger, *et al.*, 1980). Muir (1989) reviewed that during milk storage and transport, there are three factors; growth, desegregation of bacterial clumps and contamination, which can result in a significant increase in bacteria count, and in an equally important change in the type of flora in the milk. The milk is transferred by pumping several times between the farm and its ultimate destination. Such robust physical treatment tends to break up bacterial clumps and, as a result, there is a significant increase in total bacterial count without growth being necessary. Furthermore, during transport and storage, the raw milk comes into contact with a number of surfaces and there is ample scope for contamination. Therefore, it is not surprising that milk, initially of excellent bacteriological quality, may have over 1×10^6 bacteria/ml, mainly psychrotrophs, by the time it is processed at dairy factory (Flückiger, *et al.*, 1980; Kessler, 1981). For good quality ex-milk it can be expected that in practice the maximum storage period may be 2 days at about 5°C if manufacturing problems are to be avoided. Alternatively if longer storage periods are necessary for economic reasons, storage temperatures well below 5°C may be essential (Flückiger, *et al.*, 1980).

2.3.2 The main groups of microorganisms in raw milk and contamination sources

The groups of microorganisms which may occur in raw milk are given in Table 2.1. To what extent representatives of the different groups occur in raw milk is determined by the degree of infection from the different sources (Stadhouders, 1975). In low count raw milk, < 5,000 colony forming units (cfu)/ml, the minimal bacterial contamination

from the exterior of the udder and from milking equipment is reflected in the predominance of nonthermoduric *micrococci* (including *staphylococci*) and *streptococci*. As total colony counts increase, then the proportions change; generally, increase in gram-negative rod-shaped bacteria occur at the expense of the micrococci. Considerable variation in the incidence of thermoduric organisms and psychrotrophs in fresh raw milk has been reported. Some differences may be regional or seasonal, and others associated with methods of cleaning and disinfecting equipment on individual farms (Bramley & McKinnon, 1989; Muir, 1989).

Table 2.1 The composition of the microflora of raw milk (Stadhouders, 1975).

	Name
1	Pathogenic bacteria
2	Non-thermoresistant micrococci
3	Non-thermoresistant coryneforms
4	Yeasts and moulds
5	Gram-negative rod-shaped bacteria <i>Pseudomonas</i> , non-thermoresistant, <i>Achromobacter</i> , <i>Alcaligenes</i> species, <i>Flavobacterium</i> , <i>Enterobacteriaceae</i>
6	Non-thermoresistant lactic acid bacteria
7	Thermoinsistant bacteria

Group 1, pathogenic bacteria, comes from the milker (particularly the hands) and from the skin and the udder of the cow (Stadhouders, 1975; Burgess, *et al.*, 1994). Within pathogens, only the spore-forming *clostridia* and *bacillus* can survive pasteurization, and only *Bacillus cereus* can survive pasteurization and grow at refrigeration temperature. Pathogens in raw milk do not pose a significant hazard when the milk is subsequently

heat-treated. In contrast, the enzymes associated with the psychrotrophic flora of raw milk can cause significant spoilage (Muir, 1989).

Groups 2, 3 and 4 are from the udder of the cow and from the environment. As far as the number of bacteria is concerned, the milking materials, the transport and storage tanks are the main sources of infection (Stadhouders, 1975).

According to Burgess, *et al.* (1994), group 5, Gram-negative rod-shaped bacteria are common in the environment (particularly in water) and proliferate on inadequately cleaned surfaces. The Enterobacteriaceae and the genera *Alcaligenes*, *Achromobacter*, *Flavobacterium* and particularly *Pseudomonas* are psychrophilic Gram-negative rods. They are still able to develop at 5°C and often dominate the microbial population (Stadhouders, 1975; Burgess, *et al.*, 1994).

Langeveld & Cuperus (1980) found that *Pseudomonas* has a doubling time of 5.5 hours at 5°C in pasteurized milk. Assuming the same value for the growth in raw milk, one can calculate that even at a low initial number (for instance 1/ml) the storage time cannot be lengthened to more than 4 days without greatly affecting the total number in the raw milk (Stadhouders, 1975).

McKinnon & Pettipher (1983) observed that the incidence of spores of psychrotrophic strains of *Bacillus spp.* in individual producer's milk was low, and seldom exceeds 10/ml. The species found include *Bacillus coagulans*, *Bacillus circulans*, *Bacillus cereus* and *Bacillus subtilis*. Some of these psychrotrophs, when growing in refrigerated milk, produce extracellular heat-resistant lipase (more details will be reviewed in section 2.6), as well as proteinases which may degrade casein. These organism particularly destructive if high numbers are present. Prolonged cold storage may also lead to increased enzyme production as a result of stress in the bacteria (Bramley & McKinnon, 1989; Burgess, *et al.*, 1994). The Gram-negative psychrotrophs are killed by pasteurisation, but their enzymes are not inactivated. As a consequence these bacteria are of considerable importance in manufactured milk products though their source is often post-pasteurisation contamination (Bramley & McKinnon, 1989).

The coliform/enteric group are also Gram-negative, rod-shaped bacteria. This group of bacteria commonly contaminate raw milk. They can rapidly build-up in moist, milky residues in milking equipment, and then become the major source of contamination of the milk produced. As they do not survive pasteurization, this group is frequently used as an indicator of inadequate processing or post-process contamination. As this group ferment lactose and many strains are psychrotrophic, they are also responsible for spoilage (Burgess, *et al.*, 1994; Bramley & McKinnon, 1989).

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 inadequate udder cleaning before milking (Bramley & McKinnon, 1989).

Representatives of Groups 6 and 7 can gain access to the raw milk. Cleaning and disinfection are effective ways to reduce their numbers (Stadhouders, 1975).

Burgess, *et al.* (1994); Bramley & McKinnon (1989) and Muir (1989) defined and reviewed those bacteria which survive laboratory pasteurization (that is heating to 63°C for 30 minutes) and are classed as thermophilic organisms, such as *Bacillus* spores, *Clostridium* spores, *Microbacterium*, *Micrococcus* and *Alcaligenes*. The most important types are those bacteria producing heat resistant spores, i.e. *Bacillus* and *Clostridium spp*, which may be frequently present in raw milk, and large numbers are often related to poor farming practices and poor cleaning-in-place (CIP) techniques (Burgess, *et al.*, 1994). Spore-forming bacteria of the genus *Bacillus* form the most important class of organisms capable of surviving pasteurization, and of further growth in milk. It has already been noted that there is a seasonal variation in the numbers of spore-forming bacteria in raw milk (Muir, 1989).

Micrococci and *Microbacterium spp* are derived almost exclusively from milking equipment. Most thermophilic organisms do not multiply appreciably in raw milk even at ambient temperatures, and thus a high thermophilic count in milk up to 24 hours old is reliable evidence of gross contamination from milking equipment (Bramley & McKinnon, 1989).

2.4 Effect of heat processes on microflora

2.4.1 Thermization

Thermization is the treatment of milk at temperature of 60 to 65°C for 10-20 seconds. It is a mild continuous heat treatment, immediately followed by cooling, such that the raw milk properties are almost unchanged while the activity of the bacteria, especially the psychotrophic bacteria, are considerably reduced (van den Berg, 1984, Muir, 1989). At low initial counts, the number of survivors is too low to cause rapid deterioration of the thermized product, but, if the initial bacterial load of the raw milk exceeds 5×10^6 cfu/ml its effectiveness is significantly reduced (Muir, 1989).

Anon (1978) reported that thermization of milk at temperature between 60 to 65°C and holding time of 15 to 20 seconds, the number of active psychotrophic bacteria can be reduced, and an increase in plate-count can be prevented, during 3 days storage of milk at 5-7°C. It was found that a heat shock for 10 seconds at 65°C stimulates the germination of *Bacillus cereus* spores in milk. If the milk subsequently was kept at 37°C for one hour, 85% of the spores had turned into a vegetative form. So thermization can be used to improve the keeping quality of raw milk and can be regarded as a pre-treatment instead of a heat treatment in the usual sense because the milk properties are only slightly changed by this treatment (van den Berg, 1984).

The inactivation of bacterial enzymes during thermization is slight, respectively estimated by the increase in free fatty acids and free tyrosine in the thermized milk. Nevertheless, an effect of practical significance was only achieved with heating temperatures in the range 62-72°C (Muir, 1989; van den Berg, 1984).

2.4.2 Pasteurization

There are three pasteurization methods:

- (1) bath heating at 62-65°C for 30 minutes;
- (2) short time heating at 71°C for 15-40 seconds;
- (3) high temperature heating at 85-90°C for 1-4 seconds (Kessler, 1981).

Muir (1989) and van den Berg (1984) have stated that pasteurization not only kills pathogenic organisms in raw milk but also destroys a wide range of other bacteria. The important groups of bacteria which can survive pasteurization are aerobic spore formers, *Streptococcus thermophilus*, *Enterococci*, *Micrococci*, *Microbacterium* and *Alcaligenes tolerance*. The main class of spoilage organisms which survives pasteurization are the spore-forming bacteria. The vegetative cells of such bacteria are destroyed by pasteurization, but the spores are very heat-resistant. After heat treatment, the spores germinate in the product grow and multiply and, eventually, cause spoilage via their degradative enzymes.

If HTST pasteurized milk which has not been re-infected, is stored at temperatures above 7°C, it will deteriorate by the growth of rapidly germinating spores of *Bacillus cereus* (Stadhouders, 1975).

Griffiths (1991) showed growth of *Bacillus spp.* in pasteurized milk can lead to a number of defects. The most well known fault caused by *Bacillus cereus* is bitty cream. The effect is due to the enzyme phospholipase C, synthesized by *Bacillus cereus*. This enzyme degrades the milk fat globule membrane of native milk fat globules. *Bacillus Cereus* is also associated with a sweet-curdle defect in pasteurized products due to production of a proteolytic enzyme by the organism. Frequently the first sign of this defect is the appearance of small 'buttons' or 'pellicles' on the bottom of the milk container. The growth of psychrotrophic *Bacillus spp.* is associated with bitter flavour development due to the action of proteinases on the milk proteins. However, other off flavours, including fruity, unclean or putrid, rancid, yeasty and sourness have been described in milks that have supported growth of psychrotrophic *Bacillus spp.*. These off flavours arose within 2-12 days in milks inoculated with a heavy inoculum and stored at 7.2°C. When HTST-pasteurized milk is re-infected and stored at < 7°C, Gram-negative rods, particularly *Pseudomonades*, will predominate and spoil the milk (Stadhouders, 1975).

Coryneform bacteria may form a substantial proportion of the flora of heat-treated milk, but they grow very slowly at refrigeration temperatures. Only rarely have thermoduric micro-bacteria been associated with defects in dairy products (Muir, 1989).

Micrococci form another minor group of thermoduric bacteria in raw milk. They may be as numerous in freshly collected milk as the aerobic spore-forming bacilli, but they are rapidly overgrown at 7°C. The inability of micrococci to grow in raw milk is to some extent probably due to the presence of lactenins, a group of natural inhibitors (Muir, 1989).

Many strains of *Streptococcus spp.* (*Str. thermophilus*, *Ent. faecalis* and *Str. brevis*) are also thermoduric. But at temperatures above 80°C they can not survive (van den Berg, 1981). *Streptococci* grow very slowly at refrigeration temperatures, and thus pose no great threat to pasteurized products (Muir, 1989).

Kessler (1981) concludes that there is no correlation between the number of originally present in raw milk and that of those surviving after pasteurization, and there is also no correlation between the number of surviving organisms and the storage life of pasteurized milk. The bacterial flora which survives pasteurization grows only slightly in cooled milk. Exceptions are the psychrotrophs which are the usual cause of the spoilage of milk which has been recontaminated. *Psychrotrophic streptococci* and *enterococci* which are not usually considered as organisms introduced by recontamination can, under unfavourable circumstances, survive short time heat treatment and act as spoilage organisms of pasteurized milk.

2.4.3 Sterilization and ultra-high temperature treatment (UHT)

The typical conditions for sterilization are 109-115°C for 20-40 minutes and for ultra-high temperature treatment are 135-150°C for 2-6 seconds.

Sterilization produces destruction of the whole of the microflora and spore formers (except for some heat resistant spores, e.g. *Bacillus stearothermophilus*) and complete

enzyme inactivation (except for some heat resistant lipase and proteinases derived from *Pseudomonas* strains). But sterilization also produces browning reaction and a cooked or caramelized flavour in milk. UHT produces less discolouration and flavour changes (Kessler, 1981).

However microbiologically caused changes may also occur in sterilized products. The organisms relevant to spoilage in sterilized products are the spore-forming types of bacteria. Spore-forming microorganisms, if they survive sterilization, lead to protein destruction (*Bacillus subtilis*, *Bacillus licheniformis*), explosion of cans (*Clostridium sporogenes*), or 'sweet coagulation' (*Bacillus coagulant*, *Bacillus stearothermophilus*, *Bacillus cereus*). The reinfection, in the case of improper sealing, induces lactic acid formation and coagulation (lactic acid-producing *streptococci* and *lactobacilli*). If changes of a microbiological nature are found, the first step should be to check the sterilization regime in the production process (Carić, 1994; Muir, 1989).

For evaporated milk, it must be commercially sterile in order to maintain quality at room temperature. This means that it must not contain organisms which will grow, and probably produce defects, under the normal storage conditions. If a residual organism is prevented from growing by the lack of oxygen in the environment (a highly aerobic organism), or if it is an obligately thermophilic organism which will grow only at elevated temperatures such as 45°C, the product may be 'commercially sterile' (Nelson, 1989).

2.5 Microbial defects in evaporated milk

Evaporated milk, sometimes called 'unsweetened condensed milk', is whole milk from which about 60% of the water has been removed by evaporation. The manufacture of evaporated milk consists of; first standardization, preheating, pasteurization, evaporation, homogenization, cooling, second or final standardization, packaging, sterilization and storage (Carić, 1994; Kalogridou-Vassil, 1990; Lampert, 1975).

The heating processes (preheating, pasteurization) are sufficient to destroy all non-spore-forming bacteria and also many of the less resistant types of spore-forming bacteria. Only heat resistant types could survive, but the temperatures employed at this stage are too high to make thermophilic growth a problem. However, temperatures employed at evaporation are almost ideal for development of thermophilic bacteria, which may become a problem after prolonged operation (Kalogridou-Vassiliadou, 1990; Nelson, 1989). The sterilization of evaporated milk is designed to result in a commercially sterile product. It is generally assumed that all bacteria originally present will be destroyed during the manufacture of evaporated milk, but spoilage may occur as a result of the incidence of heat-resistant spore forms in the raw milk or be caused by contamination during processing (Kalogridou-Vassiliadou, *et al.*, 1989 ; Lampert, 1975).

Kalogridou-Vassiliadou, *et al.* (1989) and Nelson (1989) reviewed that the organisms can be divided into spore-forming organisms which are of high heat resistance and survive a slightly inadequate heat treatment, and non-spore-forming which are usually of low heat resistance and the presence of these organisms indicates post-sterilisation contamination, frequently as the result of a leaky container. Most of the heat resistant forms are species of the genus *Bacillus*, but species of the genus *Clostridium* have occasionally been encountered. Generally the growth of the spore-forming bacteria in evaporated milk usually produces several physico-chemical and sensory changes, which affect the shelf-life of the milk and which seems to strongly depend on the type of microorganisms present in raw milk.

Bacillus coagulant, *Bacillus cereus* and *Bacillus stearothermophilus* may cause a cheesy flavour and odour, and develop an increase in acidity to cause acid coagulation if the milk is held in storage at high temperature. These organisms grow best at 37°C and above, and high storage temperatures and/or inadequate cooling are factors in this type of spoilage. *Bacillus subtilis* causes a non-acid curd, which may then be digested to a brownish liquid with a bitter taste (Nelson, 1989). Lampert (1975) claimed that microorganisms are rarely found in cans of bitter evaporated milk; they probably do not survive the storage time needed to develop the off-flavour.

The coagulum formed by *Bacillus megaterium* is accompanied by some gas and a cheesy odour. Gas production associated with putrefaction and a smell of hydrogen sulphide have been reported as caused by a *Clostridium spp.*, but this type of defect is very rare (Nelson, 1989).

It was described by Lampert (1975) that the thickening of the milk during sterilization should not be attributed to bacterial activity. However, gelation of canned evaporated milk usually is due to the presence of *Bacillus vulgatus (subtilis)*. Such milk shows no increase in acidity nor formation of gas.

Mckellar & Nchols-Nelson (1984) shown that acid coagulation in stored evaporated milk was the result of the simultaneous growth of *E. faecium* and *Bacillus subtilis*. Spoilage of evaporated milk was the result of acid and gas production, the latter in sufficient quantities to cause rupture of some cans. Nichols (1940) has observed that while gas may be produced by some *Bacillus* species in sterilized milk, this type of spoilage is normally associated with *Enterococcus spp.*

Kalogridou-Vassiliadou, *et al.* (1989, 1990) claimed that sporadic appearance of flat sour spoilage in evaporated milk has been a serious problem even when proper procedures have been followed throughout the canning operation. In flat sour spoilage, organisms ferment carbohydrates, resulting in the production of acids but without gas formation and the can ends remain flat. *Bacillus stearothermophilus* and *Bacillus coagulans* were considered to be the species typically responsible for the spoilage, *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus macerans* were also isolated from flat sour evaporated milk in considerable percentages.

2.6 Lipolysis in milk and milk products

Lipolysis is the hydrolytic degradation of milk lipids. The hydrolysis is catalysed by lipases, produces free fatty acids (FFAs) (Deeth & Fitz-Gerald, 1983), and results in the common flavour defect known as lipolytic or hydrolytic rancidity (Goff, *et al.*, 1993).

These flavours are variously described as rancid, butyric, bitter, unclean, soapy and astringent (Deeth & Fitz-Gerald, 1983).

According to Olivecrona, *et al.* (1992), freshly drawn bovine milk contains only small amounts of free fatty acids. If the milking and storage procedures are appropriate, the milk can be stored for several days with little further development of FFA. Hydrolysis of as little as 1-2% of the milk triglycerides to fatty acids gives a rancid flavour to the milk. This makes the milk unpalatable for consumption. Concentration of the reaction products in the cream may in turn impair the flavour quality of products manufactured from milk. Deeth & Fitz-Gerald (1983) pointed out that hydrolytic rancidity in milk and milk products has been a problem in the dairy industries of most countries. Although it is not considered a serious problem in many countries today, the potential for problems exists at all times and a constant vigilance is necessary to ensure effective control.

2.6.1 Lipases

The lipases involved in the hydrolysis are of two types: the endogenous milk enzyme(s), and those enzymes of microbial origin (Deeth & Fitz-Gerald, 1983).

2.6.1.1 Milk lipoprotein lipase

Lipoprotein lipase (LPL) is the principal lipolytic enzyme in bovine milk. Under normal circumstances, most of the LPL in milk is in the skim-milk fraction and about 80% of LPL is associated with the casein micelles, 10 to 20% is present in the serum, in soluble form, and only 0 to 10% is associated with the fat globule membrane (Deeth & Fitz-Gerald, 1983; Goff, *et al.*, 1993). It is noteworthy that the enzyme shows virtually no activity in milk, but when activated, it attacks the 1,3-position in tri-, di-, and monoglycerides and 1-position of glycerophosphate to give free fatty acids which cause rancidity in milk (Farkye & Imafidon, 1995).

Milk contains high concentration of lipoprotein lipase. However, little milk fat is hydrolysed in raw milk because it is protected by the fat globule membrane. Mechanical damage of the membrane or the presence of a cofactor of the enzyme in the milk or both, initiates lipolysis in raw milk (Driessen, 1989). The temperature and pH of the milk are also important conditions for lipolysis. Goff & Hill (1993) reviewed that cow's milk contains sufficient total lipase activity (mainly LPL) to release about 2 μmol of free fatty acids per minute at 37°C, but the actual activity during storage of raw milk at 4°C may be as low as 0.002 μmol of FFA min^{-1} . The optima of pH and temperature for LPL are 8.3°C and 37°C. But the pH of milk normally is 6.6 to 6.8 and the storage temperature of raw milk normally is below 4°C. These conditions are not suitable for LPL so that the rate of lipolysis in fresh milk is limited.

Deeth & Fitz-Gerald (1983) stated that in milk it is believed to be stabilized by some factor in skim fraction, possibly a heparin-like glycosaminoglycan. The casein also stabilized it with respect to heat inactivation. LPL is a relatively heat-labile enzyme. Very little (~3%) of the LPL activity of raw milk survives pasteurization (Farkye & Imafidon, 1995). It is generally accepted that HTST treatment (72°C/15 seconds) of milk almost completely inactivates the enzyme so that little if any lipolysis is caused by milk lipase in pasteurized milk (Deeth & Fitz-Gerald, 1983).

Driessen (1989) recommended that to prevent defects caused by milk lipoprotein lipase, milk is heated to at least 78°C with a holding time of 10 seconds. It is of practical importance to respect this fact, and also to prevent the mixing of homogenized milk (returns) with either raw milk or low-pasteurized milk or reconstituted low heat milk.

2.6.1.2 Microbial lipases

Milk and milk products may contain a variety of microorganisms capable of secreting lipases and proteinases which subsequently may alter these products (Driessen, 1989).

Bulk cooling and storage milk on farm for two days or longer is becoming increasingly popular. A storage temperature of 4°C is normally used but this can fluctuate especially

at milking when warm milk enters the tank and in transport of the raw milk from farm to processing depot in bulk tanks. Storage of raw milk for 24 hours or longer at the processing factory before heat treatment may also occur. Thus, three days or more can elapse between milk production and processing during which there is considerable opportunity for psychrotrophic bacteria to grow and produce enzymes, especially lipases and proteinases (Cogan, 1980; Deeth & Fitz-Gerald, 1983)

Birschbach (1994) pointed out that most microbial lipases exhibit pH optima in the 4-9 range and temperature optima at approximately 45°C.

According to Deeth & Fitz-Gerald (1983), the pH optima of the lipases are usually in the alkaline region between 7 to 9, but some have considerable or even optimal activity at the pH of milk. They generally show highest activity at 40-50°C, although there are reports of higher and lower temperature optima. It was found that only a few are inactivated under 55°C/hour heating treatment. In fat-containing media, considerable lipolysis can occur during prolonged heating at 55°C.

Driessen (1989) reviewed that bacterial lipases are most active within the temperature range of 30 to 40°C but there is still substantial activity at low temperatures. These lipases are very stable at temperature below 8°C, and at 20°C, 50% of the original activity can remain after storage of about 3 months (Andersson, 1980).

One of the most important properties of these lipases is their heat stability. This varies with the species and strain, and also with the medium in which they are heated. Many are sufficiently stable to retain at least some activity after HTST pasteurization, and even after UHT treatment (Deeth & Fitz-Gerald, 1983; Driessen, 1989).

There are relatively few reports on what proportion of the milk flora produces lipases, but results suggest that approximately one third of the isolates from cold-stored milk are lipolytic (Stadhouders & Mulder, 1958; Muir, *et al.*, 1979). The lipolysis that occurs on storage of milk is, according to Muir, *et al.* (1978a), correlated with the total count of psychrotrophic bacteria before storage.

Indirect evidence that heat resistant enzymes are involved in deterioration of pasteurization milk was obtained by Patel & Blankenagel (1972) who studied the effect that bacterial counts in the raw milk had on the flavour of pasteurized milk during storage at 7°C. Pasteurized milk which did develop flavour defects had bacterial counts of 5×10^6 /ml or greater in the raw milk; however, this was not absolutely true as there were some instances where flavour defects occurred with counts of 1,000/ml, demonstrating that types of bacteria may be more important than numbers (Cogan, 1980). Psychrotrophs are dominant organisms producing lipases in cold storage raw milk and milk products (Deeth & Fitz-Gerald, 1983).

2.6.1.2.1 Psychrotrophic bacteria

Psychrotrophic bacteria can grow at temperature below 7°C, although their optimal growth temperature may be in the range of 20 to 30°C. Psychrotrophs found in milk are mainly gram-negative, rod-shaped bacteria in the genera *Pseudomonas*, *Archromobacter*, *Alcaligenes*, *Enterobacter*, *Acinetobacter*, and *Flavobacterium*, but gram-positive psychrotrophs in the genera *Bacillus* and *Clostridium* have also been isolated (Frank, *et al.*, 1985).

Psychrotrophic bacteria are not part of the normal udder microflora. They are all common plant or soil saprophytes and get into milk mainly from improperly cleaned equipment (Cogan, 1980). The numbers of psychrotrophic bacteria in raw milk depend on sanitary conditions during production and on duration and temperature of storage before the milk is processed (Frank, *et al.*, 1985). Most psychrotrophs are destroyed by pasteurization but some of them, such as *Bacillus spp.*, can survive (Grosskope, *et al.*, 1969). Thus, the presence of psychrotrophs in pasteurized products can be attributed largely to post-pasteurization contamination (Frank, *et al.*, 1985).

Psychrotrophs are generally non-pathogenic, but when they are present in dairy products, they can cause a variety of off-flavours that include fruity, stale, bitter, putrid, and rancid, as well as physical defects (Cousin, 1982; Elliker, *et al.*, 1964). The influence of psychrotrophic bacteria on the shelf life of pasteurized milk will depend mainly on

how many are present after packaging, their rates of growth and levels of biochemical activity, and the length of storage. Even if no detectable changes occur in the product, an increase in numbers of psychrotrophs may cause problems in meeting bacterial standards (Frank, *et al.*, 1985).

The greatest problem caused by psychrotrophs is associated with the extracellular thermostable enzymes that they produce in raw milk. These thermostable enzymes can degrade milkfat in milk and milk products (Cousin, 1982; Christen, 1980; Cogan, 1980). In cultures of single lipolytic psychrotrophs, a level of approximately 10^6 or 10^7 ml⁻¹ is attained before spoilage is apparent. Muir (1978b) observed lipolysis in stored farm or factory milk with psychrotroph counts $> 5 \times 10^6$ ml⁻¹. Taints may be evident after 4-5 days at 5°C or after shorter times at higher temperature (Deeth & Fitz-Gerald, 1983).

2.6.2 Lipolysis

Lipolysis, as it occurs in dairy practice is often differentiated into spontaneous and induced (Deeth & Fitz-Gerald, 1976). Spontaneous lipolysis is initiated in some milk samples simply by prompt cooling of the fresh milk (Jellema, 1980). In contrast, lipolysis can be induced in all milk samples by rough mechanical treatment (Fleming, 1980).

The fat globule membrane protects the milk triglycerides from attack by lipolytic enzymes whose activity is further impaired by their association and/or occlusion with the casein micelles (Downey & Murphy, 1975) and possibly by the presence in milk of inhibitors to lipolysis (Deeth & Fitz-Gerald, 1975). Thus the native milk lipolytic enzymes are well partitioned from the triglycerides substrate. Hence, little or no lipolysis occurs in normal milk since for all practical purposes the lipolytic enzymes cannot make contact with their triglyceride substrate and such activity as might arise from the restricted interaction between the enzymes and substrate may possibly be further retarded by the inhibitors present (Downey, 1980).

If the raw milk or cream is subjected to excess agitation or turbulence, the fat globule membrane may be disrupted resulting the enzymes gaining access to the triglycerides which are accordingly hydrolysed. Accumulation of the reaction products, especially the FFA, as well as the mono- and diglycerides, confers a rancid or lipolysed flavour on the milk, and the concentration of the reaction products in the cream may in turn impair the flavour quality of the products manufactured from the milk (Downey, 1980).

It is generally accepted that excessive mechanical agitation and turbulence of raw milk and/or cream is the major cause of induced lipolysis. Farm milk handling, especially milking, is considered to be mainly responsible for the excess agitation (Downey & Cogan, 1975).

Heating of milk, according to (van Boekel, *et al.*, 1995), is also the cause of induced lipolysis. Heating is always accompanied by some agitation and this may lead to changes in fat globule size as a result of coalescence or disruption (Mulder, *et al.*, 1974); consequently changes in surface layers may take place: desorption of membrane material in the case of coalescence and adsorption of surface-active material from the milk plasma in the case of disruption. When foaming occurs, fat globules may come into contact with bubbles yet uncovered by plasma proteins; hence, spreading of membrane material over an (air or vapour) bubble may take place and when such a bubble disappears this material is released into the plasma; meanwhile, plasma proteins adsorb on to the partly denuded fat globules. These changes are thus not the result of heating *per se*, but of the agitation during heating, although a high temperature may promote coalescence as well as disruption. It depends on the type of apparatus used whether coalescence or disruption occurs (Mulder, *et al.*, 1974).

Fleming (1980) and Sundheim (1988) concluded that at the biochemical level, induced lipolysis is presumably equivalent to disruption of the organised structure of the fat globule membrane, this allows lipase to bind to the fat globules and hydrolyse their lipids.

2.7 Proteolysis in milk and milk products

The process by which protein is broken down is called proteolysis. The major enzymes concerned are proteinases, e.g. rennin, pepsin and trypsin. These enzymes degrade proteins into peptides, which are then degraded by various peptidases to smaller peptides and free amino acids (Bylund, 1995).

Proteinase-producing psychrotrophic Gram-negative bacteria are often isolated from milk. The best represented genus is *Pseudomonas*, with *Ps. fluorescens* as the most common species (Law, 1979).

Psychrotroph proteinases may act first during refrigerated handling of raw milk before heat treatment, and Law, *et al.* (1977, 1979) have shown that strains of *Pseudomonas* and *Acinetobacter* spp., growing to approximate 10^7 colony-forming units (cfu/ml) and above produce sufficient proteinase to degrade β - and κ -casein to an extent detectable by polyacrylamide-gel electrophoresis (PAGE) and starch-gel electrophoresis (SGE). Bengtsson, *et al.* (1973) produced evidence that UHT-sterilized milk coagulated in the presence of heat-resistant *Pseudomonas* proteinase; κ -casein degradation was detected as the release of sialic acid into the milk during storage. Whey proteins were degraded only slowly. Adams, *et al.* (1976) also reported that casein was degraded by proteolytic species of *Pseudomonas*. κ -casein degradation was detectable even before the population had reached 10^4 cfu/ml. β -casein was degraded more than α -casein by most strains, though this was only detected at much higher populations (10^6 - 10^7 cfu/ml). Some strains also degraded the whey proteins, β -lactoglobulin and α -lactalbumin.

Although the proteinases appear to cause some protein breakdown in raw milk, their action in stored heat-treated milk and milk products is likely to be of greater overall economic importance. Many of the psychrotroph proteinases possess a remarkable capacity to survive severe heat treatment even for UHT-sterilization (Law, 1979).

Cogan (1980) concluded that to minimize the effects of heat-resistant enzymes on quality deterioration of milk and its products, factors which support the growth of bacteria especially psychrotrophs should be curtailed. These were summarized:

- (1) Increase the frequency of milk collection on the farm.
- (2) Ensure that milk is not stored above 4°C.
- (3) Ensure adequate cleaning of the milking machine, bulk tank and ancillary equipment.
- (4) During transport of the milk to the factory, ensure that the temperature does not increase and that the tanker and its ancillary equipment are adequately cleaned.
- (5) Pasteurization or "Thermisation" (65°C for 10-15 seconds) of the milk on arrival at the factory is useful as it generally destroys or markedly reduces the psychrotrophic microflora. Such a treatment would be especially useful if the raw milk has been stored for several days on the farm.

2.8 On-farm concentration of milk

Milk which has been pre-concentrated 2- or 3-fold clearly should be cheaper to transport to the processing plant than the equivalent quality of normal milk. Similarly, all the other volume dependent handling and processing costs should be favourably affected by concentration. For example, pump and milk storage capacities are reduced proportionately, as is cheese vat capacity or evaporator loads in milk powder plants. Robertson (1987) pointed that on-farm concentration of milk using either membrane processing or mini-evaporators may well be a concept which be of rapidly increasing importance in the decade ahead. It might be thought that the greatest savings in transport costs can be achieved by concentrating milk on farms at greatest distance from the dairy factory. But there is not much published information found about the study of on-farm concentration of milk.

Anon (1992) reported that an on-farm concentrator can halve water content in milk. The trials were carried in New Zealand. The *Milcon* on-farm milk concentrator was used. It is a small-scale mechanical vapour recompression evaporator, similar in basic

technology to some of the large new milk concentration units at some dairy factories. It concentrates the milk at temperature between 61°C and 65°C, at a rate of 200 litres of wholemilk an hour. The developers believe the unit has potential to increase milk solids level to 36%. Their thinking is a mixing regime at the factory of 10% concentrate and 90% normal milk — which would normally come from farms closer to the factory. Blending of concentrated milk with wholemilk at the factory allows standardisation of milk solids for downstream processing. But Zall & Chen (1984) reported that milk concentrates up to about 2-fold can be skimmed efficiently using the conventional milk separators commonly found in dairy plants.

Slack, *et al.* (1982) demonstrated on-farm concentration of milk by ultrafiltration. The results showed that raw whole milk ultrafiltrates containing 24% total solids, 9.8% protein and 11.5% fat can be obtained using spiral wound membranes, and the concentrate was stored at 4.5°C, with negligible rancidity, and without pasteurization, up to four days after milking. Zall & Chen (1984) reported the dairy products such as cottage and cheddar cheeses, yogurt and cultured milk, ice cream and beverage milk can be made in a satisfactory manner using membrane concentration systems.

However, there still are some disadvantages of on-farm concentration of milk. Anon (1992) and Slack, *et al.* (1982) found milk fat in concentrated milk was damaged to some extent. Robertson (1987) concluded that the actual or potential disadvantages of pre-concentration are mainly a matter of whether the use of such milk gives entirely normal product and whether new equipment needs to be developed to handle the changed physical characteristics and behaviour during milk processing. Moderate changes in concentration can strongly affect factors such as gel strength, and moisture retention in cheesemaking. Obviously, factors like increased lactose retention can have a profound effect on cheese pH, and maturation. These should be investigated and overcome before the on-farm concentration systems are used.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and equipment

3.1.1 Fresh raw milk

Fresh raw milk used in the trials was collected immediately after milking from No. 4 dairy farm of Massey University. An example of the compositions of raw milk is shown in Appendix I.

3.1.2 Chemicals

All chemicals used were of analytical grade. Their sources were: BHD Chemicals Ltd. (Dorset, UK), Bevaloid Chemicals Ltd. (Levin, New Zealand), Polychem (Auckland, New Zealand), and Sigma Chemical Company (Missouri, USA).

3.1.3 Microbiological media

The following media were used in this study:

(1) Standard plate count agar

Composition (g/l): yeast extract 2.5; pancreatic digest of casein 5.0; glucose 1.0; agar 15.0. pH 7.0 ± 0.2 .

Source: Unipath Ltd., Hampshire, England.

(2) Violet red bile agar

Composition (g/l): yeast exact 3.0; peptone 7.0; sodium chloride 5.0; bile salts No.3 1.5; lactose 10.0; natural red 0.03; crystal violet 0.002; agar 12.0. pH 7.4 ± 0.2 .

Source: Unipath LTD., Hampshire, England.

3.1.4 Equipment and instruments

- (1) A Centritherm evaporator (CT1B-2, ALFA-LAVAL) in the pilot plant at the Food Technology Department of Massey University was used.
- (2) A single tube falling film evaporator in the pilot plant made by Food Technology Department of Massey University was used.
- (3) Master Sizer/E: Malvern Instruments Ltd. Malvern, U.K., held in New Zealand Dairy Research Institute.
- (4) Ferranti-Shirley Viscometer System: Ferranti Instrumentation LTD. Moston, Manchester, UK.
- (5) Kjeltec system (1026 distilling unit, digestion system 6/12): Perstorp company, science and technology (NZ) LTD.
- (6) PAGE system (mini Protean II equipment): Bio-Rad Laboratories, Richmond, USA.
- (7) Other instruments used included a LASER densitometer, an analytic balance, a dry oven, a refrigerator, an incubators, a water bath, an autoclave, and a refractometer.

3.2 The evaporators used in the experiment

A Centritherm evaporator and a falling film evaporator were used in this study. Most of the work was carried out on the Centritherm, which is similar to the proposed evaporator used for the on-farm evaporator system. Some trials were conducted on the falling film evaporator. A comparison of the results obtained from these two evaporators was made.

3.2.1 The Centritherm evaporator

As shown in the Figure 3.1 the Centritherm evaporator has an externally heated rotating rotor in the form of a truncated cone. The general processes occurring in the Centritherm evaporator can be briefly described as follows: the liquid to be concentrated is preheated and fed into the inner surface of the rotating cone. Under the centrifugal field, the liquid is immediately spread on the inner surface in a form of film, which moves very fast in a radial direction. Since the film is very thin, its heat transfer resistance is small, consequently vapour is released very rapidly. Vapour passes out via the vapour outlet to a condenser. The steam for heating is fed into the steam jacket and condenses on the outer surface of rotating cone. The condensate from the steam is impelled off by the action of the centrifugal force from the outer surface of the cone as soon as the condensate is formed, which results in more areas being exposed for steam to be condensed. Therefore the overall heat transfer coefficients on the rotating cone surface can be very high.

3.2.2 The falling film evaporator

The falling film evaporator is the dominant type used in the dairy industry. The general processes occurring in the falling film evaporator can be briefly described as follows: The liquid to be concentrated is preheated and pumped into the top of the evaporator where it flows through the distributor. The liquid then flows down on the inside of heating tubes as a thin boiling film. The heat flux which is released by condensing steam in the steam jacket outside the heating tube transfers to heating tube walls and conducts through the walls, then the heat issues from the inside of tube walls to make a portion of the falling film evaporate. The downward movement of falling film caused by gravity is assisted by the vapour produced which likewise flows downward. The vapour is separated from concentrate in a separator placed at the base of the heating tube and goes to the condenser.

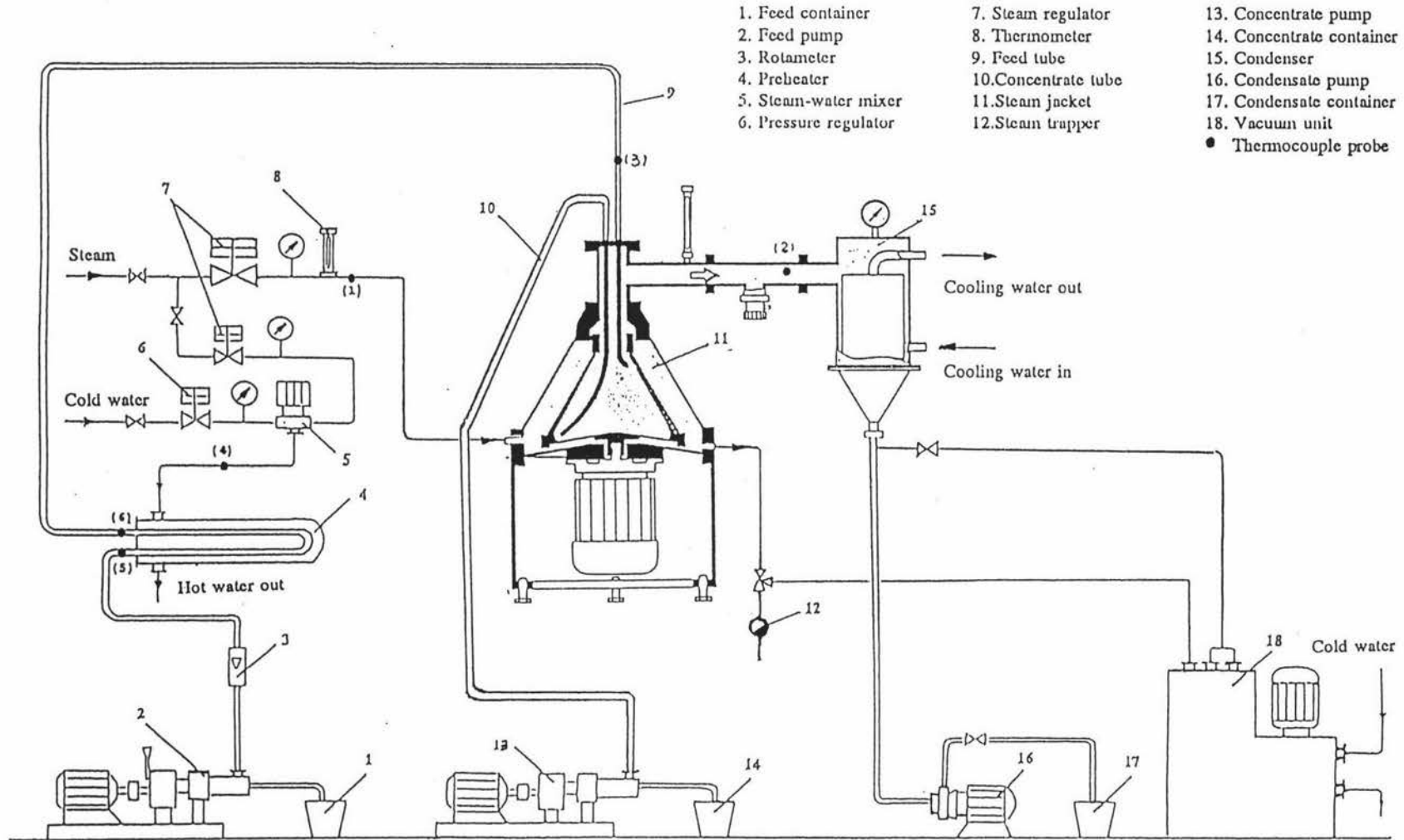


Fig. 3.1 The schematic diagram of the Centritherm evaporator

3.2.3 Processing conditions

The processing conditions used in the experimental work are shown in Table 3.1.

Table 3.1. The selected processing conditions

Evaporating temperature:	50°C, 60°C and 70°C,
Temperature differences:	15°C
Concentrations of milk:	30, 35, 40, 45, 50% TS
Storage temperature on farm:	5°C
Storage time on farm:	3 days maximum
Transportation time:	3-4 hours
Temperature rising during Transportation:	from 5°C to 10°C
Storage temperature:	5°C and 10°C
Storage time:	2 days maximum

The evaporating temperatures of 50°C, 60°C and 70°C, and the temperature difference of 15°C have been chosen for the experiments. The highest evaporating temperature is limited by the heat sensitivity of milk because the denaturation of whey proteins will be severe when the temperature is over 70°C. The lowest evaporating temperature is limited by the economical operation of the vacuum and cooling system.

To obtain the desired milk concentrate, 30 kg raw milk was used for each trial and the milk was recycled in the evaporator. The concentrated milk samples were taken at concentrations of about 30, 35, 40, 45 and 50% TS. All samples were held at 5°C for three days.

The transport of milk from the farm and subsequent storage at the dairy factory may result inevitably in further contamination and raising of milk temperature and increase of the bacterial growth rate. During transportation and storage, the growth of bacteria

in milk depends on both the storage temperature of milk and the environment temperature, the duration of transport and the contamination of the milk (types and numbers of organisms in initial raw milk). The rise in temperature up to the time of arrival at the factory depends on the temperature of the environment, on the size and heat conductivity of the tanker vessel and on the time taken for transport (Flückiger, *et al.*, 1980). The data collected from Longborn dairy factory of Tui Milk Cooperate Company of New Zealand shows that the raw milk transportation takes about maximum 4 hours from a farm to reception area at the factory and the temperature of raw milk increases to a maximum of 10°C. Therefore, in this study, the transportation time and temperature increase were specified as 5°C to 10°C for 4 hours.

A shaking water bath was used to imitate the transportation process. The storage temperatures at the dairy factory were set at 5°C and 10°C. The contamination was imitated by adding raw milk into concentrated milk before the simulated transportation.

3.3 Analysis methods

3.3.1 Microbiology

3.3.1.1 Sampling

To prevent microbial contamination, the evaporator was cleaned using the clean-in-place (CIP) techniques, which are the same as those in the dairy factory, before and after each trial. The bucket containing recycled milk was cleaned thoroughly with cold water first and then hot water (temperature was over 80°C) before use, and it was covered with a lid to prevent contamination from air while the evaporation process was undertaken.

Sampling instruments and containers that include metal dippers and glass screw-capped bottles were sterilised before use. Hands were washed and dried before sampling. Thoroughly mixed milk was sampled and the samples were immediately cooled to and maintained at 5°C.

3.3.1.2 Total bacteria content

The Standard Plate Count Method (MFA, 1978) was used.

Medium: Standard plate count agar.

Procedures: Aseptically add 1 ml of the appropriate dilution and than 10-12 ml of sterile standard plate agar (at 45-46°C) to a sterile labelled Petri dish, mix, and allow to set. Invert plates and incubate at $30\pm 1^\circ\text{C}$ for 72 ± 3 hours. Report as colony forming units/g (cfu/g). Count only the plates which have between 30 and 300 colonies on them.

3.3.1.3 Coliforms

Coliform enumeration direct plating method (MAF, 1978; Hartman & LaGrange, 1985) was used.

Medium: Violet red bile agar.

Procedures: To 1 ml inoculum in Patri dish carefully add 10 ml of molten VRBA at 45°C , mix, and allow plate to set. Then add 5 ml of molten VRBA as an overlay.

Incubate at 30°C for 24 hours. Count red colonies and report as number of coliforms/g product, or negative or positive in given weight of product.

3.3.1.4 Thermophilic Count

Medium: Standard plate count agar.

Procedures: Aseptically transfer 1 ml of the appropriate dilution and than about 20 ml of sterile standard plate count agar into a sterile labelled dish. Mix promptly and allow to set. Overlay the agar with about 5 ml sterile standard plate count agar and allow to set again. Invert plates and incubate at $55\pm 1^{\circ}\text{C}$ for 48 ± 2 hours (MFA, 1978).

3.3.1.5 Psychrotrophic bacteria

Medium: Standard plate count agar.

Procedures: Prepare dilutions of product and then plate as described for the Standard Plate Count, except incubate at $7\pm 1^{\circ}\text{C}$ for 10 days. Take special care that melted medium is cooled to $45\pm 1^{\circ}\text{C}$ before pouring plates to prevent destruction of some psychrotrophic bacteria. At temperatures above 7°C , other organisms may grow, possibly yielding misleading results (Frank, *et al.*, 1985).

3.3.2 Chemistry and physics

3.3.2.1 Total solids

Method: Gravimetric method (MFA, 1979).

The details of principle, procedure and calculation are given in Appendix II.

3.3.2.2 Total protein content

Method: Kjeldahl method (MFA, 1979; Case, *et al.*, 1985).

The details of principle, procedure and calculation are given in Appendix III.

3.3.2.3 Casein content

Method: Kjeldahl method (MFA, 1979).

The details of principle, procedure and calculation are given in Appendix IV.

3.3.2.4 Denatured whey proteins

Method: PAGE method (Hillier, 1976).

Principle: Any charged ion or group will migrate when placed in an electric field. Since proteins carry a net charge at any pH other than their isoelectric point, they too will migrate and their rate of migration will depend upon the charge density (the ratio of charge to mass) of the proteins concerned; the higher the ratio of charge to mass the faster the molecule will migrate. The application of an electric field to a protein mixture in solution will therefore result in different proteins migrating at different rates towards one of the electrodes. Protein bands are stained by dyes, and then scanning densitometer is used to determine the absolute amount of proteins (Grappin, 1992; Hames, 1990).

Procedure:

- (1) Make Native-PAGE gel (Native-page gives a measurement of the undenatured monomeric protein).
- (2) Dilute concentrated milk samples to the same total solid as raw milk with distilled water.
- (3) Adjust the pH of samples to 4.6 with 1.0 M HCL.
- (4) Centrifuge the samples at 5,000 rpm.
- (5) Withdraw the serum, and mix with sample buffer in 1:5.
- (6) Apply 10 μ l of each sample to the gel.
- (7) Run the gels at 200 V for about 45 minutes until the dye disappeared from the bottom of the gel.

- (8) Stain the gels for 1 hour in Coomassie blue dye solution in a closecontainer with continuous vibrations.
- (9) Destain the gels by two destaining steps of 1 hour, and 19 hours, in destaining solution.
- (10) Keep the finished gels in distilled water until scanning.
- (11) Scan the gels on an Ultrascan XL model laser densitometer.
- (12) Calculate the areas under the peaks from the scanning, and determine the percentage of whey denaturation.

3.3.2.5 Fat content

Method: Roess-Gottlieb method (MFA, 1980; Case, *et al.*, 1985).

The details of principle, procedure and calculation are given in Appendix VI.

3.3.2.6 Free fatty acids content

Method: Titration method (MFA, 1979).

Principle: Free fatty acids are ether extracted and determined by titration against alcoholic potassium hydroxide using alpha-naphtholphthalein indicator.

Procedure:

- (1) Pipette 10.0ml of well mixed sample at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ into a Mojonnier extraction flask.
- (2) Carry out a blank using 10.0 ml of water.
- (3) Add 3 drops of bromophenol blue indicator.
- (4) Add 0.5M sulphuric acid dropwise until the colour changes to greenish/yellow (usually 0 - 1.5 mls required).
- (5) Add 5 ml of neutralised ethanol, stopper and shake vigorously for one minute.
- (6) Add 15 ml of mixed solvent, stopper and again shake vigorously for one minute.

- (7) Centrifuge for 5 minutes.
- (8) Pipette 10.0 ml of the clear supernatant ether layer into a 100 ml Erlenmeyer flask.
- (9) Add 10 ml neutralised methanol.
- (10) Titrate against 0.01M alcoholic KOH to the first sign of a greenish tinge in the yellow solution.

Calculation: Free fatty acid content m.Mols/litre = 1.35 (T - B)

where T = Sample titration figure

B = Blank titration figure

3.3.2.7 Fat globule size distribution

It was measured by Master Sizer/E in New Zealand Dairy Research Institute.

Principle: The Particle Sizers are all based on the principle of laser ensemble light scattering. They fall into the category of a non imaging optical system due to the fact that sizing is accomplished without forming an image of the particle onto a detector. The MasterSizer/E employs two forms of optical configuration to provide its unique specification. The first is the well known optical method, called "conventional Fourier optics" because it is employed by Malvern in all such instruments prior to the launch of MasterSizer/E. The second is a new optical configuration, called "reverse Fourier optics", used in order to allow the measurement size range to be extended down to 0.1 μ m.

Procedure: The details are available in the manual.

3.3.2.8 Viscosity

Measured by Ferranti-Shirley Viscometer System in the lab of Food Technology Department.

Principle: Ferranti-Shirley viscometer is a cone and plate viscometer. The fluid is held by its own surface tension between a cone of small angle that just touches a flat surface. The torque caused by the drag of the fluid on the cone is measured as one of the members is rotated while the other member remains stationary.

The special feature of the cone and plate viscometer is that the shear rate is uniform at all points in the fluid, provided that the angle of the cone is small. This makes the cone and plate viscometer of particular use for non-Newtonian fluids because the true rate of shear can be obtained comparatively easily. Other features of this type of viscometer are (1) end effects are negligible, (2) a small amount of fluid is needed (usually less than 2 ml), and (3) the thin layer of fluid in contact with temperature-controlled metal base plate enables measurements to be made at high rates of shear without the need to compensate for the heating effect of the high shear rate (Bylund, 1995; Dinsdale and Moore, 1962).

Procedure:

- (1) Set up the viscometer system following the manual.
- (2) Adjust the water bath temperature to 5°C and keep the water recycle through the viscometer cone and plate.
- (3) Measure the viscosity following the manual.

Calculation: Viscosity = Shear stress/ Shear rate

Where Shear stress = Scale reading $\times K_T$ (Pa)

Shear rate = $\Omega \times K_D$ (S^{-1})

Cone speed $\Omega = 100$ rpm

$K_T = 0.6646$

$K_D = 18.7$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Microbiological properties of concentrated milk evaporated by the Centritherm evaporator

The total solids of the samples taken from the trials with different evaporating temperatures are given in the Table 4.1. During the trials, the concentration of milk was steadily increased and determined using a refractometer. This method can only give an approximate reading which was close to the design value. The exact concentrations of milk were measured by weight-oven method after the all samples were taken.

Table 4.1 The total solids of samples from the trials with different evaporating temperature

Evaporating temperature	Sample	Total solids (%)
50°C	S ₅₀ 1	30.3
	S ₅₀ 2	36.4
	S ₅₀ 3	41.0
60°C	S ₆₀ 1	29.4
	S ₆₀ 2	36.1
	S ₆₀ 3	40.5
70°C	S ₇₀ 1	30.3
	S ₇₀ 2	37.1
	S ₇₀ 3	41.1

4.1.1 Changes in microbiological properties of concentrated milk during storage on farm for three days

In this part of the work, the content and growth of total bacteria, psychrotrophic bacteria, coliform bacteria, and thermophilic bacteria were examined.

4.1.1.1 Total bacteria content

Figures 4.1, 4.2, and 4.3 are total bacteria content (standard plate count--SPC) changes with storage time (at 5°C) for same concentration milks evaporated at different temperatures. It can be seen that at similar concentrations, the standard plate counts were reduced as the evaporating temperature was increased from 50°C to 70°C.

SPC changes with storage time (at 5°C) for different concentrations of milk evaporated at 50°C, 60°C and 70°C are shown in Figures 4.4, 4.5, and 4.6. It should be kept in mind that at the same evaporation conditions to obtain the higher total solid of milks require the longer evaporating time. When the evaporating temperature was 50°C, the standard plate count decreased gradually as the total solids increased. When the evaporating temperature were 60°C and 70°C, the standard plate counts were decreased significantly as the concentration increased to 30% TS, then only slightly further reduced as the concentration increased to 41% TS. The final standard plate count reduced as the evaporating temperature was increased. This indicated that when the evaporating temperature was beyond 60°C, it greatly reduced the standard plate count.

From Figures 4.4, 4.5, and 4.6, it also can be seen that when storage time (at 5°C) was increased, the standard plate count of raw milk and the samples of milk concentrated at 50°C increased, but that of samples of milk concentrated at 60°C and 70°C were nearly constant. This phenomenon can be explained by the results of the testing of psychrotrophic bacteria count¹.

¹ Refer to section 4.1.1.2.

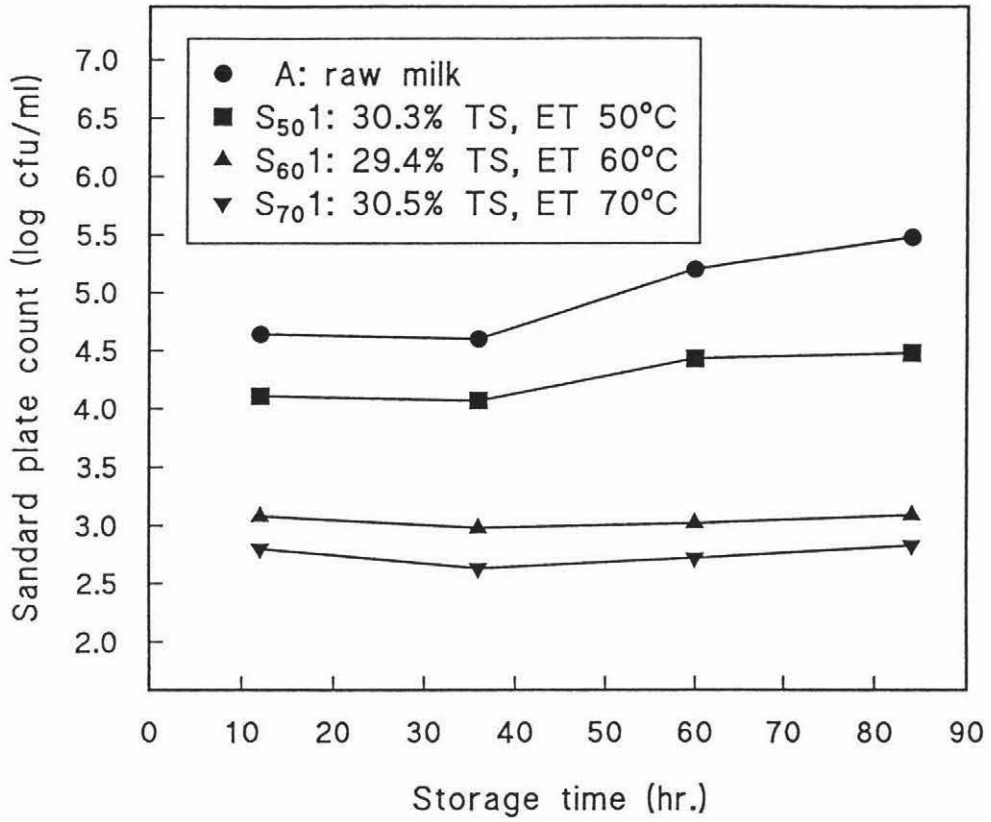


Fig. 4.1 Total bacteria change with time for raw milk and concentrated milk (~30%TS) evaporated at different temperatures when stored on farm at 5°C.

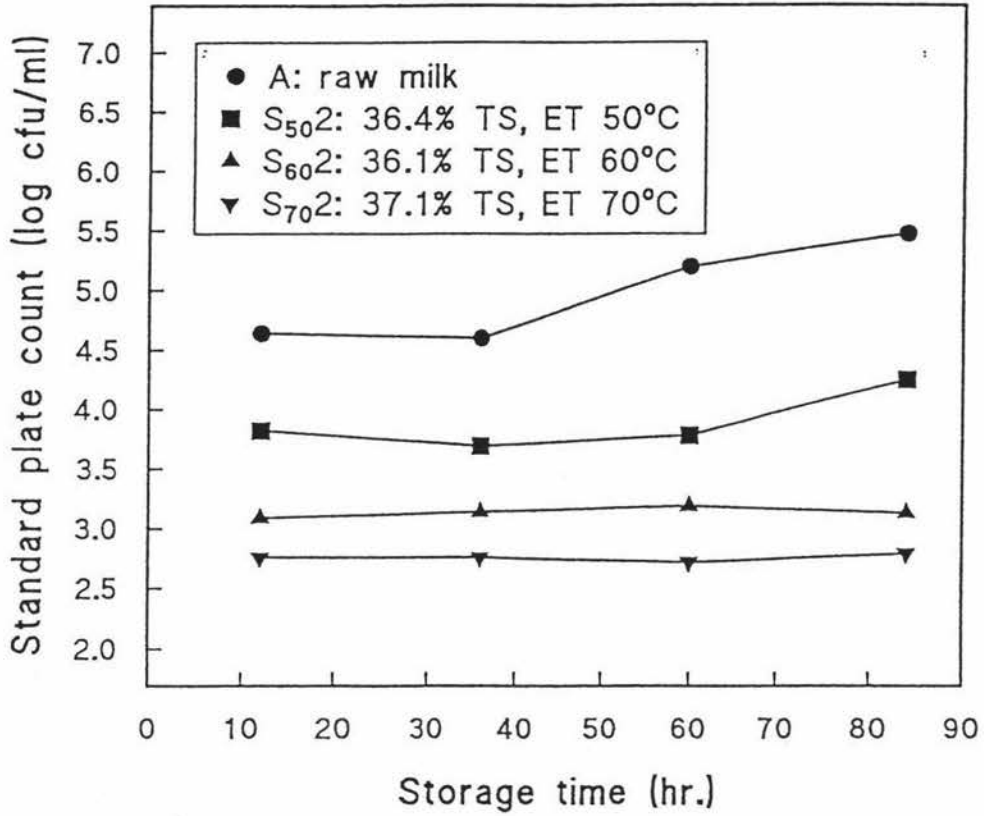


Fig. 4.2 Total bacteria change with time for raw milk and concentrated milk (~36%TS) evaporated at different temperatures when stored on farm at 5°C.

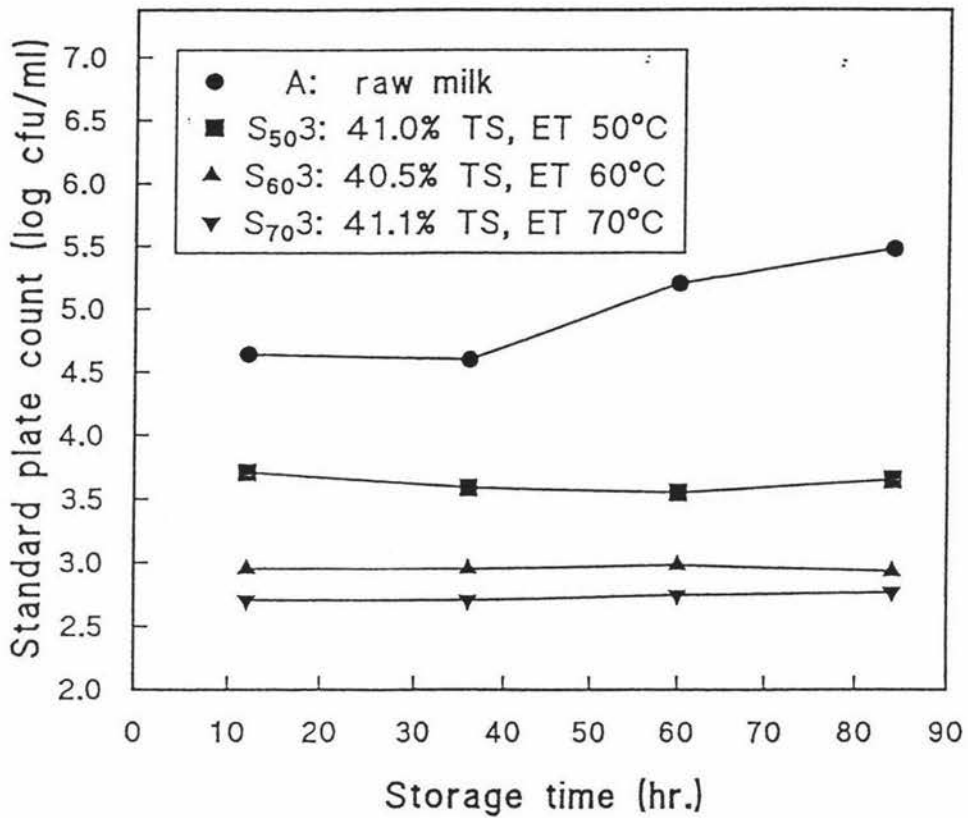


Fig. 4.3 Total bacteria change with time for raw milk and concentrated milk (~41%TS) evaporated at different temperatures when stored on farm at 5°C.

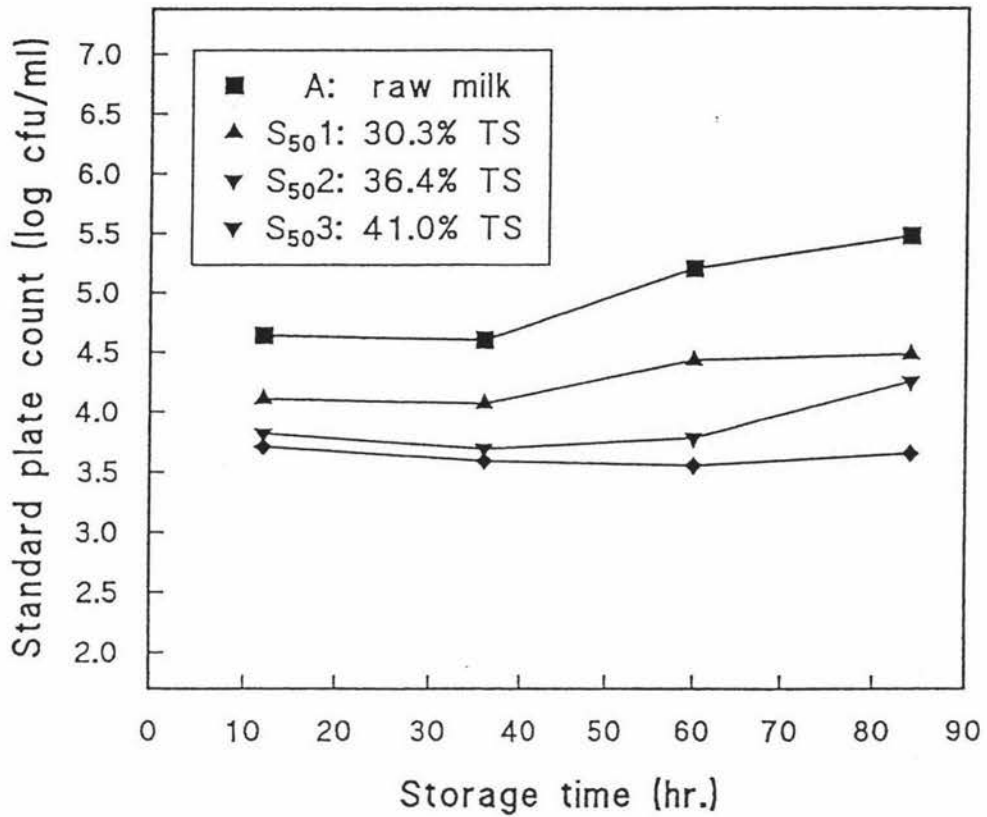


Fig. 4.4 Total bacteria change with time for raw milk and concentrated milk evaporated at 50°C when stored on farm at 5°C.

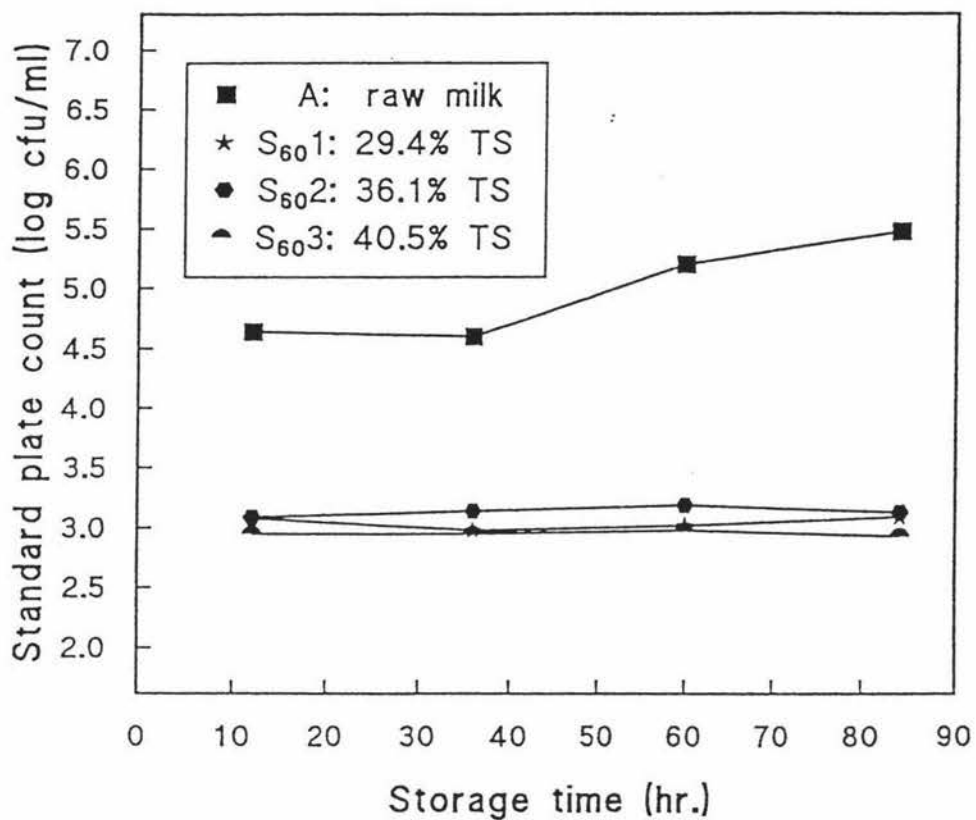


Fig. 4.5 Total bacteria change with time for raw milk and concentrated milk evaporated at 60°C when stored on farm at 5°C.

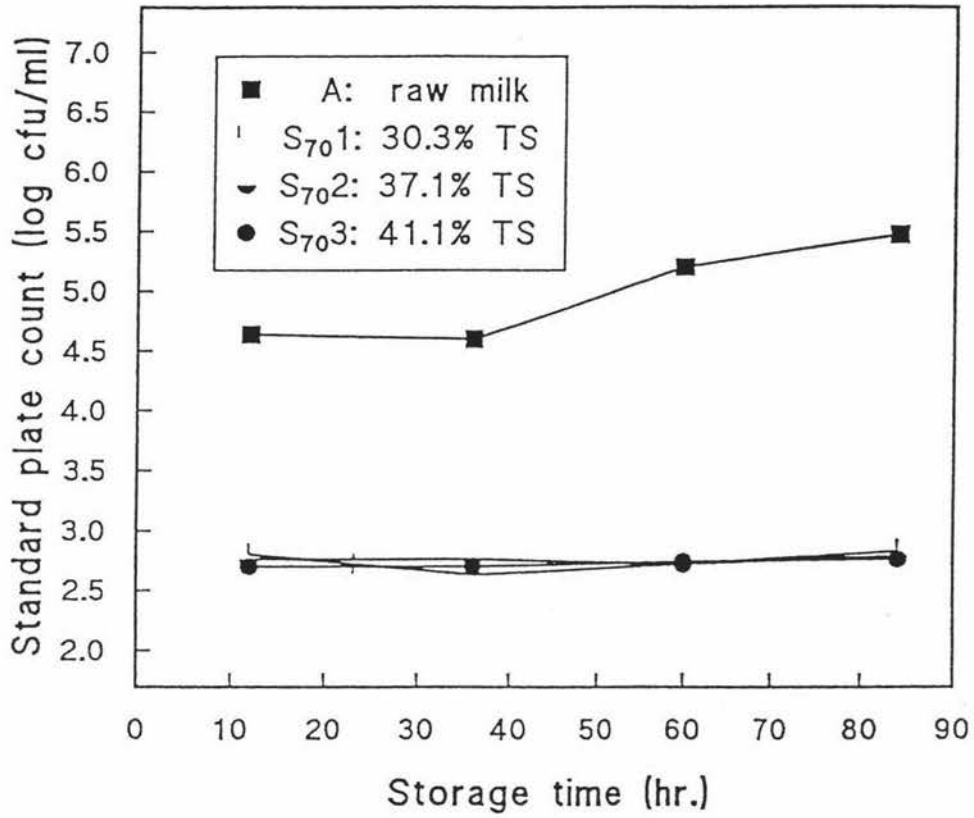


Fig. 4.6 Total bacteria change with time for raw milk and concentrated milk evaporated at 70°C when stored on farm at 5°C.

4.1.1.2 Psychrotrophic bacteria count

Figure 4.7 shows that there were many psychrotrophic bacteria in the raw milk and the samples of milk concentrated at 50°C. The numbers of psychrotrophic bacteria increased slightly during storage at 5°C and this reflected in the standard plate count.

There were no psychrotrophic bacteria detected in the samples of milk concentrated at 60°C and 70°C. Psychrotrophs are all common plant or soil saprophytes and contaminate milk mainly from improperly cleaned equipment (Cogan, 1980). The group contains heat-resistant bacteria, such as *Bacillus Spp.*, which can survive after pasteurization. Since no psychrotrophs were found in the concentrated milk samples evaporated at 60°C and 70°C, there were probably no heat-resistant psychrotrophs in the raw milk.

4.1.1.3 Coliform bacteria count

The results of examining coliform bacteria in the raw milk and concentrated milk stored at 5°C are presented in Table 4.2.

Table 4.2 The results of coliform bacteria count

Samples	Colonies (cfu/ml)	
	Storage time: 12 hours	Storage time: 36 hours
Raw milk	8,000	9,000
S ₅₀ 1	1,230	1,320
S ₅₀ 2	250	470
S ₅₀ 3	210	240
S ₆₀ 1	0	
S ₇₀ 1	0	

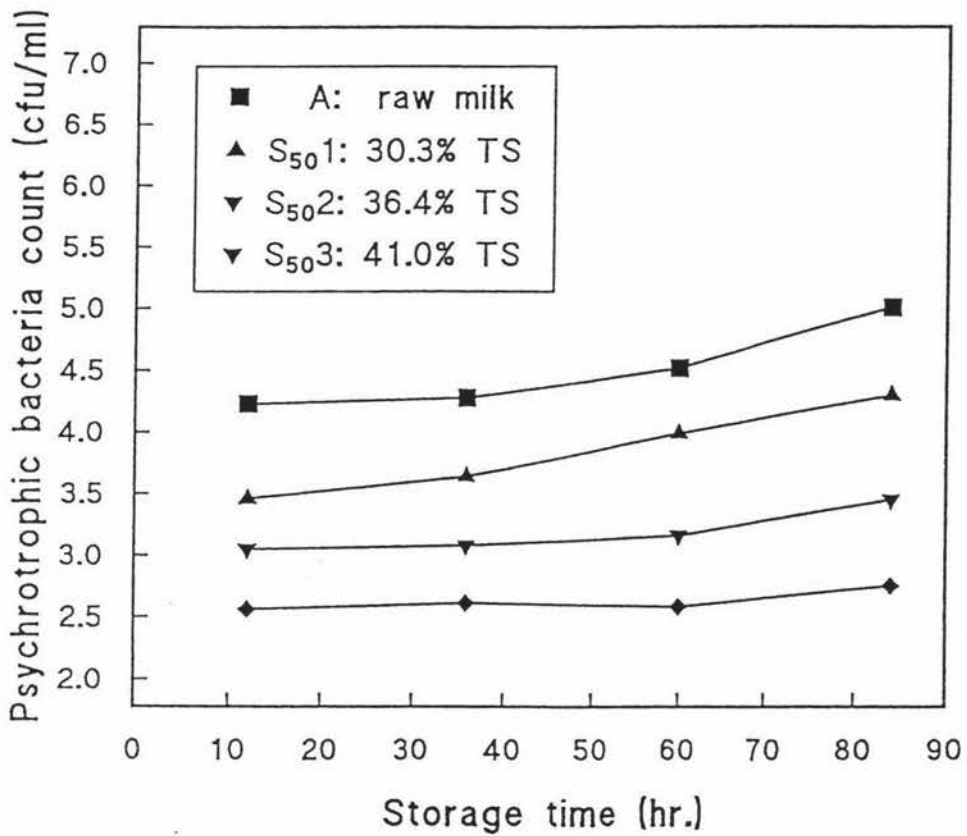


Fig. 4.7 Psychrotrophic bacteria change with time for raw milk and concentrated milk evaporated at 50°C when stored on farm at 5°C.

From above Table 4.2, it can be seen that there were about 8,000 cfu/ml coliform bacteria in raw milk sample. Numbers of these bacteria were gradually reduced when the milk was evaporated at 50°C and can be killed at 60°C and 70°C. The content of coliform bacteria increased with the storage time when the storage temperature was at 5°C. The results suggested that the concentrated milk evaporated at 60°C and 70°C would not contain coliform bacteria unless the milk was recontaminated after evaporation.

4.1.1.4 Thermophilic bacteria count

The results of thermophilic bacteria counts in the raw milk and concentrated milk are presented in Table 4.3.

Table 4.3 The results of thermophilic bacteria count

Samples	Colonies (cfu/ml)	Samples	Colonies (cfu/ml)
Raw milk	6	S ₆₀ 2	3
	4		2
S ₅₀ 1	5	S ₆₀ 3	3
	4		2
S ₅₀ 2	3	S ₇₀ 1	0
	0		0
S ₅₀ 3	0	S ₇₀ 2	4
	2		1
S ₆₀ 1	2	S ₇₀ 3	3
	2		1

From above Table 4.3, it can be seen that the raw milk had only few thermophilic bacteria. This part of experiment was designed to investigate the effect of the evaporating temperature on the growth of the thermophilic bacteria. However, due to the small number of the thermophilic bacteria in the raw milk, no conclusion can be

drawn from the results presented above except that evaporation failed to reduce their numbers.

4.1.2 Microbiological changes during the simulated transportation and storage at the dairy factory for two days

The results from microbiological properties of concentrated milk during storage on farm² and free fatty acids content³ suggested that the milks evaporated at 60°C and 70°C gave the better microbiological properties and lower free fatty acids content than that at 50°C. But the results from denatured whey proteins⁴ showed a small portion of whey proteins could be denatured at the evaporation temperature of 70°C. To minimise the denaturation of whey protein but maximise the destruction of bacteria, the optimum temperature should be 60°C. The results of apparent viscosity of concentrated milk⁵ suggested that the concentration can be raised to 40% - 50% TS without gelling occurring.

Based on these results, the evaporation temperature of 60°C and milk concentration of 40% - 50% TS were chosen in the experimental work to study the microbiological changes in the concentrated milk samples during transportation to the dairy factory and storage there for two days. At first, the samples of concentrated milk were stored at 5°C for three days to imitate the procedure of storage at the farm. Then the samples were put on the shaking water bath and controlled the temperature rose gradually from 5°C to 10°C during four hour period to imitate the process of transportation from a farm to a dairy factory. Because contamination may occur in the transportation, 5ml raw milk samples were mixed with 195ml concentrated milk samples individually in order to imitate the contamination in practice. Lastly, the samples were stored at 5°C and 10°C

² For more details, refer to section 4.1.1.

³ For more details, refer to section 4.2.3.

⁴ For more details, refer to section 4.2.4.

⁵ For more details, refer to section 4.2.1.

(assuming the worst storage condition) for two days to imitate the procedure of storage in the dairy factory. The microbiological changes, i.e. total bacteria, psychrotrophic bacteria, and coliform bacteria numbers during these processes were determined.

The total solids of the samples taken from the trial for the study of the transportation and storage at the factory are given in Table 4.4.

Table 4.4 The total solids of samples taken from the trial for the study of the transportation and storage at processing plant

Sample	S ₆₀ 4	S ₆₀ 5	S ₆₀ 6	S ₆₀ 7	S ₆₀ 8
Total Solids (%)	40.4	43.5	48.0	51.1	54.4

4.1.2.1 Total bacteria count

Figure 4.8 shows the total bacteria number changes in the raw milk and concentrated milk with and without contamination during the whole storage and transportation process, i.e. the storage on the farm at 5°C from 0 to 76 hours, transportation from 76 to 80 hours (temperature rise from 5 to 10°C) and storage at the dairy factory at 5°C from 80 to 150 hours.

From Figure 4.8, it can be seen that:

(1) The total bacteria count in raw milk was higher than that in the concentrated milk. This is because when the milk was evaporated at 60°C, some non-heat resistant bacteria were killed.

(2) The total bacteria count in the non-contaminated concentrated milks remained nearly constant when the concentrated milks were stored at 5°C on farm, and transported to the factory and stored at 5°C. This result indicated that psychrotrophs were killed in the concentrated milk, few bacteria could grow when the milk samples were stored at 5°C.

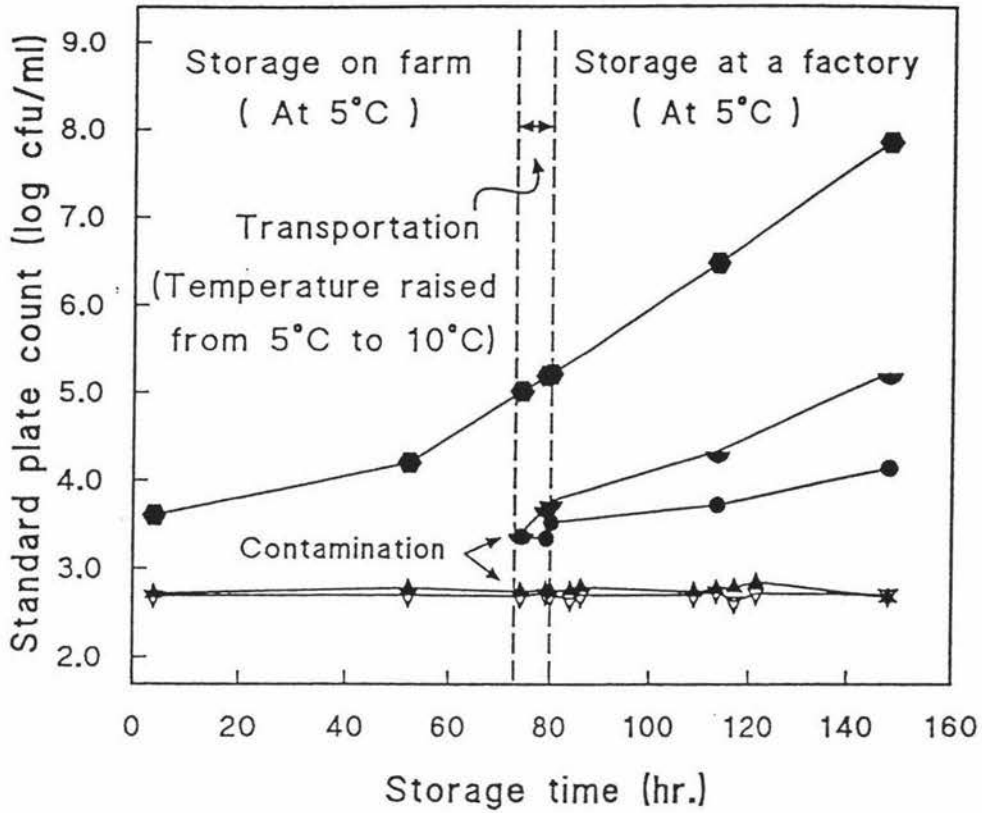


Fig. 4.8 Total bacteria change with time for raw milk and concentrated milk evaporated at 60°C during storage on farm, transportation and storage at a factory.

- Raw milk
- ▼ 40% TS milk contaminated by 5% raw milk
- 50% TS milk contaminated by 5% raw milk
- ▲ S₆₀₄ 40% TS milk
- ▽ S₆₀₇ 50% TS milk

(3) When the concentrated milk was contaminated by some raw milk, different results were found, i.e. during transportation and storage at the factory, the total bacteria counts in the contaminated concentrated milk were increased. This means that when the concentrated milk is contaminated by raw milk, psychrotrophs from raw milk can grow in the concentrated milks at low temperature.

(4) There was nearly the same total bacteria count in 40% and 50% TS concentrated milk after being contaminated by raw milk, but after transportation and storage at the factory, the total bacteria count in 40% TS concentrated milk was higher than that in 50% TS concentrated milk. This phenomenon suggested that the higher total solids could restrain bacteria from growth, so higher concentration should be better from the point view of microbiological quality of milk.

Lowering the temperature of milk decreases the rate of bacterial growth but, at the same time, it favours those species which metabolize best at lower temperatures. Thus storage of milk at 5°C not only decreases the overall bacterial growth rate but also changes the microbial balance in favour of those organisms, the psychrotrophs, which multiply at this temperature (Flückiger, *et al.*, 1980).

Figure 4.9 shows changes in the total bacteria numbers in the concentrated samples with storage time when the storage temperatures were 5°C and 10°C at a dairy factory. It can be seen that when the storage temperature was 10°C, the growth rate of total bacteria was quicker than that at 5°C in every sample. Storage temperature is one of the most important conditions for the microbiological quality of milk. It also can be seen that in higher total solids milk sample, rate of total bacteria growth is lower.

There is no published literature found about the effect of storage for two further days at the dairy factory of then on-farm pre-concentrated milk, but there were a lot of investigations in which the effect of cold storage on bulk collected raw milk for two further days at the dairy factory was studied. Atherton & Bradfield (1957) found that samples of good quality alternate day collected milk, taken from the tankers on arrival at a creamery, could be held at 3.3°C for up to three days without any serious

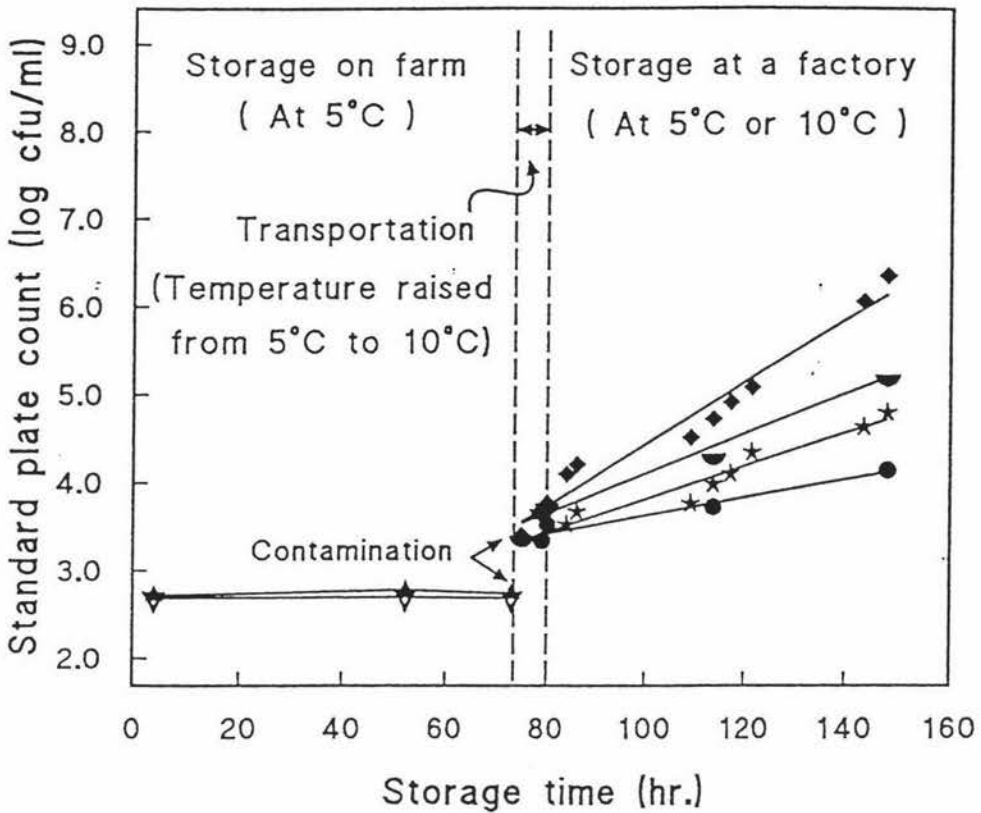


Fig. 4.9 Total bacteria change with time at different storage temperature of 5°C and 10°C for concentrated milk with contamination by adding 5% raw milk during the storage at a factory.

- ◆ 40% TS milk with contamination stored at 10°C
- ▼ 40% TS milk with contamination stored at 5°C
- ★ 50% TS milk with contamination stored at 10°C
- 50% TS milk with contamination stored at 5°C
- ▲ 40% TS milk
- ▽ 50% TS milk

deterioration in bacteriological quality. Similarly, Crawford (1967) observed that alternate day collected milk of good bacteriological quality could be held at 5°C at the dairy factory for one day with very little increase in the bacterial content, but on holding at this temperature for a second day, the bacterial numbers increased sharply. On the other hand, the results obtained by McLarty & Robb (1968) showed a marked increase in the incidence of high total psychrotrophic and coliform colony counts when samples of poorer quality milk taken from road tankers at the creameries, were held for 24 hours at 5°C. Bockelmann (1970) made daily examinations for up to three days of raw milk stored at 5°C in tanks at a dairy factory. It was concluded that milk containing 1×10^6 bacteria/ml, with less than 50 per cent of these being psychrotrophs, can be satisfactorily cold-stored at 5°C for two to three days before pasteurization. He also observed that in milk cold-stored (at 5°C) at the plant, 18 per cent of the total bacterial content on the first day and 65 per cent on the third day were psychrotrophs. However, he did not mention the examination of heat resistant enzymes in the milk after pasteurization, which is likely to cause problems in the further processing and the final products.

It was suggested that alternate-day milk collection followed by plant storage for one to two days at 4°C or collection every three days followed by storage at 4°C for one day are both satisfactory systems. Longer storage periods than these are inadmissible unless the milk is heat-treated on arrival at the factory (Flückiger, *et al.*, 1980).

From the results of this study, there is no doubt that the microbiological quality of concentrated milk is much better than that of raw milk. Therefore, concentrated milk can be stored at a dairy factory longer than raw milk when they are under the same transportation and storage conditions.

4.1.1.2 Coliform bacteria count

Table 4.5 shows the results of coliform bacteria count for raw milk and concentrated milk evaporated at 60°C. As shown previously that there were no coliform bacteria in the milks concentrated at 60°C. 5% raw milk was added into the concentrated milks

before transportation. The changes of coliform bacteria count were examined during the transportation and storage at different temperatures of 5°C and 10°C at the factory.

Table 4.5 The results of coliform bacteria count from the trial for the study of the transportation and storage at processing plant

Storage time (hr.)	Raw milk		40% TS milk contaminated by 5% raw milk		50% TS milk contaminated by 5% raw milk	
	Storage temp. 5°C	Storage temp. 10°C	Storage temp. 5°C	Storage temp. 10°C	Storage temp. 5°C	Storage temp. 10°C
	Colonies (cfu/ml)					
4	11					
52	116					
76	5,500		210		185	
80	6,800	6,800	245	245	177	177
104	35,000	81,000	880	1850	330	410
128	430,000	520,000	4800	6800	600	930

It can be seen from above Table 4.5 that (1) during the transportation, there was no obvious change in the coliform bacteria count. (2) During the storage at the factory for two days, the coliform bacteria count increased at both storage temperatures of 5°C and 10°C, but the growth rate of coliform bacteria at 10°C is quicker. This indicates some of the coliform bacteria are psychrotrophic bacteria which can still grow at 5°C, but more coliform bacteria can grow at 10°C. (3) After the storage at factory, for the 40% TS concentrated milk with contamination, the coliform bacteria count increased from 245 to 4,800 cfu/ml at 5°C, from 245 to 6,800 cfu/ml at 10°C; but for the 50% TS concentrated milk with contamination, the coliform bacteria count increased from 177

to 600 cfu/ml at 5°C, from 177 to 930 cfu/ml at 10°C. This also suggested the higher the total solids of the milk, the lower the growth rate of coliform bacteria.

4.2 Chemical and physical properties of concentrated milk evaporated in Centritherm evaporator

4.2.1 Apparent viscosity

The apparent viscosity of the concentrated milk may increase during the storage period, the phenomenon is called age-thickening. Snoeren, *et al.* (1984) reported that the age-thickening of skim-milk concentrate appears to be dependent on the degree of concentration, the storage temperature and variations in salt balance.

However the viscosity of skim-milk concentrate was found by Bloore and Boag (1981) to be influenced by five principal factors: temperature, total solids, age, protein content and preheat treatment. There is usually an inverse relationship between viscosity and temperature and a direct nonlinear relationship between the concentration of a solution and viscosity at constant temperature (Dinsdale and Moore, 1962).

Cooled raw milk and concentrated milk are non-Newtonian fluids. Their apparent viscosity is dependent on shear rate (Goff & Hill, 1993). In this study, the shear rate was fixed at the same rate during the measurement of viscosity.

The on-farm pre-concentrated milk was proposed to be stored on farm and at a dairy factory for five days at 5°C. The viscosity of concentrated milk should not be too high to be pumped before processing.

Figures 4.10, 4.11, and 4.12 show the changes of apparent viscosity for raw milk and concentrated milks with storage time at different concentrations and different evaporation temperatures. It can be seen: (1) the viscosity were obviously increased with the increase in concentration, (2) the viscosity of raw milk did not change with storage time, (3) the viscosities of 30% TS and 35% TS concentrated milk were increased slightly with storage time, but there was obvious increase in the viscosity of 41% TS

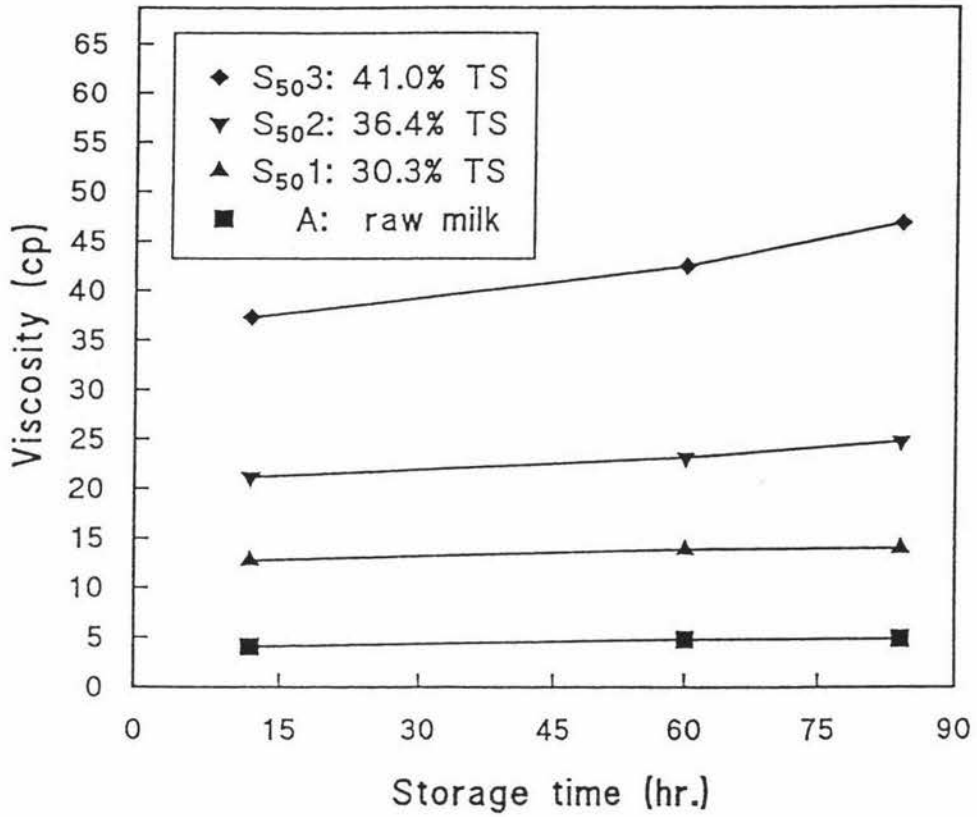


Fig. 4.10 Viscosity changes with time for raw milk and concentrated milk evaporated at 50°C when stored on farm at 5°C.

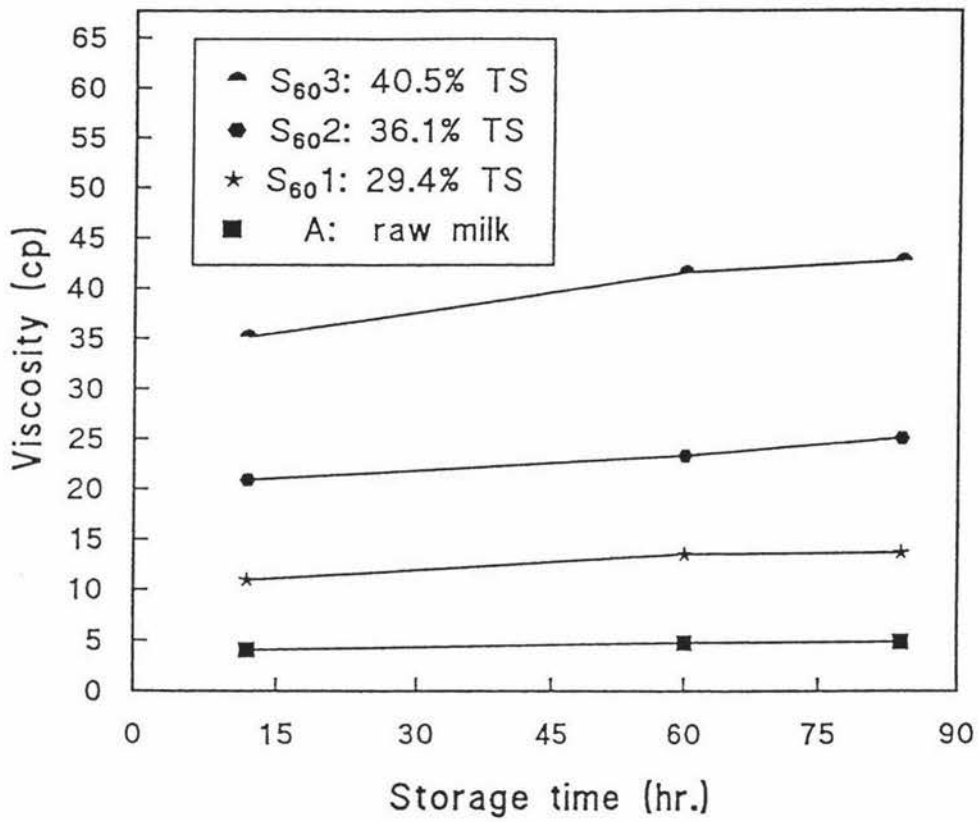


Fig. 4.11 Viscosity changes with time for raw milk and concentrated milk evaporated at 60°C when stored on farm at 5°C.

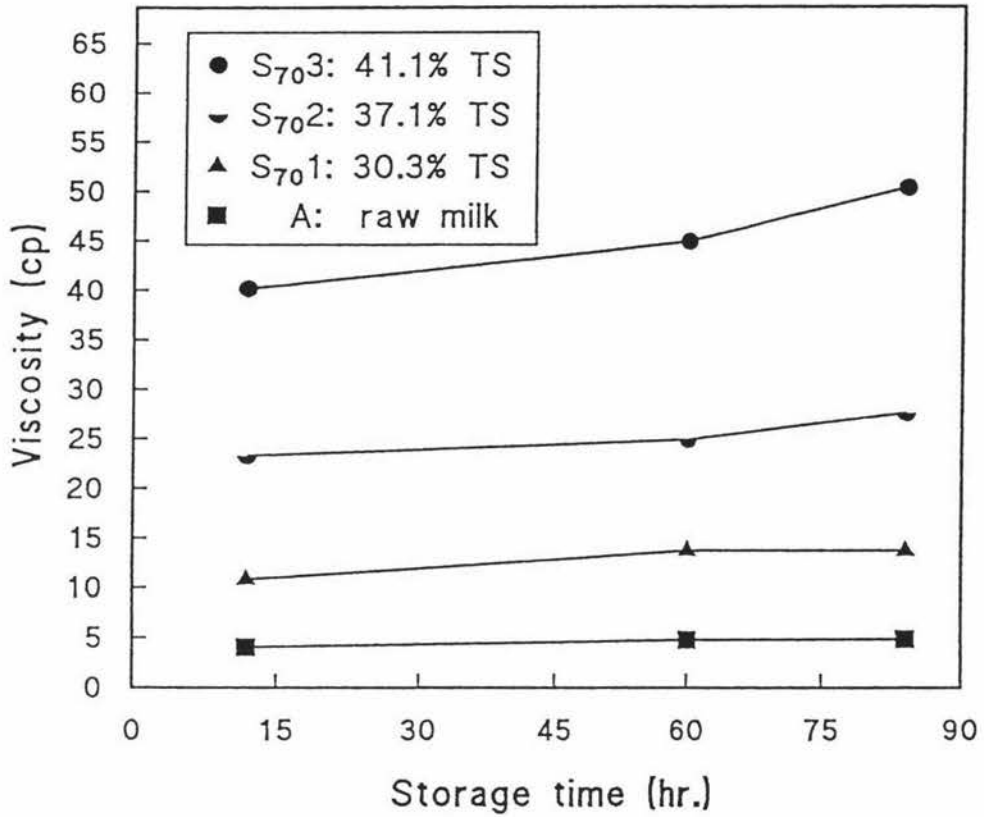


Fig. 4.12 Viscosity changes with time for raw milk and concentrated milk evaporated at 70°C when stored on farm at 5°C.

concentrated milk, and (4) the effect of evaporation temperature on viscosity of same concentration (below 41% TS) was not significant.

The changes in the viscosity of concentrated milk (40-50% TS) during five days storage at 5°C are shown in Figure 4.13. It can be seen that the viscosities of the concentrated milk with total solids in the range of 40-51% increased slightly with storage time; the rates of increase in viscosity are slightly increase from 40% TS to 51% TS. For 51% TS concentrated milk sample, the viscosity changed from 193 cp to 225 cp at 5°C from the second day to the fifth day, but no gel appeared. Thus, it appears that the fresh whole milk can be concentrated up to about 50% TS on farm and stored at 5°C for five days. When the total solids was increased to 54% TS and stored at 5°C for five days, the viscosity of the samples increased very sharply and the samples gelled on the fourth day.

It was noticed that lactose crystals were formed in some samples of concentrated milk of above 50% TS after stored at 5°C for three days. This indicated that the saturated concentration of lactose was reached in these samples. But no further experimental work was carried out on this aspect due to time limitation.

4.2.2 Fat globule size distribution

Figures 4.14, 4.15 and 4.16 show the fat globule size distribution in the concentrated milks evaporated at 50°C, 60°C and 70°C at different concentrations. It can be seen that as the concentration of milk increased, the volume (%) of the large size globules (around 4 μm) were reduced rapidly before the concentration reached about 30% TS, and then the volume (%) kept nearly constant. The decrease of the large size fat globules is mainly due to disruption of fat globules caused by the recycling of milk in the evaporator. One problem with the damaged fat globules is that the milk fat will be released from the fat globules membrane and, more important is that the released milk fat can be easily attacked by the lipolytic enzymes, which exist naturally in milk or may be produced by some bacteria. This problem will be discussed in the following section.

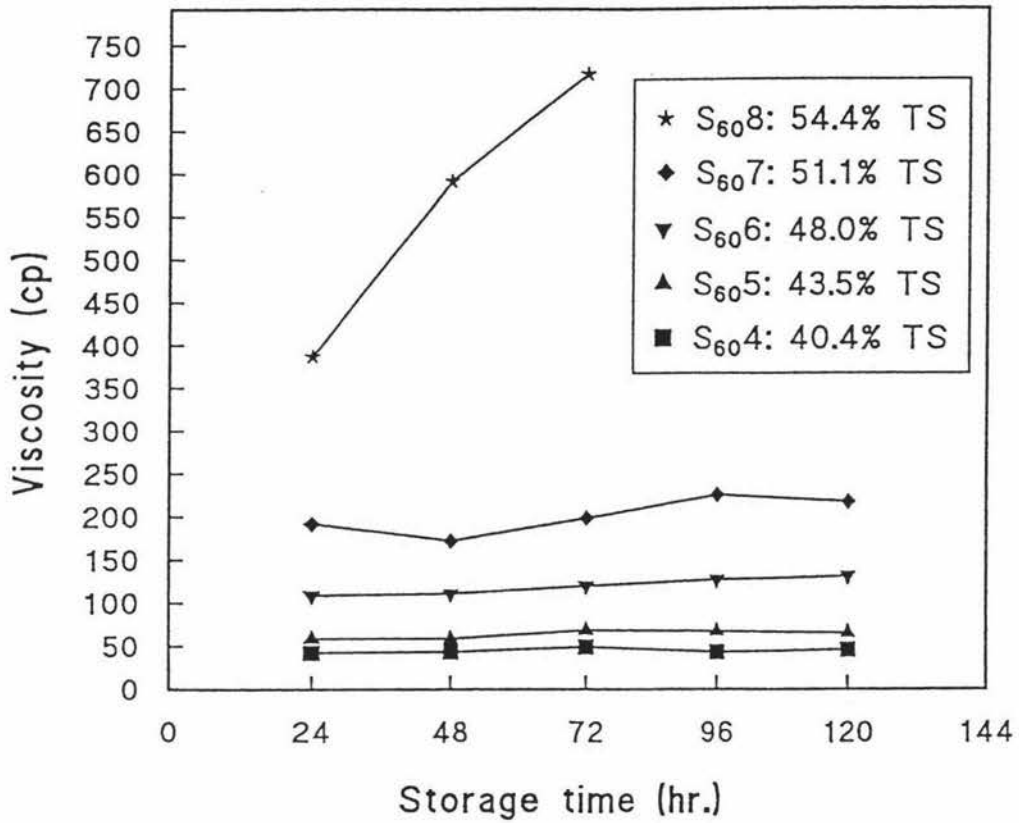


Fig. 4.13 The effect of concentration of milk on viscosity when milk evaporated at 60°C and stored on farm at 5°C.

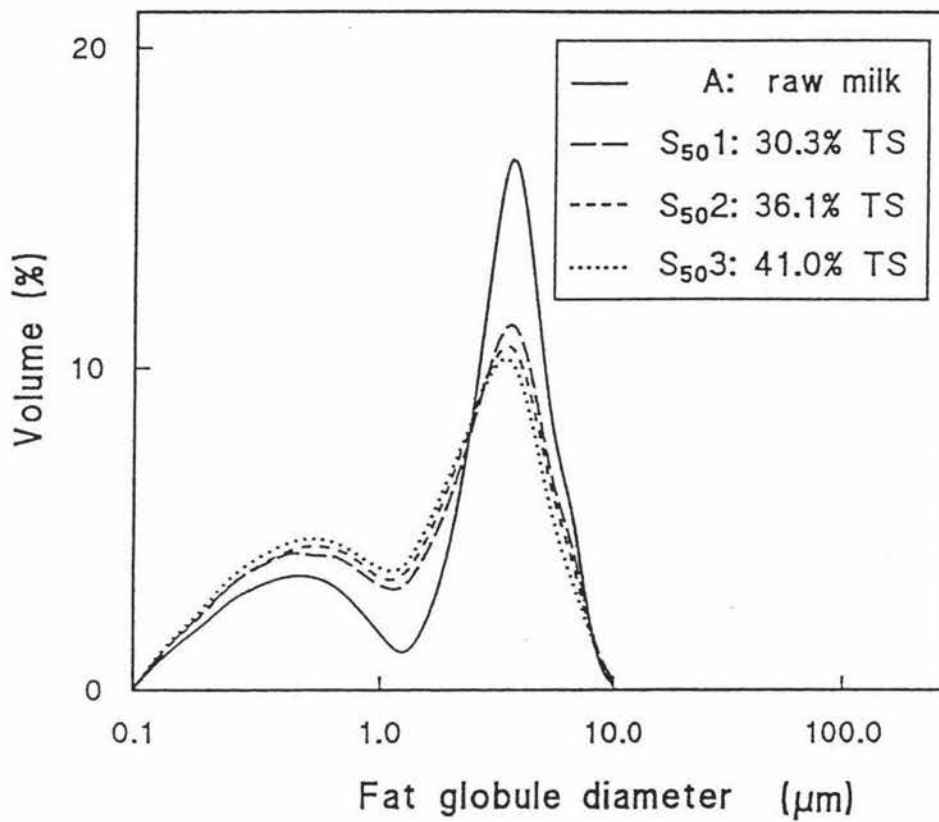


Fig. 4.14 Fat globule size distribution of raw milk and concentrated milk evaporated at 50°C.

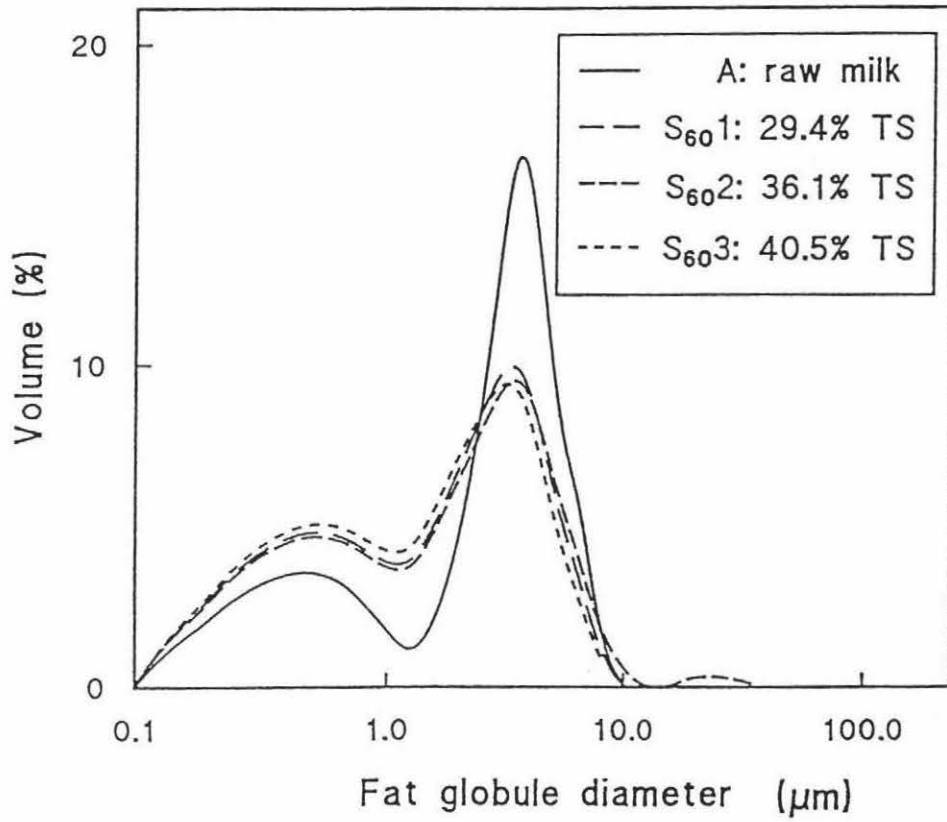


Fig. 4.15 Fat globule size distribution of raw milk and concentrated milk evaporated at 60°C.

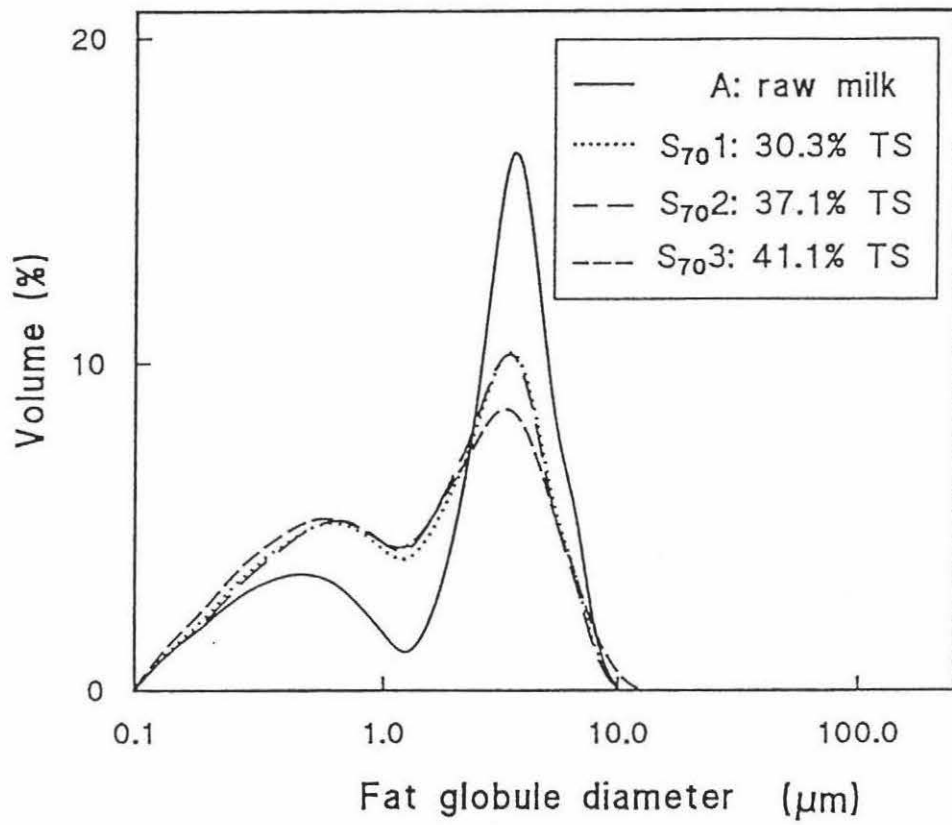


Fig. 4.16 Fat globule size distribution of raw milk and concentrated milk evaporated at 70°C.

4.2.3 Free fatty acids content

It can be seen from Figure 4.17 that the free fatty acids contents of concentrated milks were highest when evaporation temperature was 50°C, and that of the other concentrated milks and raw milk were nearly same.

Unlike milk itself where the intrinsic lipolytic enzymes are largely responsible for lipolysis, enzymes from at least three different sources may contribute to elevated free fatty acids levels in dairy products. Firstly, elevated free fatty acids levels in dairy products may be due to pre-manufacture lipolysis, arising from the uptake from milk of free fatty acids liberated by the native milk enzyme (Downey, 1975). Alternatively, the free fatty acids levels may develop in the products themselves due to post-manufacture lipolysis, caused either by microbial growth in the actual products themselves or the activity of heat-resistant, microbial enzymes produced in bulk cooled milk/cream by psychrotrophic bacteria (Cogan, 1977).

Milk fat exists in milk as minute globules surrounded by a thin protective layer, usually called a membrane. The main components of membrane are protein (almost half of the membrane material) and phospholipids (roughly one-third). The membrane functions to prevent the fat globules from flocculation and coalescence, and it protects the fat against enzyme action (Welsstra & Jenness, 1984). Agitation of whole milk can cause either aggregation (clumping, churning) or disruption (splitting, dispersion) of the milk-fat globules (Mulder & Walstra, 1974). The temperature of the milk, and hence the physical state of the milk-fat, and the severity of agitation determine which of aggregation and disruption effect predominates. However, in both cases the fat lose its natural protection and becomes free and thus susceptible to lipases attack (Deeth & Fitzgerald, 1978). Lipases act upon emulsified fat (triacylglycerides). During this hydrolysis, di-and mono-glycerides are produced together with free fatty acids. The build up of free fatty acids may produce a rancid or soapy flavour (Driessen, 1989).

When milk is evaporated, some agitation always accompanies this process. That means some of the fat in the concentrated milk may have lost its natural protection and allow

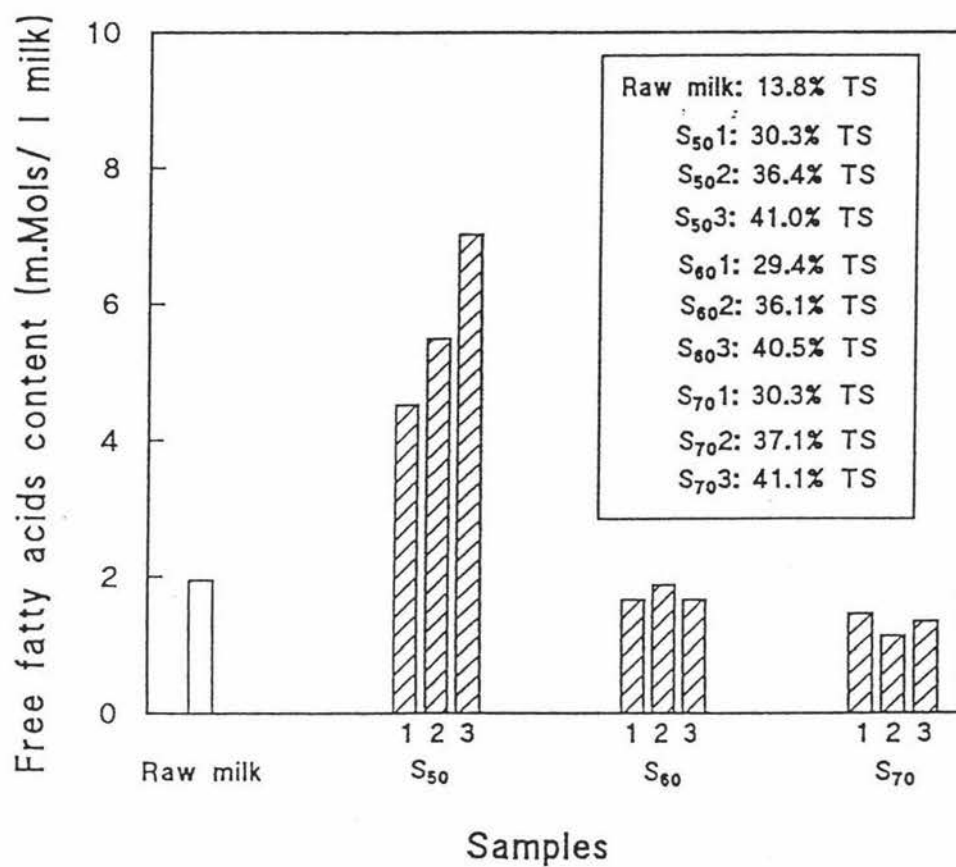


Fig. 4.17 The free fatty acids content in all samples after storage for 3 days at 5°C.

easy accessibility of lipases from milk or micro-organisms to the glyceride of the fat globule. From the results of psychrotrophic bacteria testing⁶, it has shown that there was a high population of the psychrotrophic bacteria in the raw milk and the samples of concentrated milk evaporated at 50°C and psychrotrophic bacteria still multiplied when the samples were stored at 5°C. It is well known that some psychrotrophic bacteria, such as *Pseudomonas sp.*, can produce lipases (Stepaniak & Sorhang, 1995; Fairbairn & Law, 1986; Griffiths, 1983; Law, 1979). So the damage to natural fat protection due to the fracture of the globule membrane during evaporation, and the increase of lipases from the high population of psychrotrophic bacteria result in the increase of free fatty acids content in the concentrated milk evaporated at 50°C.

4.2.4 Denatured whey protein

The results of denatured whey proteins are presented in Tables 4.6 and 4.7. There was no detectable denaturation of whey proteins in the samples (30-40% TS) concentrated at 60°C for 40 to 90 minutes. But when the evaporation temperature was 70°C, about 50% of the BSA was denatured as concentration was measured beyond 40% TS.

Table 4.6 Denaturation of whey proteins in concentrated milks prepared at evaporation temperature of 60°C

Concentration	30% TS	35% TS	40% TS
α -La	0	0	0
β -Lg	0	0	0
BSA	0	0	2%

⁶ Refer to Figure 4.7 in section 4.1.1.2.

Table 4.7 Denaturation of whey proteins in concentrated milks prepared at evaporation temperature of 70°C

Concentration	30% TS	35% TS	40% TS
α -La	0	0	0
β -Lg	0	0	0
BSA	44%	47%	51%

These results agree with the statement of Walstra and Jenness (1984). They pointed out that whey proteins started to noticeably denature above 60°C. The results reported by Larson and Roller (1955) also shown that the whey proteins were not sensitive to heat when the heating temperature was below 60°C.

4.3 Some properties of concentrated milk evaporated by falling film evaporator

4.3.1 Total bacteria count

Figure 4.18 shows the comparison in the survival of total bacteria for concentrated milks (about 30% TS) evaporated at 50°C, 60°C and 70°C in the Centritherm evaporator and the falling film evaporator.

From Figure 4.18, it can be seen that the survival of total bacteria came down obviously after the milk was evaporated at 50°C, 60°C and 70°C in both evaporators. But the survival of total bacteria decreased more sharply when the raw milk was evaporated in the falling film evaporator compared with the milk evaporated in the Centritherm evaporator. This means when the evaporation temperature and concentration are same, more bacteria can be destroyed in the falling film evaporator. This is because the milk contacts the heating surface longer in the falling film evaporator than in the Centritherm evaporator.

4.3.2 Fat globule size distribution

Comparing Figure 4.19 with Figures 4.15, 4.16 and 4.17, it can be seen that the fat globule size distribution in the concentrated milk evaporated by the falling film evaporator was similar to that in the concentrated milk evaporated by the Centritherm evaporator. This means that there is a similar effect of the operation on the fat globules using the two evaporators. It is likely that the main disruption of fat globules is not on the evaporating surface but in pumps feeding milk to, and removing concentrate from the evaporators.

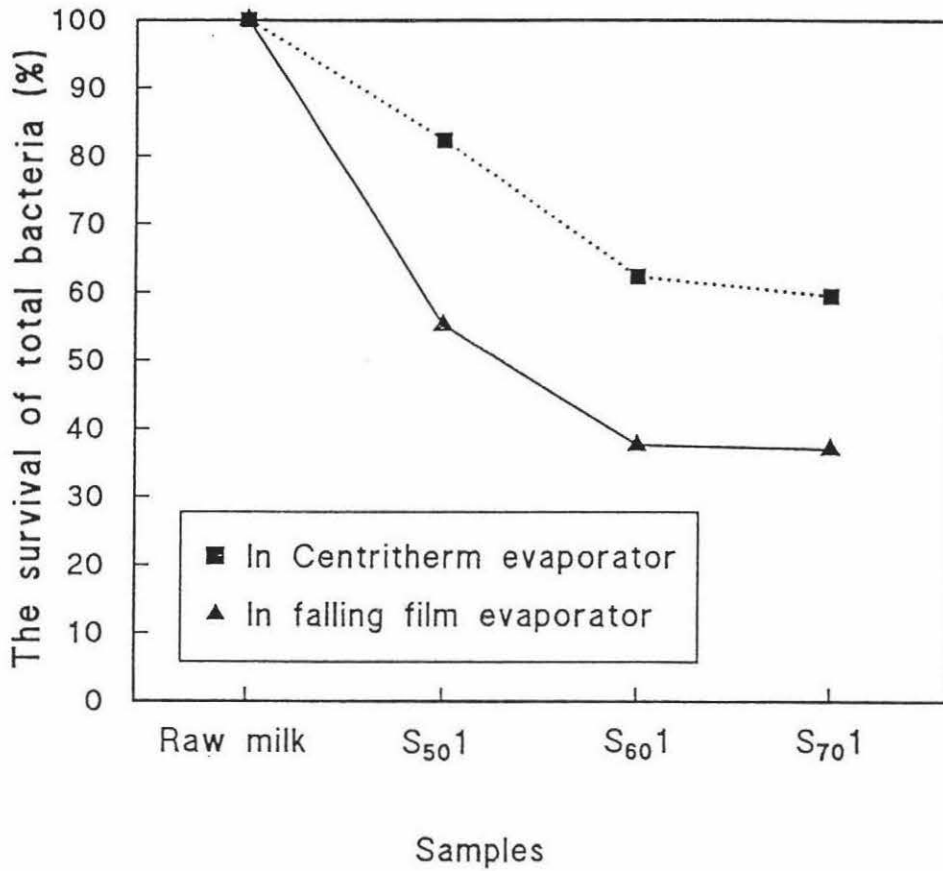


Fig. 4.18 The comparison in the survival of total bacteria for concentrated milk (~30%TS) evaporated in the Centritherm evaporator and a falling film evaporator.

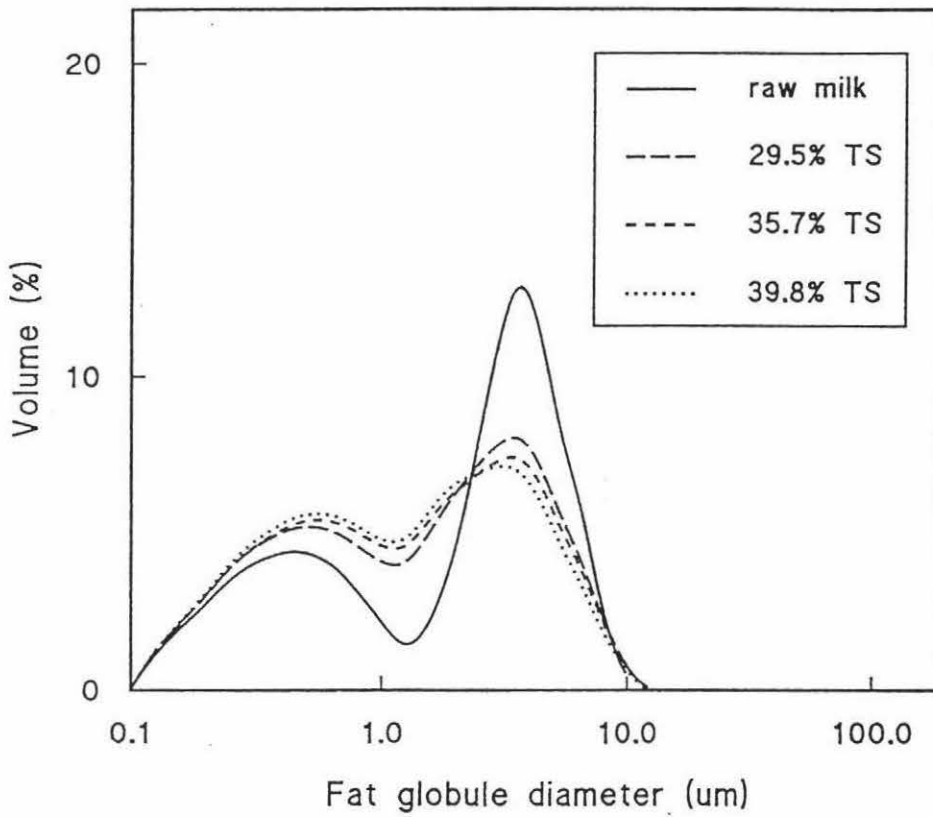


Fig. 4.19 Fat globule size distribution of raw milk and concentrated milk evaporated in a falling film evaporator at 60°C.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the experimental results, it was determined that suitable conditions for evaporation would be an evaporating temperature of 60°C, and the concentration of milk could be 40% to 50% TS. The storage time of three days at the farm and two days at the factory could be acceptable if the temperature of concentrated milk were kept at 5°C, and concentrated milk can be stored at a dairy factory longer than raw milk when they are under the same transportation and storage conditions.

The following conclusions could be draw from the experimental results:

(1) It was found that in general the numbers of total bacteria were reduced as both the evaporating temperature and the concentration of milk were increased. When the evaporating temperature was 50°C, the total bacteria count decreased gradually as the concentration of milk rose. When storage time on farm (at 5°C) was increased, the standard plate counts of raw milk and the samples of milk concentrated at 50°C increased. However, when the evaporating temperature was above 60°C, the numbers of total bacteria were decreased significantly with the increase of the concentration of milk and when storage time on farm (at 5°C) was increased, the standard plate counts of the samples of milk concentrated at 60°C and 70°C were nearly kept constant.

(2) There were many psychrotrophic bacteria in the raw milk and the samples of concentrated milk evaporated at 50°C. Whereas there were no psychrotrophic bacteria detected in the samples of concentrated milk evaporated at 60°C and 70°C, which indicated that there were probably no heat-resistant psychrotrophs in the raw milk used in this study.

(3) It was also found that the numbers of coliform bacteria were greatly reduced in the samples of milk evaporated at 50°C and were totally killed when evaporating temperature was over 60°C.

(4) It was found that the total bacteria count in the concentrated milk without contamination did not change during the simulated transportation and storage at the plant for two days, whereas the numbers of total bacteria in the concentrated milk with contamination by adding 5% raw milk were increased during the simulated transportation and storage. The increased bacteria during transportation and storage at the plant were mainly the psychrotrophic bacteria, but the increasing number of bacteria in the sample of 40% TS is higher than that in samples of 50% TS, which confirmed that the higher concentration of milk, the lower the rate of bacteria growth.

(5) The apparent viscosity of concentrated milk increased gradually as the concentration rose up to 50% TS but only slightly increased with the storage time. However, a great increase in the viscosity with concentration and storage time occurred in the samples of about 54% TS concentration.

(6) For the fat globule size distribution in the samples of concentrated milk, it was found that as the milk concentration increases, the volume (%) of the large size globules (around 4 μm) were reduced rapidly before the concentration reached about 30% TS, and then the distributions of fat globules size were nearly kept constant.

(7) The contents of free fatty acids in the raw milk and milks concentrated at 60°C and 70°C were nearly the same (less than 2 m.Mols/l milk), whereas the content of free fatty acids in the milk concentrated at 50°C was higher and increasing with storage time (4.5-7.0 m.Mols/l milk). This was ascribed to both the damage of natural fat protection due to the fracture of the globule membrane during evaporation, and the increase of lipases from the high population of psychrotrophic bacteria in the samples of milk concentrated at 50°C.

(8) The denaturation of whey protein was not found in the samples of milk (30-40% TS) concentrated at 60°C for 40 to 90 minutes. However when the evaporation temperature was at 70°C, there was about 50% the BSA was denatured as concentration of milk was increased over 40% TS.

(9) The results obtained from the falling film evaporator shown that the reduction of total bacteria count and the psychrotrophic bacteria count with the increasing of concentration and evaporating temperature was quicker than that in Centritherm evaporator. The fat globules size distribution is nearly the same in both evaporators under the similar experimental conditions.

5.2 Recommendations

To make the pre-concentrated milk being accepted by the dairy factory for further processing, the following aspects of research work may need to be investigated.

1. The use of pre-concentrated milk in further milk processing should be investigated. One method could be to mix proportion of pre-concentrated milk with the fresh raw milk to produce the products in order that the present specification of the products could be satisfied.
2. Lactose crystallization was noticed in some samples of concentrated milk of above 50% TS after stored at 5°C for three days. Due to time limitation, no experimental work was conducted on this aspect. The lactose content in the raw milk could be tested, then the saturated concentration of lactose in the samples would be calculated, which may limit the concentration of milk to be done at on-farm evaporation system in certain seasons.
3. As the reduction of bacteria numbers in the falling film evaporator was greater than that in the Centritherm evaporator, it can be deduced that the denaturation of whey protein would also be more severe in the falling film evaporator than in the Centritherm evaporator. Further experimental work could

be carried out to determine the difference of denaturation of whey proteins in each evaporator.

4. Psychrotrophs in milk may produce heat-resistant enzymes, which can break down milk fat and proteins. The lipolysis of milk fat has been explored by testing the change in free fatty acids content during the work. The proteolysis in milk during on-farm concentration should be investigated.

APPENDICES**Appendix I**

An example of the compositions of raw milk used in the study

Total Solids	13.8%
Fat	4.6%
Total proteins	3.3%
Casein	2.5%
Whey protein and non protein-nitrogen	0.8%

Appendix II

The method of measuring total solids in milks

1. Method: Gravimetric method (MFA, 1979)
2. Principle: A known weight of sample is dried under given conditions to constant weight. The weight after drying constitutes the weight of total solids.
3. Procedure:
 - (1) Condition the dishes with lids open in the oven for 30 minutes and allow to cool to room temperature with lids on in the desiccator and weigh (W_1).
 - (2) Pipette about 3 ml of well mixed sample at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ into a dish, replace the lid and weigh (W_2)
 - (3) Remove the lid and place the uncovered dish on the boiling water bath for 30 minutes.
 - (4) Transfer the dish and its lid to the drying oven, insulating the base of the dish from the shelf. Dry for 2 hours and allow to cool to room temperature in the desiccator and weigh (note weight).
 - (5) Return to the oven and dry for further 1 hour allow to cool as before and reweigh.
 - (6) Repeat step 5 until successive weighing differ by not more than 0.5mg (W_3).
4. Calculation:

$$\text{Total solids \% (m/m)} = (W_3 - W_1) \times 100 / (W_2 - W_1)$$

Appendix III

The method of measuring total protein content in milks

1. Method: Kjeldahl method (MFA, 1979; Case, *et al.*, 1985)

2. Principle: Weighed samples are catalytically digested with sulphuric acid, converting the organic nitrogen into ammoniacal nitrogen. The ammonia is released by the addition of sodium hydroxide, distilled and absorbed in boric acid, and then titrated. The percentage nitrogen content is multiplied by the factor 6.38 to convert to percent protein.

3. Procedure: Do one blank determination without sample, for each new batch of reagent.
 - (1) Weigh 5 ml sample in a weighing funnel on the balance to 4 decimal places.
Digestion:
 - (2) Transfer quantitatively to the Kjeldahl flask, rinsing funnel with a little water.
 - (3) Add 10.0g potassium sulphate, 0.20g copper sulphate and anti-bumping material.
 - (4) Add 20 ml sulphuric acid and swirl gently to mix contents.
 - (5) Heat the flask gently in the digestion apparatus, rotating the flask in the stand at frequent intervals for the first fifteen minutes until frothing has stopped.
 - (6) Continue heating the flask more vigorously until the liquid becomes clear.
 - (7) Continue boiling the solution for 90 minutes.
 - (8) Allow the flask to cool in the stand to room temperature.Distillation:
 - (9) Add carefully about 100 ml of warm water to the flask.

- (10) Fit the flask into the distillation apparatus.
- (11) Add 25 ml boric acid solution to the beaker, from a measuring cylinder.
- (12) Add 4-5 drops of mixed indicator to the beaker.
- (13) Put the beaker under the Liebig condenser so that the outlet tip is just below the solutions surface.
- (14) Distil the sample following the distilling unit manual.
- (15) Titrate the distillate with 0.1M hydrochloric acid; the titration is complete when the indicator turns from green to grey/blue.

4. Calculation:

The percentage of protein can be calculated as follows

$$\% \text{ Total Protein} = [(T - B) \times M \times 1.40 \times 6.38] / W$$

Where T = Titre of hydrochloric acid

B = Titre of the blank

W = Weight of sample

M = Molarity of the hydrochloric acid

Appendix IV

The method of measuring casein content in milks

1. Method: Kjeldahl method (MFA, 1979)
2. Principle: The total nitrogen content of the sample is determined. The casein is then precipitated with acetic acid--acetate buffer and filtered off. The nitrogen content of the filtrate is determined. The casein content is calculated from the two nitrogen determinations made according to Kjeldahl method in Appendix III.
3. Procedure: (1) Determine the Total nitrogen (TN) content of the sample according to the procedure in Appendix III.

Precipitation:

- (2) Pipette 10 ml well mixed sample at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ into a 100 ml volumetric flask.
 - (3) From a measuring cylinder add 75ml water at 40°C and add by pipette 1.0 ml acetic acid solution.
 - (4) Gently mix the contents of the flask and stand 10 minutes.
 - (5) Add from a pipette 1.0 ml sodium acetate solution and mix again.
 - (6) Allow the contents of the flask to cool to 20°C before diluting to volume with water. Mix by gentle inversion.
 - (7) When the precipitate of casein and fat has settled, filter through a dry pleated filter paper into a dry flask.
 - (8) Determine the nitrogen content of the clear filtrate (non casein nitrogen, NCN) using 50 ml according to the Kjeldahl method.
4. Calculation:
 - (1) Calculate % TN to three significant figures i.e. $\text{Total Protein}/6.38$

(2) Calculate % NCN to three significant figures and multiply % NCN by 0.994.

(3) Casein content (%) = $6.38 \times (\text{TN} - \text{NCN})$

Note:

The factor 0.994 corrects the NCN determination for the volume of precipitate. No error of significance will arise if this factor is used for all wholemilks. However, if the method is being applied to skimmilk the correct factor is 0.998.

Appendix V

Reagents and buffer solutions of PAGE

1. Acrylamide 30%: stock solution made up from 30 g Bis/acrylamide mixture 1:37.5 to 100 ml with purified water.
2. Tris [Tris(hydroxymethyl)aminomethane]
3. 6M HCL
4. Glycerol
5. 0.1% (w/v) bromophenol blue
6. Glycine
7. Ammonium persulphate (100 mg to 1.0 ml with distilled water for stock solution. Make up fresh each day.)
8. Temed (Tetramethylethylenediamine)
9. 1MHCL
10. Resolving gel buffer
To a 200ml beaker add 72.6 g Tris base. Add about 180ml distilled water. pH to 8.8 with 6M HCL. Bring to 200ml volume. Store at 4°C.
11. Stacking gel buffer
To a 100ml beaker add 6.0 g Tris base. Add about 60ml distilled water. pH to 6.8 with 1M HCL. Bring to 100ml volume. Store at 4°C.
12. Sample buffer
To a 500ml beaker add 100ml stacking gel buffer, 300ml distilled water, 10ml 0.10% bromophenol blue and 40ml glycerol. Check pH. Should be about 6.8. Store at 4°C.
13. Electrode buffer
To a 500ml beaker add 7.5 g Tris base, 36.0 g glycine. Add about 400ml distilled water. pH to 8.3. Bring to 500ml volume. Store at 4°C.

Appendix VI

The method of measuring fat content in milks

1. Method: Roess-Gottlieb method (MFA, 1980; Case, *et al.*, 1985)
2. Principle: Fat is extracted from an ammoniacal alcoholic solution of the sample with diethyl ether and petroleum ether, the solvents evaporated, and the residue is weighed and calculated as fat content.
3. Procedure: At the same time as the determination, carry out a blank determination on the reagents using the method described below, but omitting the sample. If the blank exceeds 1 mg the reagents should be checked.
 - (1) Heat the flasks containing 3 glass beads in the oven for 30 minutes.
 - (2) Cover the flasks and allow them to cool on a bench next to the balance for 1 hour.
 - (3) Weigh the flasks (W_1). Handle the flasks at all times with tongs.
 - (4) Accurately weigh about 10 ml of the sample into a Mojonnier flask (W_2).
 - (5) Add 2 ml ammonia solution and mix.
 - (6) Add 2 drops of phenolphthalein solution.
 - (7) Add 10 ml ethanol and mix.
 - (8) Add 25 ml diethyl ether, stopper the flask and shake with inversion for 1 minute.
 - (9) Remove the stopper carefully and add 25 ml petroleum ether using the first millilitres to rinse the stopper and inside of the neck of the flask allowing the rinsing to run into the flask. Stopper the flask and shake with inversion for 1 minute.
 - (10) Centrifuge the flask for 5 minutes.

- (11) Remove the stopper, rinsing it and the neck of the flask with a little petroleum ether as before. Carefully pour the ether layer into the preweighed flask from step 3.
- (12) Carefully add sufficient water to raise the top of the aqueous layer to the narrow part of the extraction flask.
- (13) Repeat the extraction (steps 9-12 inclusive) twice.
- (14) Carefully evaporate the ether layer on a water bath in an adequately ventilated cupboard. If the flask is of small capacity some of the ethers may be removed after each extraction.
- (15) When evaporation is completed, wipe the outside of the flask with a clean dry towel. Blow clean dry air into the flask to remove any last remaining fumes.
- (16) Heat the flask in the oven for 1 hour.
- (17) Cover and cool the flask as in step 2 for 1 hour. Weigh the flask (W_3).

4. Calculation:

$$\% \text{ fat in the sample} = (W_3 - W_1 - W_b) \times 100/W_2$$

Where W_1 = weight of empty flask

W_2 = weight of sample

W_3 = weight of (flask + fat)

W_b = weight gain (or loss) of reagent/humidity blank

Appendix VII

Reagents for measuring free fatty acids content

1. Bromophenol blue indicator, 0.5% aqueous solution.
2. Sulphuric acid, 0.5M
3. Mixed solvent, two parts of diethyl ether to one part petroleum spirit.
4. Alpha-naphtholphthalein indicator, 1% solution in ethanol.
5. Neutralised ethanol.
6. Neutralised methanol.

To neutralise reagent 5 or 6, add 0.5 ml of reagent 4 to 100 ml of the alcohol, neutralise with alcoholic potassium hydroxide to the first sign of greenish tinge in the yellow solution.

7. Alcoholic potassium hydroxide, 0.01M in ethanol. Standardise frequently against benzoic acid dissolved in the mixed solvent.

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