The Effect of Milk Fat Globule Membrane Damage in the Absence of Air on Fouling in Heat Exchangers

A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Master of Technology in Food Technology of Massey University

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乳脂球膜的破损在无空气介入时对换热器中牛乳结垢的影响

该论文是新西兰梅西大学食品技术专业
硕士学位的必要组成部分

方砾

一九九八年二月

To my dear wife and daughter

Thanks for their understanding and moral support!

Abstract

The fouling by whole milk of processing plant surfaces, especially in heat exchangers, is a serious problem, but is incompletely understood despite extensive past investigations. While milk fat has generally been thought to play a minor role in fouling, the results of some previous work suggest that this is not always the case. The state and form of the fat, as well as processing conditions, may have effects on milk fouling behaviour.

Careless mechanical handling of whole milk is known to cause fat damage. The present study set out to investigate the effect on fouling of damage to the milk fat globule membrane (MFGM) by mechanical stresses in the absence of air.

Pasteurised non-homogenised whole milk was deliberately stressed to differing degrees by passing it through a cavitating pump a variable number of times. The extent of damage was measured using four different techniques: a free fat (FF) test (a modified extraction method), a lipolysable free fat (LFF) test (free fatty acid determination after incubation of the sample with pig pancreatic lipase, a technique developed during this work), particle size distribution (PSD) measurement by laser light scattering, and confocal laser scanning microscopy (CLSM).

The fouling behaviour of both damaged and undamaged milk was investigated by heating the milk from about 4°C to about 94°C in a custom built double pipe heat exchanger, which could be disassembled easily to access the fouling layer. Milk flowed in the annulus, with a Reynolds number range of about 220-310. The fouling rate was measured and expressed as the rate of increase of the overall resistance to heat transfer, normalized using the overall heat transfer coefficient determined at the start of a run.

The fouling rate exhibited by damaged milk (normalized by the rate for undamaged milk, to account for batch-to-batch variation) was found to increase significantly with the extent of cavitation treatment. There was also a clear positive relationship between both the FF and LFF contents of milk and the extent of cavitation treatment, suggesting strongly that the observed increases in fouling rate were the result of increased MFGM damage.

PSD measurement and CLSM both showed that cavitation caused the appearance in the milk of some large, irregularly shaped fat globules, presumably the result of coalescence. The FF results, and observation by CLSM, indicated that only a small proportion (< 6°C) of the total milk fat had to be measurably damaged to cause extensive fouling.

The fat contents of the fouling layers were found to be very high (>45% on a dry weight basis). Although some of the experimental conditions, especially the low Reynolds numbers, may have contributed to this result, other fouling investigations made in New Zealand have produced similar results.

It is hypothesised that large globules formed by the coalescence of native globules whose membrane have been damaged could migrate more easily to the stainless steel heating surface. There, they could act as anchor points for the build-up of a fouling layer with a continuous protein phase. This hypothesis is supported by CLS micrographs of the fouling layer. Further investigation is warranted.

Recommendations are made for improving the methods used to measure MFGM damage, fouling and fouling rate, and the structure of the fouling layer.

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R_F: heat transfer resistance of fouling deposit (m^2KW^{-1})
U: heat transfer coefficient after fouling formation ($Wm^{-2}K^{-1}$)
U_o: heat transfer coefficient prior to fouling formation ($Wm^{-2}K^{-1}$)
d_{vs}: volume surface average diameter of fat globules (μm)
$N_i/\Delta d$: number frequency ($ml^{-1}\mu m^{-1}$)
d_o: outlet diameter of inside tube (mm)
d_i: inlet diameter of inside tube (mm)
D_o: outlet diameter of outside tube (mm)
D_i: inlet diameter of outside tube (mm)
Q': flow rate of milk ($L\ hr^{-1}$)
t_m: milk outlet temperature (°C)
T_m: milk inlet temperature (°C)
t_w: hot water outlet temperature (°C)
T_w: hot water inlet temperature (°C)
ρ_{milk}: milk density (Kgm^{-3})
μ_{milk}: milk viscosity (Pa s)
c_p: heat capacity ($KJKg^{-1}K^{-1}$)
S': area of milk cross section (m^2)
d_e: equal diameter of milk cross section (mm)
V': milk velocity ($m\ s^{-1}$)
Re: Reynolds number
ΔT_m: logarithmic temperature difference (°C)
Q_1 (Q_m): energy needed to heat up milk (W)
Q_2 (Q_w): energy given by the hot water (W)
A: heat exchange area (m^2)
Q'_{water}: flow rate of hot water ($L\ hr^{-1}$)
ρ_{water}: water density (Kgm^{-3})
R_FU_o: fouling rate (min^{-1})

Chapter *1* Introduction

The dairy industry is one of the main industries in New Zealand, with a turnover of 6.1 billion NZ dollars in 1996 (New Zealand Dairy Board, 1998). The amount of milk processed was nearly 9.3 billion litres, and whole milk powder production was about 298 thousand tons, in the year 1995/1996. Therefore, every 1% of needless loss costs the dairy industry millions of dollars.

The work reported here sought to establish quantitative causal relationships between some current milk handling practices causing structural damage to milk, and the operation of milk powder processes.

Milk can be damaged during handling, in particular by mechanical stress, air inclusion, temperature cycling and microbial enzymes. The effects of all these reverberate throughout the milk process.

Milk fouling (i.e. fouling of process plant by milk) is a very important phenomenon and is very common during milk handling especially in heat exchangers. A "fouling factor" is allowed for in designing heat exchangers (Palen, 1986). Excess surface is required to ensure sufficient heat transfer where there is fouling formation; in other words, the heat exchanger has to be oversized. Nonetheless, fouling is still troublesome because it increases production costs by reducing plant availability, increasing energy losses and

necessitating regular cleaning. Also, unavoidable product losses owing to milk solids deposition on plant surfaces reduce the output of milk powder.

Although milk fouling has been studied extensively, it is still an unsolved problem. The cost related to milk fouling is still huge. Also, previous work has mostly considered proteins and minerals; fat has previously been thought to play a minor role in fouling owing to the small amounts found in deposits (about 4-8% as reported). Actually, nobody seems to have studied the role of fat in fouling deposit formation.

The present work was to be an exploratory study targeting the effect of fat globule membrane damage by mechanical forces on one aspect of plant operation: fouling. Three areas of investigation were to be tackled:

1. Methods of damaging the milk fat globule membrane (MFGM)
2. Measurement of milk fat globule membrane damage
3. Measurement of fouling (both rate and structure/composition)

Since air entrainment can also damage the MFGM, but has other additional effects on the milk proteins, it was excluded from the present study. Thus, this work was to concentrate on MFGM damage by mechanical forces without air entrainment.

This study set out to attempt to answer the question of whether MFGM damage does have an effect on milk fouling. The mechanisms by which MFGM damage affects fouling, the fouling rate, and the structure and composition of fouling layers was to be investigated to various extents within the time available for the study. The study's most

important aim, perhaps, was to demonstrate that damage caused to milk by improper handling can cause large losses later in the process.

Chapter *2*: Literature Review

This review covers the following topics, which were relevant to the present research:

--- Milk fouling, including types, mechanisms, kinetics, foulant compositions and measurements, and factors thought to affect milk fouling, are discussed in Part A (Sections *2.1* to *2.5*).

--- Damage to the milk fat globule membrane (MFGM), its likely causes and its measurement are discussed in Part B (Sections *2.6* to *2.7*).

Part A

2.1 Milk Fouling

Milk fouling refers to the unwanted deposition of dissolved or suspended solids from process streams on to equipment surfaces (Fig. 2.1). Fouling deposits are poor thermal conductors and increase resistance to flow, thus reducing production efficiency. According to a survey by Steinhagen et al. (1990), the food industry suffers from fouling more severely than other industries in New Zealand. The most troublesome milk fouling deposits are normally found in heat exchangers, which are widely used in the modern dairy industry. Steinhagen et al. (*op. cit.*) estimated that the cost of fouling would be expected to be about NZ$141 million per year in New Zealand.

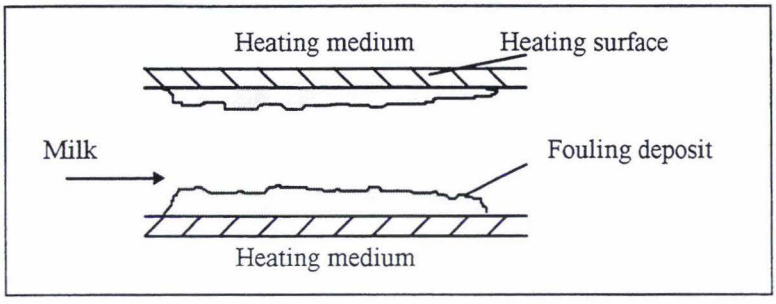


Fig. 2.1: Fouling deposit on heating surface

Milk fouling deposits have been classified into two types defined by different temperature ranges: Type A is described as a spongy, creamy white, curd-like deposit which is formed at 70-90°C. Type B is a compact, crystalline, grey, brittle deposit which is formed at 110-140°C (Burton, 1968). Other authors have identified different temperature ranges for Type A and Type B fouling, for example, 90-100°C and 120-140°C respectively (Yoon and Lund, 1989). Thus the classification of fouling into Types A and B is based only on temperature range. Flow configurations may also have an effect. No data are presented in the literature to aid discussion of this issue.

Substantial work has been done on compositions of deposits, mechanisms of fouling, monitoring of fouling and techniques of cleaning in place (CIP).

There are only a few papers on fouling by whole milk at low temperature (below 100°C). Most of the descriptions of milk fouling in this review relate to skim milk or whey protein solutions heated at ultra high temperature (UHT). Fouling of whole milk at low temperature will be indicated specifically.

2.2 Foulant Composition

Most workers agree that proteins and minerals are the main components of milk fouling deposits. β-lactoglobulin has been found to represent nearly half of the protein content of the deposit (Belmar-Beiny and Fryer, 1993). Milk fouling increases rapidly at 70°C (Jeurnink, 1996). This temperature matches the onset of β-lactoglobulin denaturation (Belmar-Beiny and Fryer, 1993; Delplace, et al., 1994). Casein (molecular and micellar) is the other major protein type in the deposit (Yoon and Lund, 1989). Calcium phosphate is the most important mineral in the deposit. More than 80% of the ash consists of calcium and phosphate, most likely in the form of $Ca_3(PO_4)_2$ (Jeurnink et al., 1989).

Typical deposit compositions are presented in the literature. Type A deposit consists of 50-60% protein, 30-35% mineral and 4-8% fat. Type B consists of about 70% mineral, 15-20% protein and 4-8% fat (Burton, 1968). There might be other materials which have not been measured, as the sum of the percentages of protein, mineral and fat is sometimes less than 100%.

The distribution of proteins and minerals is not homogeneous; minerals are found to concentrate at the interface between the fouling layer and the heated surface, whereas proteins form the main bulk of the deposit (Belmar-Beiny and Fryer, 1993).

The proportions of minerals and protein vary with the type of deposit, the location of deposition or the prior treatment of the milk (e.g. preheating in UHT treatment). Lactose

hardly contributes to the deposit unless it participates in the Maillard reaction or caramelises under high temperature (i.e. >100°C) (Jeurnink, 1996).

Fat has usually been thought to play a minor role in milk fouling, and, mostly the fat content of deposits has been reported to be relatively low (Jeurnink, 1996; Burton, 1968). However, there are some "abnormal" results. Unpublished data obtained at the New Zealand Dairy Research Institute (NZDRI) showed a high fat content of up to 40% in the deposit from recombined whole milk (T. Truong, personal communication). Burton (1968) stated that 34% fat had been found in the deposit on the unheated holding tube surface after direct-heating in UHT treatment. Other workers found that the deposit from milk sterilised with homogenisation at 138°C had 25-35% fat, and that deposit fat content varied from section to section of a plant (Lalande et al., 1984). For example, in the sections of a heater where the temperature was between 100-105°C, the deposit had 15-20% fat (Skudder et al., 1986). Most recently, Jeurnink (1996) obtained a deposit with 60% fat from recombined milk that had been made up from skim milk powder and anhydrous milkfat homogenized at 130 bar. However, the author gave no explanation for the high fat content in the deposit. These data showing higher than usual fat contents in fouling layers may or may not be related to particular anomalies in handling. The role of fat in milk fouling has not been studied systematically.

2.3 Fouling Mechanisms

2.3.1 Phases of Fouling

Fouling is arbitrarily divided into three phases for convenience of investigation and description (Delplace et al., 1994). Fig. 2.2 shows a typical fouling resistance - time (R_F - t) curve which shows the three stages of fouling clearly. R_F is the resistance to heat transfer of the fouling deposit. It can be calculated as:

$$R_F = 1/U - 1/U_o \quad (m^2KW^{-1}) \qquad (2.1)$$

where U = the heat transfer coefficient after fouling formation ($Wm^{-2}K^{-1}$),

and U_o = the heat transfer coefficient prior to fouling ($Wm^{-2}K^{-1}$).

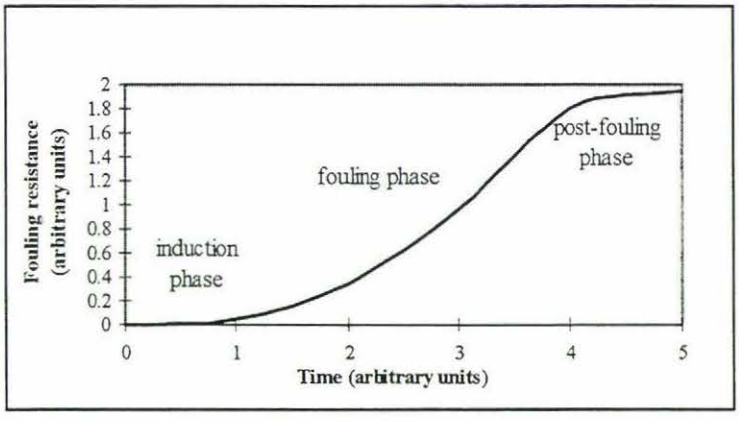


Fig. 2.2: A typical fouling resistance curve, showing the three phases of fouling (Adapted from Fryer and Slater, 1987)

(1) ***Induction phase*** The fouling deposit is very thin, and there is no fouling resistance. Fryer and Slater (1987) stated that the length of the induction phase was a complex function of both the nature of the surface and flow velocity. Not all activated molecules which reach the surface can attach; a certain orientation is needed for the reaction (Jeurnink, 1996). This phase is

very important, because extension of it would prolong the operating time of the plant (Belmar-Beiny and Fryer, 1993). Fast deposition will take place readily in the vicinity of irregularities in the thin layer formed in the induction phase.

(2) ***Fouling phase*** The fouling resistance increases almost linearly with time. Both denatured protein and precipitated calcium are formed in the bulk fluid and then they move to the surface during this period (Jeurnink, 1996). Fig. 2.3 illustrates schematically a possible mechanism of deposit formation from whey and milk fluids. The experiments of Belmar-Beiny et al.(1993) indicated that the mineral content of the deposit was uniform along the heating tube, while the protein content increased along the tube as the foulant layer became thicker. Belmar-Beiny et al. (1993) concluded that the rate of fouling is primarily controlled by protein reactions. This agrees with the assumption of Fryer (1989) that surface bond formation is governed by protein-protein interaction during the fouling phase.

(3) ***Post-fouling phase*** The rate of increase of the fouling resistance is much lower.

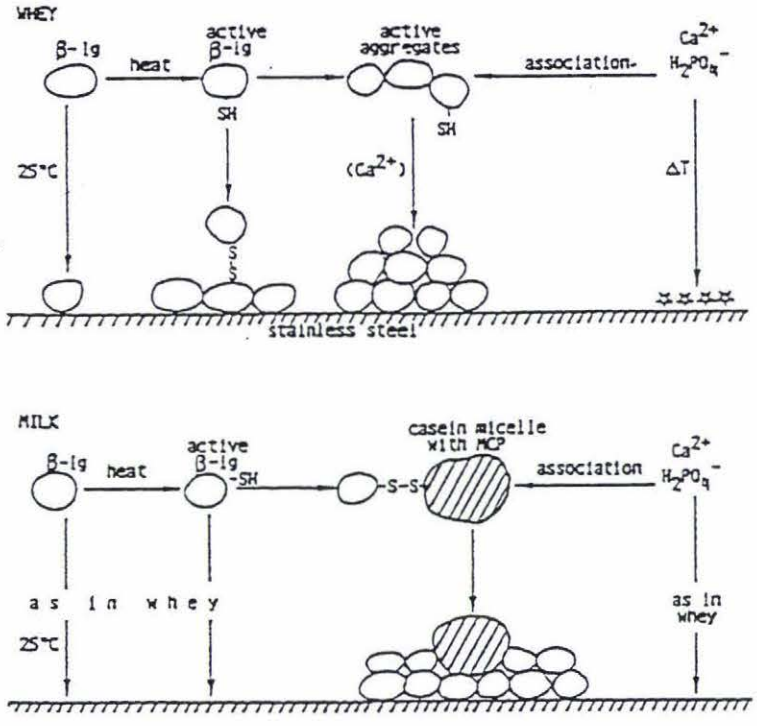


Fig.2.3: Schematic representation of the fouling mechanisms during heating of whey and milk (Jeurnink, 1996)

2.3.2 Outline of Fouling Mechanisms

During the induction phase, a monolayer of protein adsorbs to a hot stainless steel surface as soon as a protein-containing milk fluid contacts the surface (Fig. 2.3). Further deposits will adsorb on to this monolayer (Fryer and Slater, 1987; Delsing and Hiddink, 1983). Fryer (1989) thought the formation of surface bonds was probably governed by protein-surface or protein-salt interaction. Thus, the whole fouling layer is built up by two different types of deposit: a dense, compact sub-layer near the metal surface, and a spongy , porous upper layer weakly bound to the sub-layer (Jeurnink and Brinkman, 1994; Truong et al., 1996) (Fig. 2.4).

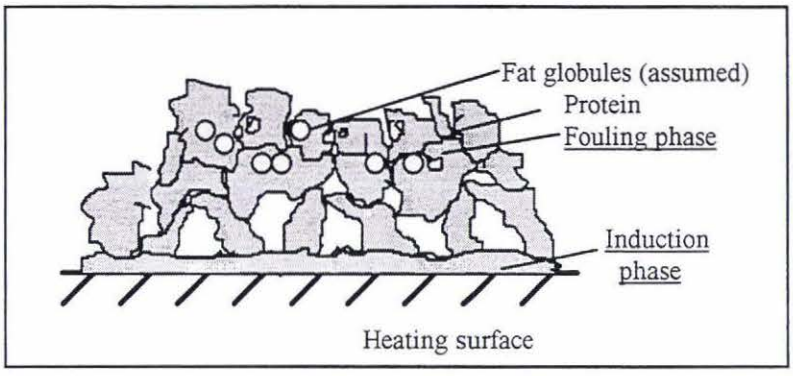


Fig. 2.4: Structure of fouling deposit from fresh whole milk on heating surface (Redrawn from microstructural photographs by Truong et al.,1996)

A number of workers have tried to determine which component deposits on to the heating surface first, but there is still no overall conclusion. The observation that a thin induction layer forms first on the surface suggests that proteins deposit first. Delsing and Hiddink (1983) supported this concept and thought that a protein film changed the properties of the surface; their research also showed that if the fluid contained no calcium, no visible fouling was observed. Foster and Green (1990) suggested that mineral and protein parts of the layer are built simultaneously. They found that the distribution of the components changed with depth, and no distinct layering was observed. Surface analysis by X-ray photoelectron spectroscopy (XPS), suggested that proteins were most likely to be the first species to adhere to the stainless steel surface (Belmar-Beiny and Fryer, 1993). Calcium ions can promote protein aggregation in the bulk fluid (Belmar-Beiny and Fryer, 1993).

It appears that the fouling phase, during which the fouling resistance increases significantly, cannot begin until the initial layer is formed (Fryer and Slater,1987). Fouling is thought to proceed by a nucleation and growth process (Bradley and Fryer,

1992). Those proteins which are already unfolded, denatured and aggregated, and have exposed reactive sites, require a shorter adhesion time than native proteins (Bradley and Fryer, 1992). Thus fouling by protein is controlled by the denaturation reaction taking place in the bulk fluid rather than by the reaction at the surface. Fryer (1989) stated that heavy fouling occurs in the first effects of multiple-effect milk evaporators, where the temperature is highest, and in the last effects, where the liquid is most concentrated.

Foster et al. (1989, 1990) studied fouling deposition by whole milk and skim milk at 100°C and 140°C, and reported that deposits containing a higher proportion of protein were easier to remove. They also reported that protein was concentrated on the outsides of deposits, while calcium phosphate was concentrated close to the heating surface.

Jeurnink et al. (1996) summarised three possible mechanisms of fouling.

(1) Fouling in heaters is the result of surface reaction controlled by wall temperature. In other words, fouling is a process which takes place in the thermal boundary layer.

(2) Reactive molecules or particles formed in the bulk of the solution diffuse to the surface where they can deposit. Jeurnink et al. (*op. cit.*) further stated that this concept was supported by the fact that the fouling occurs in holding tubes where the wall temperature is lower than the bulk temperature. There is a general assumption that only proteins unfolded in the thermal boundary layer are able to adsorb to heated surfaces (Delplace et al., 1994).

(3) Air bubbles formed at the heated surface act as nuclei for reactive species to deposit.

The widely accepted mechanisms underlying fouling are the denaturation of whey proteins (mainly β-lactoglobulin), and mineral precipitation (mainly of calcium salts). These two phenomena are popular targets of investigation.

2.3.3 Protein Denaturation and Aggregation

Most studies on the protein contribution to milk fouling focus on the behaviour of β-lactoglobulin. β-lactoglobulin is heat unstable; its denaturation above 70°C causes fast fouling. Lalande et al. (1984) found that β-lactoglobulin was so sensitive that more than 94% β-lactoglobulin was denatured after heat treatment comprising pre-pasteurisation (75°C - 85°C, 20% denatured) and sterilization (70°C - 120°C, 74% denatured) in their experiment. Yoon and Lund (1989) also claimed that β-lactoglobulin played an important role in fouling at the normal pH (6.76) of milk in the temperature range of 88-120°C. Some researchers claimed that the principal protein in the deposit was β-lactoglobulin, while the major milk protein, casein micelles, played a minor part compared with β-lactoglobulin (Lyster, 1965; De Jong et al., 1992). Jeurnink (1996) further stated that " if serum proteins are (nearly) absent in the milk, fouling reduces by two-thirds".

Denatured β-lactoglobulin was also thought to behave as a bridge between other milk components and the heating surface in fouling formation. When β-lactoglobulin is partially unfolded, it exposes reactive sulphydryl (-SH) groups, and then aggregates either with other β-lactoglobulin or with other proteins like casein micelles. Bradley and

Fryer (1992) found that the rate of fouling is relatively low when there is a low level of free -SH groups (i.e. a low level of denaturation).

2.3.4 Mineral Precipitation

Calcium phosphate constitutes more than 80% of the mineral fraction of fouling deposits. It can precipitate directly on to the heating surface or contribute to a deposit via precipitation on deposited protein aggregates. One study showed that calcium and phosphorus were essential for fouling formation: if no calcium was available in the testing fluid, there was no serious fouling except for the first thin layer of protein (Delsing and Hiddink, 1982). Phosphorus seems necessary to the formation of a firm fouling layer (Delsing and Hiddink, 1982).

2.3.5 Effect of Milk Fat

In the literature, the amount of fat in fouling deposits has normally been reported to be small. Many researchers concluded that fat played only a minor role in deposition (Burton, 1968; Jeurnink, 1996). Although there is little work specifically targeting fat involvement in fouling, some facts have been reported, and assumptions have been made, by several authors:

- Experiments showed an increase in the fat content of fouling in a sterilizer when the milk was not homogenized. It was assumed that the amount of fat in the foulant from unhomogenised milk could be affected by the size of the fat globules (Lalande et al., 1984).

- A high temperature driving force lowers the proportion of fat in the deposit. It is assumed that the fat in the deposit was entrapped by protein. When the temperature driving force is high, the deposition of the proteins can be so fast that less fat could be entrapped (Jeurnink et al., 1989).
- Little difference was found between deposits from whole milk and from skim milk. Not only the fat, but also the mineral and protein, concentrations were similar. Based on these observations, Foster et al. (1989, 1990) suggested that the role of fat in the deposition process is minor.
- A high percentage of fat (up to 60%) in the deposit occurred in the case of recombined milk homogenised at 130 bar (Jeurnink, 1996). Since homogenised milk has a different fat globule membrane to fresh milk, this behaviour in fouling probably relates to the nature of the fat globule membrane (Jeurnink, 1996).
- Phospholipids could be the key to seasonal variation of the fat content in fouling. The phospholipids are surface-active and their concentrations vary seasonally (Burton, 1967).

2.3.6 Other Parameters Affecting Fouling

Two groups of factors, product-related and process-related, can be argued to affect fouling.

Product Related Factors

Product related factors include the initial pH, the milking season (or stage of lactation), the milk's mineral content, the age of the milk, the breed of cow, etc. (Burton, 1967).

The pH of fresh milk is 6.68-6.77, and the rate of fouling increases as the pH decreases. Yoon and Lund (1989) and Foster et al.(1989) reported that the amount of fouling increases with milk acidity in the pH range 6.3-6.8. With increasing pH, the mineral content of the deposit increases while the proportions of protein and fat decrease (Calvo and De Rafael, 1995). The relation between pH and amount of deposit is not linear; the effect of pH becomes greater as the pH falls (Burton, 1968). Skudder et al. (1986) stated that a comparatively higher milk pH would increase the running time of a UHT plant with respect to fouling formation.

Normally, the amount of fouling increases with the age of milk (Burton, 1968; Swartzel, 1983). But, an opposite result was found by Burton (1968). When fresh whole milk was stored for a comparatively short time, for example, 24 hours refrigerated storage, the amount of deposit formed was only 50% of that when the milk was fresh (Burton, 1968).

Process Related Factors

Process related parameters include preheat treatment, temperature of heating surface, temperature of bulk fluid, velocity, Reynolds number etc.

Preheat treatment reduces the amount of fouling in UHT plant. Experiments showed that preheating became more and more effective as the preheating temperature approached the temperature of the main heating surface. Preheating causes the

denaturation of soluble proteins and the precipitation of calcium phosphate from the milk (Burton, 1968; Skudder et al., 1981). The higher the preheating temperature and the longer the holding time, the less the deposit formed. The preheating temperature affects the amount of fouling deposit more than the holding time (Yoon and Lund, 1989).

Although it has been found that preheating has a great influence on reducing fouling of the main heating section, no description of changes in fouling in the preheat section have been reported. As mentioned before, the fouling is proposed to be a surface reaction, and the writer thus believes that there may be less fouling in terms of the total amount of deposit in the whole heating system because of the smaller surface in the preheat section.

The studies of Foster et al. (1989, 1990) indicated that a higher processing temperature caused more fouling deposition. Also, the proportion of minerals in the deposit increased with surface temperature in the range 100°C - 140°C.

Increased flow velocity will reduce fouling deposition. Experiments have shown that as the Reynolds number increased, the total amount of fouling decreased (Belmar-Beiny et al., 1993).

Early studies found that air incorporated in heated milk affected fouling through bubbles which adhered to the heating surface (Burton, 1968). The air bubbles act as nuclei for fouling solids and encourage deposit formation. Their contribution depends on the amount of air in the milk, the temperature difference between the surface and the bulk milk, the operating pressure and the wall shear stress (Jeurnink, 1995). Further detailed discussion of air and fouling can be found in Section ***2.5.***

Britten et al. (1988) found that the material of the heated surface influences the amount of deposit only slightly, but has a large effect on the adhesion strength. However, it has little effect on the structure of the fouling layer. The interfacial energy factors appear to be the main factors affecting the strength of adhesion to the surface. Britten et al. (1988) stated that the interfacial properties did not govern the kinetics of the adsorption process.

2.4 Fouling Monitoring (Measurement)

As mentioned in Section *2.1*, fouling deposits are poor thermal conductors. They also increase resistance to flow. The principal means of monitoring fouling are based on the reduction of the overall heat transfer coefficient or the increase in pressure drop necessary to maintain the product flow rate. Measuring the thickness of the deposit is another way to measure fouling formation, but this measurement is rather difficult to achieve in practice. Generally, four factors have been considered in designing fouling monitoring systems: reduction of the heat transfer coefficient, increase in pressure drop, measurement of deposit mass or thickness and chemical analysis of the deposit.

Reduction of the overall heat transfer coefficient can be calculated from measurement of the change in the temperature driving force either locally or over the whole heat exchanger. To measure pressure drop, pressure sensors can be used in-line that do not disrupt the operation of the system. But sometimes the pressure drop is not an accurate indication of fouling because blockage may be local. A very high pressure drop could

result even though little fouling deposit is formed, especially in equipment with small hydraulic diameters.

Monitoring with ultrasonic, acoustic and optical techniques, can be used for in-line detection of the thickness of fouling layers. The vibration sensor in the acoustic technique (the "noise" from a fouled heat exchanger is different to that from a non-fouled one) was found suitable in plate heat exchangers. The ultrasonic sensor (like pulse-echo and transmission techniques) performs well in tubular heating systems, and the optical sensor (i.e. a sight glass in the pipework) could be used anywhere along the processing system (Withers, 1996). Withers (1996) has recently provided a review of the development of these fouling monitors.

2.5 Milk Fouling and Air Incorporation

2.5.1 Air Incorporation

Air incorporation refers to air bubbles becoming dispersed into a continuous liquid phase by either aeration (the sucking of air into the liquid) or expansion of dissolved or pressurised air owing to pressure reduction. In many respects, a dairy foam is similar to an oil-in-water emulsion, as both are dispersions of a hydrophobic fluid in an aqueous phase, stabilised by suitable, surface-active substances (Mulder and Walstra, 1974). Foaming is the main and best known result of air incorporation in milk processing.

Miller and Puhan (1986a) studied fat damage during milking by pumping 40°C milk at 0.91 litre/min with air and 1.23 litre/min without air respectively, used a small centrifugal pump, and found that no fat damage was caused by pumping the milk for 30 minutes without air. Much foam formation and considerable fat damage occurred when a milk/air mixture was pumped for only 10 minutes. Thus, air inclusion can promote fat damage during milk handling.

Air can become incorporated into milk during milking on the farm. The total air flow is typically 20-30 times the volumetric milk flow in a high-line milking system. In such a system, air is continuously sucked into the milk for two purposes. It allows the cluster to be removed from the cow after milking, and, since the density of an air-milk mixture is much lower than that of milk alone, air entrainment helps milk flow in the milking machine. Fig.2.5 shows a typical milking system. The air is admitted via the cluster.

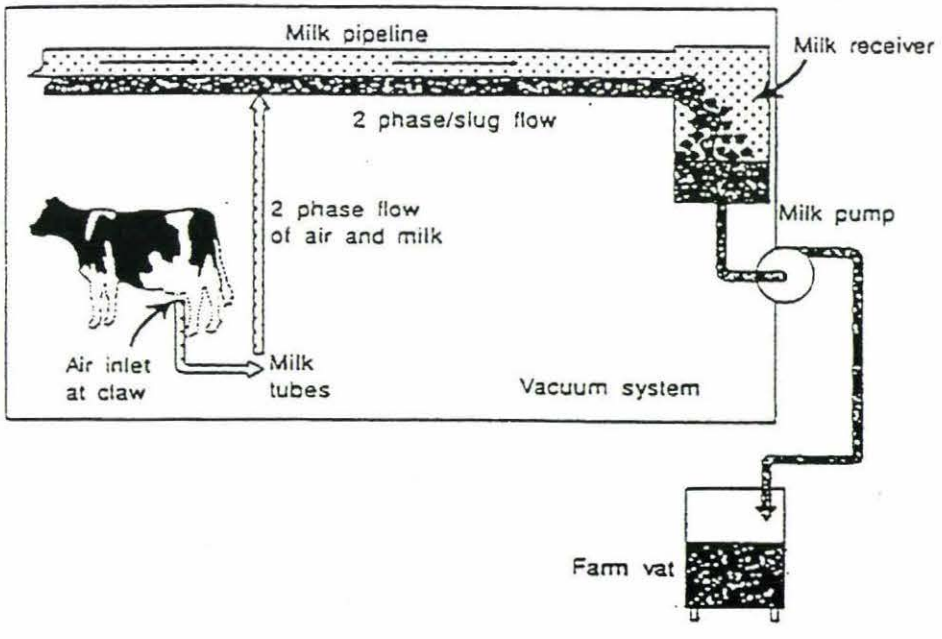


Fig. 2.5: Milk transport within conventional milking machines
(Adapted from Woolford et al., 1993)

Most New Zealand milking systems use a continually running diaphragm pump. At the end of milking, the pump may empty the receiver and start pumping air. In the case of bottom-filling vats, the milk in the vat may become aerated. A level sensor that controls the pump is essential to avoid this.

Foaming can occur whenever a pump sucks air into the milk, and when an agitator impeller is only partially immersed in the milk. Consequent lipolysis can be enhanced by fat damage resulting from air incorporation during milking.

2.5.2 Effect of Air Incorporation on Milk Fouling

Air incorporation encourages more rapid milk fouling in heat exchangers. According to Jeurnink (1996), air bubbles act as nuclei at the heating surface and enhance protein deposition by drying out of the protein membrane (mainly casein micelles in his experiment) of air bubbles. When air bubbles form at the heating surface, the surface becomes dry, and the result is a higher temperature difference between the surface and the bulk fluid; proteins coagulate and deposit on the surface owing to that high temperature difference. Eventually, the air bubble may burst apart if its membrane is carried away with the liquid flow. The process is shown schematically in Fig.2.6.

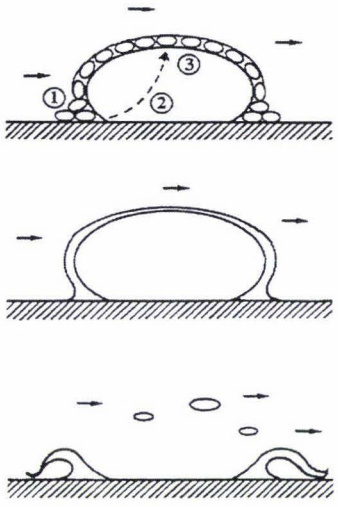


Fig. 2.6: Schematic representation of the participation of an air bubble in the fouling by milk of a hot stainless steel surface. 1. Adsorption/deposition of milk proteins at the vapour/liquid interface; 2. Evaporation of water at the vapour-liquid interface; 3. Condensation of milk protein. ⟶ : milk flow direction (Adapted from Jeurnink, 1996)

Burton (1968) found that the total amount of deposit could be reduced by between 50% and 75% if air was removed from milk before processing at 85°C in a laboratory plate pasteuriser.

Conclusion to Part A:

In Part A have been discussed previous studies on fouling types, foulant compositions, fouling mechanisms, kinetics, and fouling measurement. The roles of proteins and minerals were targets in these studies. Most fouling studies have been carried out with model fluids at ultra high temperatures. Many product and process factors can affect fouling deposition. Fouling problems are not completely understood.

Part B

As described in Sections *2.2* and *2.3.5*, the fat contents of fouling deposits has been found to be variable; it is not always low. These results lead to the question of how milk fat behaves during fouling formation. No studies of the effect of milk fat on fouling have been reported. Since the MFGM is critical to the protection of fat globules from coalescence and lipase attack, MFGM damage during milk handling might affect fouling to some extent. In Part B, previous MFGM studies are reviewed.

2.6 Damage to the Milk Fat Globule Membrane During Handling

Fat in milk is in the form of globules surrounded by milk fat globule membranes (MFGM) which contain a mixture of unsaturated phospholipids, proteins, glycoproteins and other minor components (Keenan et al., 1983). Normally, the membrane is 0.01 microns in thickness.

The stability of the milk fat globule depends on the properties both of the MFGM and of the core lipids. The membrane protects the fat in the globule from lipase activity and oxidation and prevents the fat globule from coalescing. The natural MFGM is easy to damage mechanically or chemically during milk handling. Damage to the fat globule inevitably results in damage to the MFGM.

2.6.1 Milk Fat Globule Membrane Composition

Because the MFGM is so important for the stability of the fat globule, it is important to understand the properties of the MFGM.

The proportions of membrane constituents found depends on the analytical methods used. In general, the MFGM is a lipid-protein complex consisting of 55% protein, 44% lipid and a small amount of carbohydrate. MFGM lipids contain about 27% phospholipid and 70% triacylglycerols (Kanno et al., 1991).

According to Walstra and Jenness (1984), the MFGM is made up largely of phospholipids and a wide variety of glycoproteins, like that of an outer cell membrane. In freshly drawn, uncooled milk, about 80% of the lipid phosphorus is in the fat globules, and this figure drops to only 60% quite quickly. The remainder is in the milk plasma. Most of the proteins of the membrane are highly specific. Table 2.1 summarises the MFGM composition.

Table 2.1. Composition of Bovine Milk Fat Globule Membrane

Components	Amount
Protein	25-60% of dry weight
Total lipid	0.5-1.2 mg per mg protein
Phospholipid	0.13-0.34 mg per mg protein
Phosphatidyl choline	34% of total lipid phosphorus
Phosphatidyl ethanolamine	28% of total lipid phosphorus
Sphingomyelin	22% of total lipid phosphorus
Phosphatidyl inositol	10% of total lipid phosphorus
Phosphatidyl serine	6% of total lipid phosphorus
Neutral lipid	56-80% of total lipid
Hydrocarbons	1.2% of total lipid
Sterols	0.2-5.2% of total lipid
Sterol esters	0.1-0.8% of total lipid
Glycerides	53-74% of total lipid
Free fatty acid	0.6-6.3% of total lipid
Cerebrosides	3.5 nmoles per mg protein
Gangliosides	6-7.4 nmols sialic acid per mg protein
Total sialic acids	63 nmoles per mg protein
Hexoses	0.6 μmoles per mg protein
Hexosamines	0.3 μmoles per mg protein
Cytochrome b_5 + P-420	30 pmoles per mg protein
Uronic acids	99 ng per mg protein
RNA	20 μg per mg protein
Fe	0.3 mg per 100g fat globules
Mo	0.05 mg per 100g fat globules
Cu	0.01 mg per 100g fat globules
Water	200 mg per 100g fat globules

(Adapted from Keenan et al. 1983; Walstra and Jenness, 1984)

2.6.2 Consequences of Damage to the Milk Fat Globule Membrane

The stability of fat globules is determined by properties of the membrane (and also factors such as temperature); changes in the stability of fat globules reflect changes in membrane composition or structure. Milk fat globules can coalesce and cream, depending on the extent of membrane damage. Disruption of fat globules inevitably results in membrane damage. Without the protection of the native membrane, lipases can penetrate the globule and react with the core lipid. Then, free fatty acids (FFA) will be released. When the FFA level reaches a threshold of 1.4 mmol per 100g, off-flavours will be perceived (Walstra and Jenness, 1984). MFGM damage occurs during processing and handling, and does influence product quality or production efficiency.

Milk Fat Globule Membrane Damage by Mechanical Stress

Mulder and Walstra (1974) summarised that three mechanisms for mechanical damage to the MFGM:

1. Shear, in which the velocity gradients within the fluid impose viscous forces of sufficient magnitude to deform and eventually rupture the membrane.
2. Turbulence, a mechanism similar to that of shear except that the velocity gradients are imposed by turbulent eddies. This effect is far greater and encountered more frequently than shear in practice.
3. Cavitation, the collapsing of vapour bubbles within the body of a liquid which causes high pressure gradients of very short duration.

Te Whaiti and Fryer (1975) studied the gelling of cream, found that mechanical stress like agitation at a critical temperature will do much harm to the MFGM and make the fat globule unstable. They defined the term "free fat" (FF) as the proportion of fat extracted by centrifugation at 60°C. It is synonymous with the terms "clumped fat", "churned fat" and "free oil". They also found that the maximum amount of free fat produced by shaking was produced between 27°C and 35°C. Turbulence with air present caused more free fat than that in the absence of air.

The stability of the fat globule towards stress increases with an increasing fraction of crystalline fat. Fat globules are sensitive to mechanical damage in the temperature range 20°C and 30°C. At temperatures under 20°C a shell of solid fat stabilises the fat globule and the sensitivity towards flow related stress decreases (Hinrichs, 1994).

Physical changes may occur in milk as a result of mechanical treatment. Agitation is known to affect the physical state of the fat globules and their surrounding membrane and to allow the milk lipase, which is normally associated with casein in the skim-milk, access to the fat (Mulder and Walstra, 1974).

After homogenization, the fat globule size is reduced and the MFGM composition is different. Together with the original native membrane material, serum proteins and casein micelles will adsorb on the new globule surface to form a new layer. The relative content of original membrane material will depend on the increase in surface area. The new MFMG is permeable to lipases (Mulder and Walstra, 1974).

Damage by Micro-organisms

Microbial lipase is one of two kinds of lipase (the other is the natural milk lipase) that cause hydrolytic rancidity (lipolysis). But, microbial lipase is not important compared with the native lipase unless the bacterial plate count number exceeds 1×10^6/ml (Fleming, 1991).

Damage by Foreign Matter

Foreign matter to which milk may be exposed, especially chemicals such as cleaning detergents from badly rinsed pipework, do affect fat globule membrane stability.

Damage by Air Entrainment

Foaming often occurs in farm vats and silos. Air entrainment will influence milk processability later. For example, air bubbles in the whole milk could lower skimming efficiency in separators.

When fat globules contact air bubbles, they may lose part of their membranes. The milk plasma will become enriched in membrane materials such as phospholipids. Fat globules adhere to air bubbles, some liquid fat leaves the globules by spreading, and subsequent coalescence of air bubbles causes their surface area to diminish, thereby driving the adhering globules closer together; the liquid fat acts as a sticking agent and granules of globules are formed (Walstra, 1983).

Stannard (1975) studied the effects of aeration on MFGM damage and found N_2, air and O_2 gases gave equal effects. He concluded that gas bubbles provided nuclei for fat globule clumping rather than the gas itself directly reacting with the membrane materials.

2.7 Milk Fat Globule Membrane Damage Determination

Although there are biochemical methods for determining MFGM damage, no routine method which is simple and easy to handle exists presently. Nevertheless, there are some methods which can be used to determine MFGM damage, especially to estimate trends in damage as opposed to exact quantification. Since fat core damage cannot happen without MFGM damage, some parameters used to measure fat globule damage could be used as indicators of MFGM damage. These parameters may include free fat (FF), free fatty acid (FFA), particle size distribution etc.

2.7.1 Measurements of Free Fat (FF)

Ideally, FF refers to the fat without protection of its natural MFGM. As mentioned in Section ***2.6.2***, this is not a precise definition; FF actually refers to the part of the fat that can be separated by some means from the total fat. Three methods are normally used in free fat measurement: the centrifugal method, the extraction method and the agglomerated fat test. Both centrifugal and extraction methods estimate coarsely dispersed fat, occasionally also fat from globules that are highly susceptible to physical changes (Miller and Puhan, 1986b).

Centrifugal methods rely on the density difference between "free" and membrane-covered fat. In centrifuging, the free fat will tend to be displaced towards the axis of rotation of the centrifuge, and be separated out. The best known methods of this type are the methods of Te Whaiti and Fryer (1976), and Halter et al. (1978) (Thomson, 1986).

The method of Halter et al. can measure amounts of free fat as small as 0.04% in milk using a special butyrometer.

Solvent extraction methods use organic solvents to extract fat that is outside the globule membrane or can be removed from globules whose membranes have been damaged; after evaporation of the extract, the amount of free fat can be determined. Normally, the result obtained by this extraction method is always found to be higher than that measured by the centrifugal method (Thomson, 1986). Factors like the type of solvent (carbon tetrachloride, petroleum or diethyl ether), extraction time (from 1 minute to 5 hours) and the method and intensity of shaking affect the test result.

The agglomerated fat test relies on the fact that under the effects of laminar and turbulent flow in raw milk the fat globules associate to form very stable agglomerates. Within these agglomerates the globules adhere strongly to one other as the result of the escape of liquid fat, which acts as a binding agent. The degree of agglomeration can be determined by filtration of the agglomerated particles from the milk (Thomson, 1986). Since the damaged fat can also be in the form of smaller globules, this method actually measures the extent of fat globule coalescence.

2.7.2 Measurements of Free Fatty Acids (FFA)

This method is well known and widely used to evaluate fat damage. It relies on free fatty acid being released due to the enzymatic hydrolysis of milk fat. Since lipase can attack only the core lipid that is without native membrane protection, this method is also used

to evaluate membrane damage. However, the quantity of free fatty acid produced during commercial milk handling is small.

The writer believe three factors will influence the accuracy of the measurement of MFGM damage. One is the method's sensitivity for small amounts of FFA during commercial milk handling. Another is lipase activity; optimal conditions for lipase activity do not exist during milk handling if temperatures are controlled to correct levels. Finally, the amount of active lipase may not be sufficient to attack free fat, for example, after pasteurization.

Usually, free fatty acid is extracted with a solvent. The amount of acid is determined by titration. Since different chain-length fatty acids are extracted to different extents by different solvent, results from different investigations often cannot be compared (Kuzdzal-Savoie, 1980).

The most well known method is the BDI (Bureau of Dairy Industry) method. It uses a detergent mixture to separate the free fat from the milk, then titration to get the acid degree value (ADV). It is widely accepted around the world, and other methods of FFA measurement are always tested against the BDI method. Many attempts have been made to improve this method. Driessen et al. (1977) modified the BDI method, proposing to adjust the reagent to pH 6.6 with 4 N phosphoric acid, and using thymol blue as the titration indicator instead of phenolphthalein.

Thomson (1986) used a extraction method developed by R.K. Richardson at the New Zealand Dairy Research Institute. It is similar to the standard method published by the

New Zealand Dairy Division in 1980 (see Appendix 2). It has been found to be reasonably accurate (Joerin and Bowering, 1973) and easier to use than the BDI method.

The copper soap method is a kind of colorimetric determination of the FFA content in milk (Koops and Klomp, 1977). Acidified 0.5 ml samples of milk (pH 2.5) containing complexone are shaken with 5 ml of a mixture of chloroform, heptane and methanol (49:49:2) and subsequently with a copper reagent. The copper soaps, transferred to the organic layer, are determined colorimetrically after reaction with sodium diethyl dithiocarbamate.

The gas chromatographic method separates fat by extraction through neutral, deactivated alumina. FFA is retained on the alumina. The FFA is eluted with a small volume of 6% formic acid in diisopropyl ether, then quantified by gas chromatography. This is a simple, repeatable procedure for determining the free fatty acid content of a range of dairy products (Deeth et al., 1983).

Several other methods for determining FFA were developed in the 1970s and early 1980s.

Not all methods can be applied for measuring MFGM damage. For example, the colorimetric copper soap method uses a specific acid to make a standard curve, but the free fatty acids from milk fat are of a wide range of chain lengths. Thus, the accuracy of the method is suspect.

2.7.3 Skim Milk Fat Test

No matter how efficiently milk is separated into cream and skim milk, a small amount of lipid, about 1% of the total milk lipids, remains in the skim milk. the common explanation given for this is the existence of very small fat globules, which cannot be removed from skim milk with 100% efficiency. Patton and Keenan (1975) stated that the lipids in skim milk were not fat globules but were contained in membranes, and actually sedimented in a strong centrifugal field.

Thus the amount of fat remaining in the skim milk after separation can be used to quantify the extent of mechanical damage. Deeth and Fitz-Gerald in 1978 (Leigh,1996) found that the fat content of skim milk showed good correlation between the quantities of FFA and FF in milk. However, the changes over only one pump might not be detected (Thomson, 1986). Deeth and Fitz-Gerald (1978) found a large increase in the fat content of the skim milk as a result of damage caused by a high agitation rate at high temperature in their experiments.

2.7.4 Fat Globule Size Distribution Measurement

Milk fat contains a large number of fat globules with diameters in the range 0.1 - 10.0 μm (Fig. 2.7).

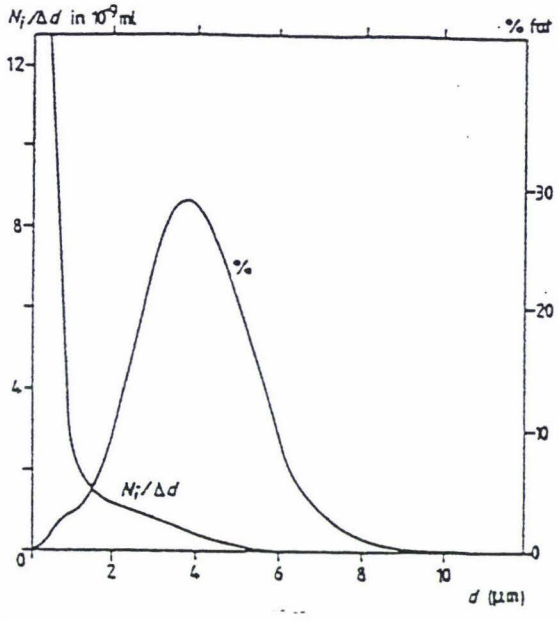


Fig. 2.7 Average size frequency distribution of the fat globules in milk of a Friesian cow (d_{vs} = 3.4 µm, fat content 3.9%). Number ($N_i/\Delta d$) and volume frequency (% of the fat) are given per micrometer class width. d_{vs} refers to the volume surface average diameter, $N_i/\Delta d$ refers to the number frequency, as per millilitre per micrometer class width. (Adapted from Walstra and Jenness, 1984)

Milk contains very many small globules which comprise a small fraction of the total fat. The total globule number is about 15×10^9/ml, and the $N_i/\Delta d$ curve in Fig. 2.7 shows that about 75% are less than 1µm in diameter. The plot of *%fat vs d* in Fig. 2.7 shows that:

(1) A large number of small globules (diameters below 1 µm) represent only a small proportion of the fat volume.

(2) Large globules with diameters above 1 µm represent a large proportion of the fat volume.

(3) The percentage of very large fat globules (diameters above 8 µm) is small (Walstra and Jenness, 1984).

The fat globule size distribution of fresh milk constitutes a "standard" distribution curve, with which one can compare globule size distributions after handling of the milk to find differences representing globule damage.

Thus, this method measures the volume distribution of the fat globule diameters. When the MFGM is damaged, fat globules can get either bigger or smaller in size. Leigh (1996) in her thesis mentioned that the sample must be compared to a "standard" fat or it must be used in a "before and after" experiment involving a treatment such as pumping. This method gives a comparable, direct measure; it may be used as a subsidiary determination of fat globule damage.

2.7.5 Fat Globule Appearance Observed by Confocal Laser Scanning Microscopy

Leigh (1996) studied the fat damage during milk handling using this technique. Images of large, coalesced fat globules showed fat damage clearly, even though this had not been revealed by the particle size distribution test. Also, micrographs showed that fat globules had been broken into many smaller ones at high shear rates. Leigh (1996) concluded that the images from confocal laser scanning microscopy gave qualitative information which supported the quantitative results from other methods of fat damage measurement.

The following description is taken mainly from the manual of the confocal laser scanning microscope made by Leica Microscopy and Scientific Instrument Group, Switzerland.

The confocal laser microscope has many advantages. It allows the user to obtain depth-selective information in the three dimensional structure of a object. The laser light can penetrate beneath the surface of most specimens, to a depth that depends on their optical density. The confocal laser scanning microscope produces an intense, monochromatic source of light and excites a fluorescent dye added to the specimen. The light emitted by or reflected from the specimen passes through the microscope's objective lens and a beam splitter to a photomultiplier through a detector pinhole. This produces a signal from which a digital image is constructed and displayed on a monitor. In order to produce a complete image, the light point is moved over the entire object, line by line by means of a mirror. The confocal principle is especially valuable in fluorescence microscopy, since it almost completely eliminates stray light not coming from the focal plane. The principle of the confocal scanning microscope is shown as Fig. 2.8.

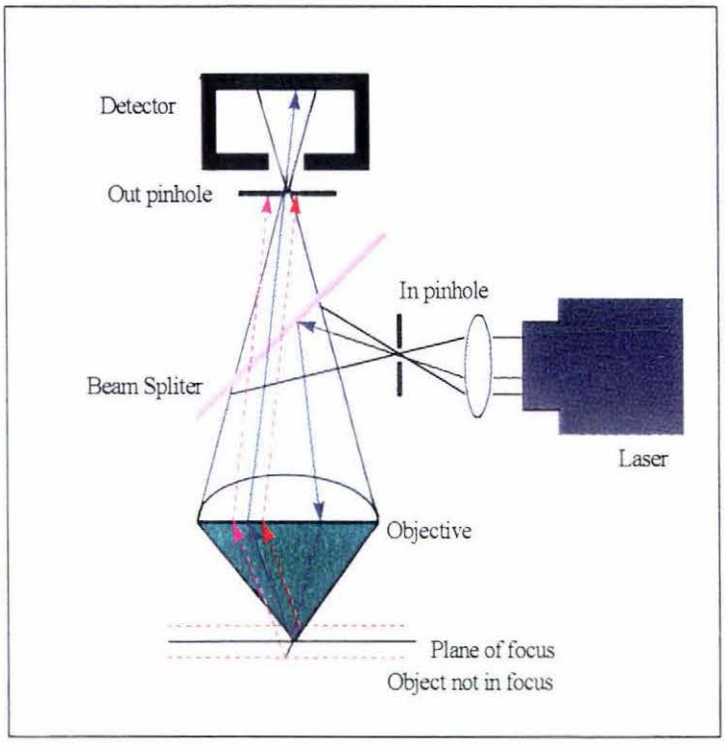


Fig.2.8: Principle of Confocal Laser Scanning Microscope

Nile Blue, which contains trace amounts of Nile Red, is the best dye to stain water-continuous systems in practice. Nile Red is freely soluble in oils and strongly fluorescent in hydrophobic environments when exited in the wavelength range 450-550 nm (Leigh, 1996). Fast Green is good for staining proteins.

In summary, the advantages of the confocal laser scanning microscope compared with light or electron microscopes are:

- Embedding of the specimens is not required
- The specimens can be subjected to non-destructive testing
- Drying of the specimens - as would be required for scanning electron microscopy (SEM) or transmission electron microscopy (TEM) - is omitted so that artefacts causes by drying are excluded.

- Optical sections up to approximately 100 μm below the surface can be observed; thus surface effects like smear caused by surface grinding are no longer significant.

2.7.6 Released Enzyme Test

Some enzymes are unique to the MFGM, so the idea of this test is based on using measurement of enzymes released from the MFGM as an indicator of damage. Stannard (1975) put forward a method of determining the extent of churning in milk by evaluating the release of xanthine oxidase and alkaline phosphatase.

Conclusion to Part B:

Part B of this review includes a discussion of damage to the milk fat globule membrane (MFGM) during milk handling. Micro-organisms, mechanical stress, foreign matter and air entrainment can all damage the MFGM to various extents in practice. Measurements of MFGM damage are also discussed. No single method is good enough to be used alone to determine MFGM damage.

Chapter *3* Materials and Methods

Several techniques had to be mastered to accomplish this research task. Firstly, finding a way to damage the milk fat globule membrane (MFGM) successfully was an important step for this study; although many factors can affect the stability of the MFGM in practice, an efficient and convenient method was required for research in a pilot plant. Secondly, an accurate and sensitive way of measuring MFGM damage was crucial; convincing conclusions could not be drawn without good measurement of the extent of MFGM damage. A third important issue in this research was the measurement of fouling. Any possible changes in fouling behaviour caused by MFGM damage had to be monitored properly for later analysis. Necessary measurements had to include fouling rate, structure of fouling layer, fouling composition etc. Lastly, a fouling experimental rig had to be designed and built. The rig had to foul rapidly, be easy to dismantle for sampling and cleaning, be convenient to control during a run and be sensitive to changes in fouling behaviour caused by MFGM damage. Also, of course, the rig had to be physically robust. In summary, the objectives of this study required:

1. A Method of damaging the milk fat globule membrane (MFGM).
2. Methods of measuring MFGM damage.
3. The design of a suitable fouling rig.

4. Methods of measuring fouling rate.

3.1 Raw Material

Approximately 1200 litres of commercially pasteurised, unhomogenized full cream milk was used in the present fouling study. Each batch of milk was stored at 4°C prior to the experiments.

3.2 Fouling Rig

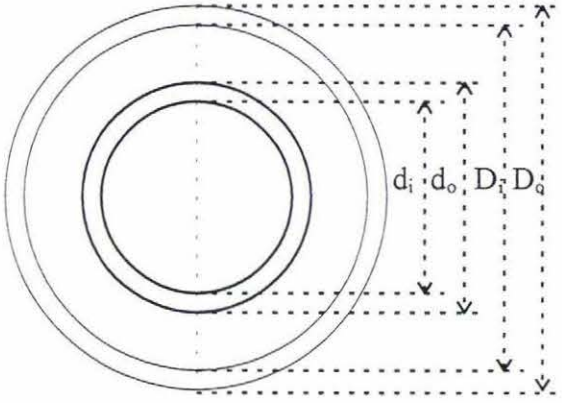
The custom built fouling rig had to meet the following requirements:

- ◊ Adequate heating
- ◊ Accuracy of temperature measurements
- ◊ Significant fouling
- ◊ Ease of sampling
- ◊ Stability and reliability in replicate trials

The custom built fouling rig is shown in Figs. 3.1 and 3.2. The rig consisted fundamentally of a double pipe heat exchanger, with hot water flowing in the inner tube and milk in the annular channel between the inner and outer tube. The whole heat exchanger was 8 metres long, made up of 7 one-metre and 2 half-metre stainless steel tubes (T316, manufactured by NZ Fasteners and Stainless Steel Co.). All the materials used here were commercial available, and of dairy hygienic standard where possible. Table 3.1 shows the dimensions of the stainless steel tubes.

Table 3.1 Dimensions of the stainless steel tubes

	outside diameter	wall thickness	inside diameter	material code
inside tube	d_o = 12.70 mm	w = 0.90 mm	d_i = 10.90 mm	T316
outside tube	D_o = 25.40 mm	W = 0.90 mm	D_i = 23.60 mm	T316



The outer tube was insulated with plastic foam (CENTURYLON AP701).

Each one metre section of the heat exchanger could be dismantled to sample the fouling layer at the end of a run. Fouling resistance was determined by measuring the temperature difference between the hot and cold sides. Type T thermocouples were used to measure temperatures throughout the experiments. They were calibrated with ice-water and boiling water.

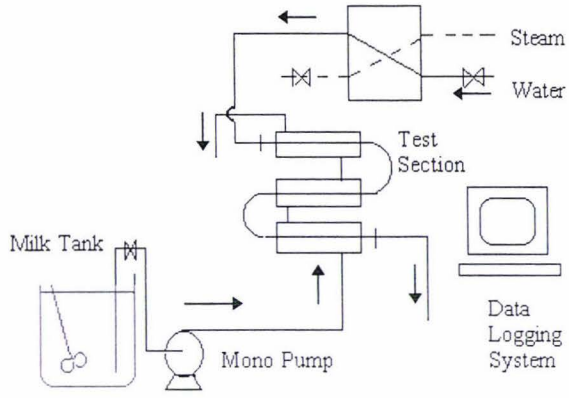


Fig. 3.1: Schematic of the custom built fouling rig

Fig. 3.2: Photograph of the fouling rig

The 2 half-metre tubes were specially designed to aid structural analysis of the fouling layers on both the heated and unheated surfaces.

1. Because milk fouls worse at higher temperatures, the last two short tubes were expected to have more fouling deposits.

2. They were easily dismantled and the fouling layer on the heated surface could be collected without structural damage.
3. If there was any fouling deposit on the unheated surface, that fouling layer also could be collected without structural damage.

A small "window" was cut in the outer tube as shown as Fig. 3.3 (a). A stainless steel cover was made to fit the "window" accurately and was kept in place with aluminium tape to ensure a smooth inside surface. At end of the run, the cover was to be removed before other parts were dismantled and dried with the fouling layer still attached at room temperature (However, no significant fouling of the (unheated) window occurred during any of the trials). Then, the inner tube was pulled out. The nine sets of tubes are connected by U shape stainless steel tubes and steam rubber hoses fastened by stainless steel hose clips. Fig. 3.3 (b) shows the connections between the tubes.

Fig. 3.4 illustrates further structural features of the custom designed heat exchanger. There were plastic stoppers on both ends of each double tube section. O-rings were used to help seal the system tightly. Heat resistant rubber tubing was used in the connections. A steel washer was welded onto each end of the inner tube to prevent the inner and outer tubes from touching. Washer diameter was just slightly smaller than the inner diameter of outside tube (D_i); the washer thus helped to keep the inside tube centred, making it easy to set up the rig. With this design, the fouling deposits on heated and unheated surfaces could be collected and analysed separately.

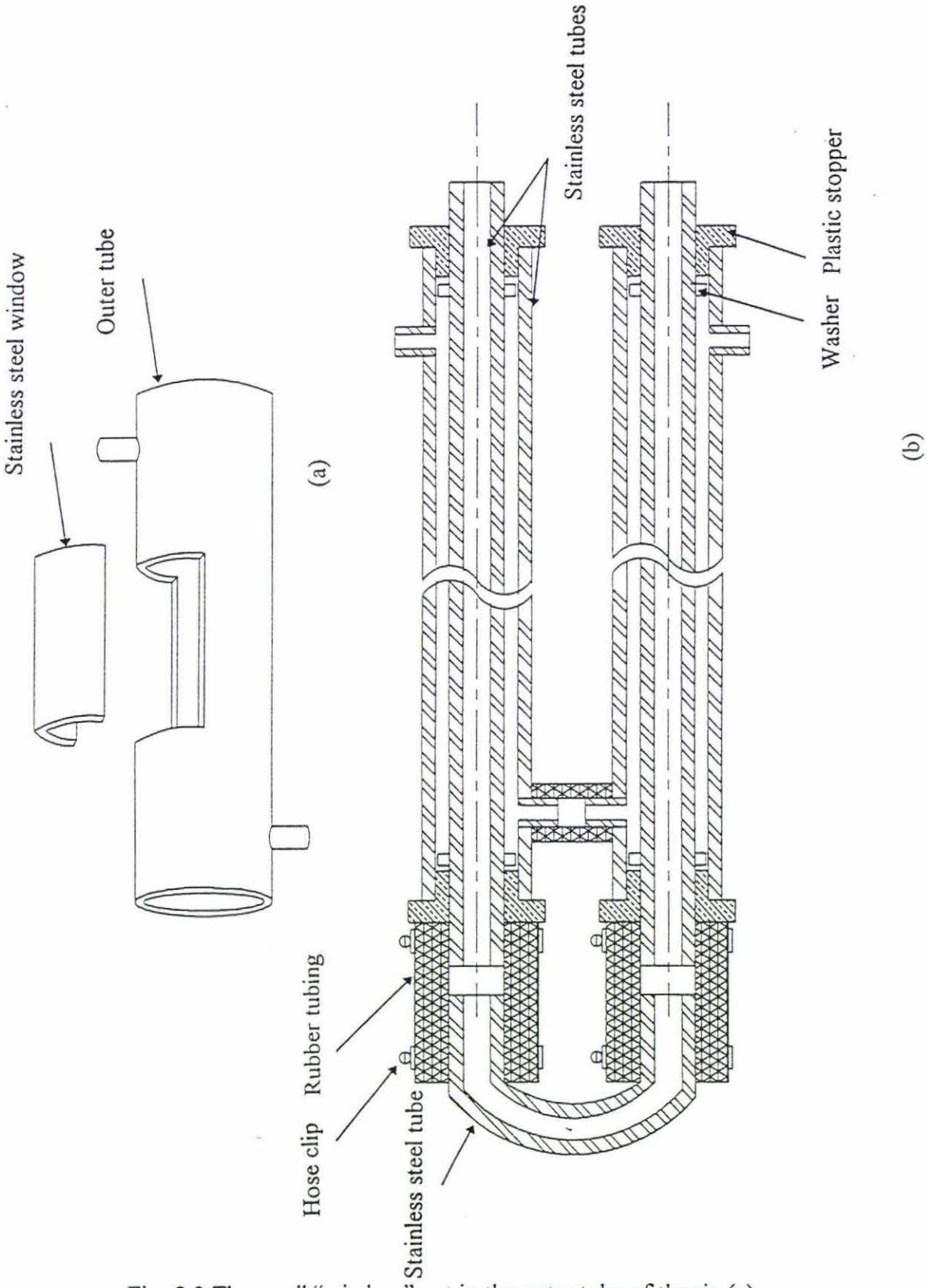


Fig. 3.3 The small "window" cut in the outer tube of the rig (a) and the method of connecting the tubes (b)

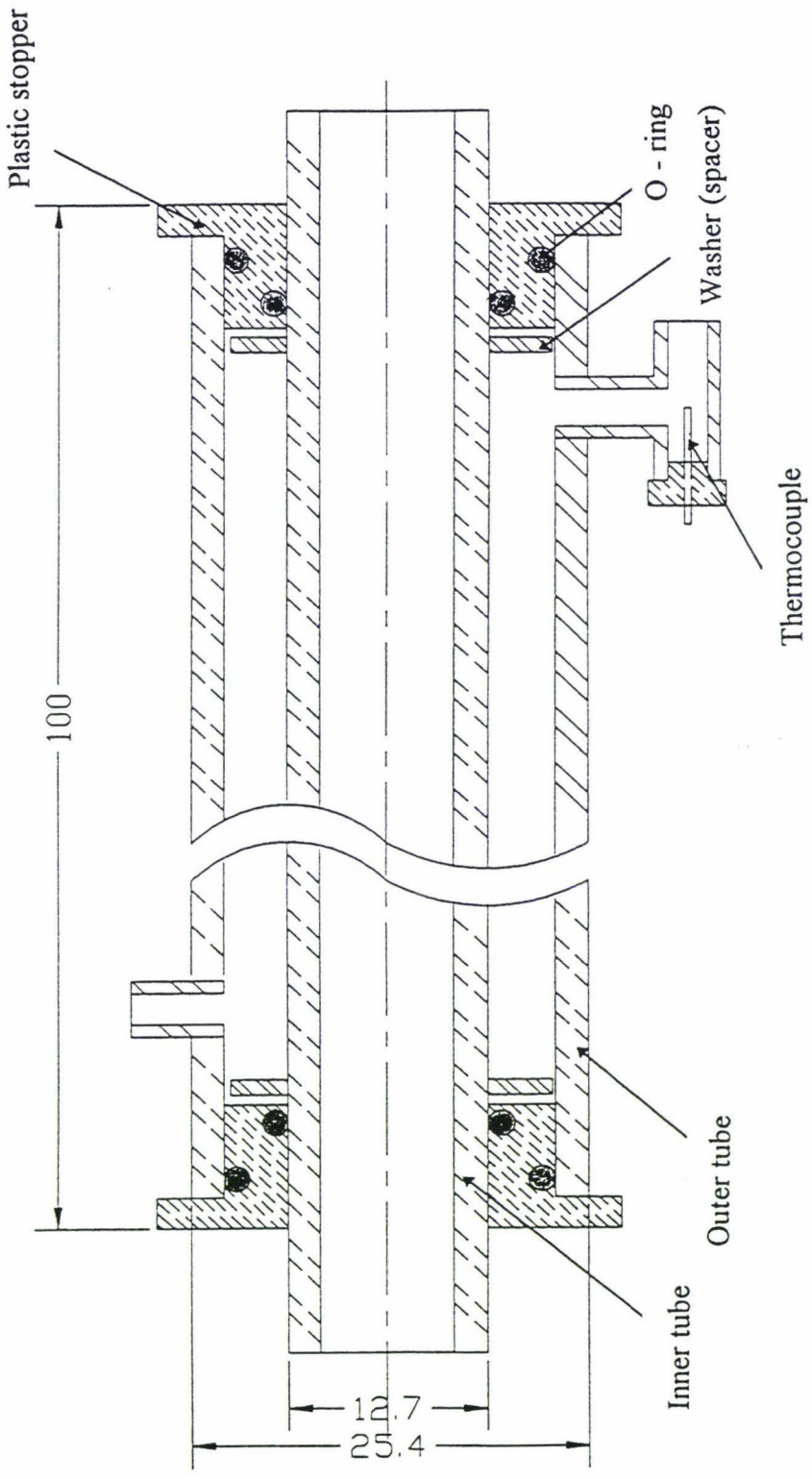


Fig. 3.4 Structural features of the heat exchanger

A positive displacement pump (Monopump Ltd., Type: SB15R5/H110, made in UK) with a speed controller (ZENZER, model: MSC-S7, made in Australia) was used to deliver milk to the double pipe heat exchanger. As the milk inlet was at the lower end of the heat exchanger, a comparatively stable milk flow rate was achieved with this pumping system.

Hot water was used as the heating medium. It was prepared in two stages. First, tap water was preheated by steam injection, then passed through a plate heat exchanger (PHE). Fresh steam was supplied to the other side of the PHE to give the required water temperature (~98°C). This process was required because available heat exchangers did not have the capacity needed to produce hot water at the required temperature and flow rate. Fig. 3.5 illustrates the process used to prepare hot water as just described.

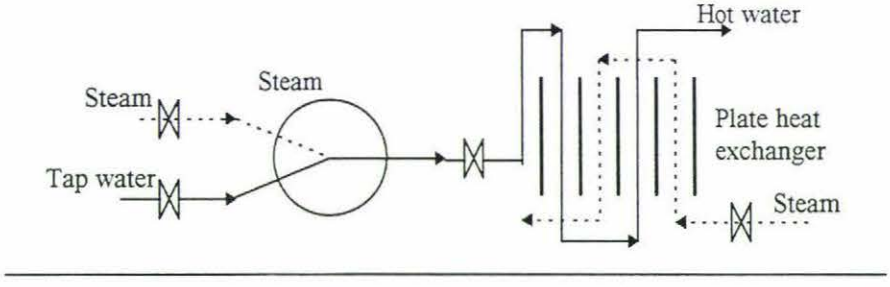


Fig. 3.5: The hot water supply system for the fouling rig

The flow rate of milk was kept as low as possible to economise on the amount of milk used. The minimum flow rate practicable in the fouling rig was 40 - 50 litres per hour. The milk was heated from about 4°C to about 93°C.

A laboratory scale agitator (Heidolph, Type: RZR 50, made in Germany) was installed in the milk tank to prevent milkfat creaming during experimental runs. The blades of the agitator were always immersed fully into the milk to ensure that air was not entrained by agitation.

3.3 Thermocouple Calibration and Locations

3.3.1 Thermocouple Calibration

Type T thermocouples were used to measure temperatures. They were mounted on the rig and connected to a computer data logging system. The software package used to display and record the signals was FIX DMACS 5.5 for Windows (Intellution Inc. USA).

The thermocouples were calibrated with ice-water and boiling water. The ice point (0°C) was supplied by the equilibrium between ice and air-saturated water. The water used was distilled to avoid error caused by contamination. Small ice particles were produced by an ice-making machine. Ice and water were mixed thoroughly in a flask to supply a constant temperature throughout. Ice was added and excess water removed periodically to maintain the temperature of the mixture as stable as possible. The temperatures read from each thermocouple were recorded. Then, the thermocouples were immersed in a kettle with boiling distilled water which was assumed to be at 100°C. The water was kept boiling during the calibration. The regression of real temperature and reading of thermocouples used was assumed to be

a straight line following the equation y=ax+b. Table 3.2 records the results of thermocouple calibration experiments.

Table 3.2 THERMOCOUPLES CALIBRATION

Thermocouples		Thermocouple reading		Regression	
Number	Location	Ice-water(°C)	Boiling water (°C)	a	b
0	MIS	-0.1	100.5	0.994	0.0994
1	MOE	0.5	101.5	0.99	-0.495
2	WIS	-0.9	99	1.001	0.9009
3	WOE	-0.5	100.2	0.993	0.4965
4	WIT	-0.6	99.7	0.997	0.5982
5	WOT	-0.5	100.5	0.99	0.495
6	WIL	-1	99.4	0.996	0.996
7	WOL	-0.9	100	0.991	0.8919
8	MIT-**O**	-1.3	98	1.007	1.3091
9	MOT-**O**	-1.3	97.9	1.008	1.3104
10	MIL-**O**	-2.4	95.4	1.023	2.4552
11	MOL-**O**	-1.8	96.6	1.016	1.8288

Symbols: M --- MILK
W --- WATER
S --- STARTING POINT
E --- END POINT
I --- INLET
O --- OUTLET
- **O** --- OUTSIDE SURFACE
T --- TEST TUBE (SHORT), THE EIGHTH TUBE FROM BOTTOM
L --- TEST TUBE (LONG), THE THIRD TUBE FROM BOTTOM
Regression equation: y=ax+b

Sample calculation:

In equation y=ax+b, y stands for the real temperature and x stands for the reading of the thermocouple. For example, for thermocouple MIS, the reading was -0.1°C for ice-water (0°C), and 100.5°C for boiling water (100°C). So, $y_1 = 0$, $x_1 = -0.1$; $y_2 = 100$, $x_2 = 100.5$, thus, $a = 100/(100.5-(-0.1)) = 0.994$, $b = -(-0.1)\times a = 0.0994$.

3.3.2 Thermocouple Locations

12 thermocouples were mounted at locations on the outer surface of the outside tube or the inner surface of the inside tube, about 5cm from the end of tube. Their locations are shown as follows:

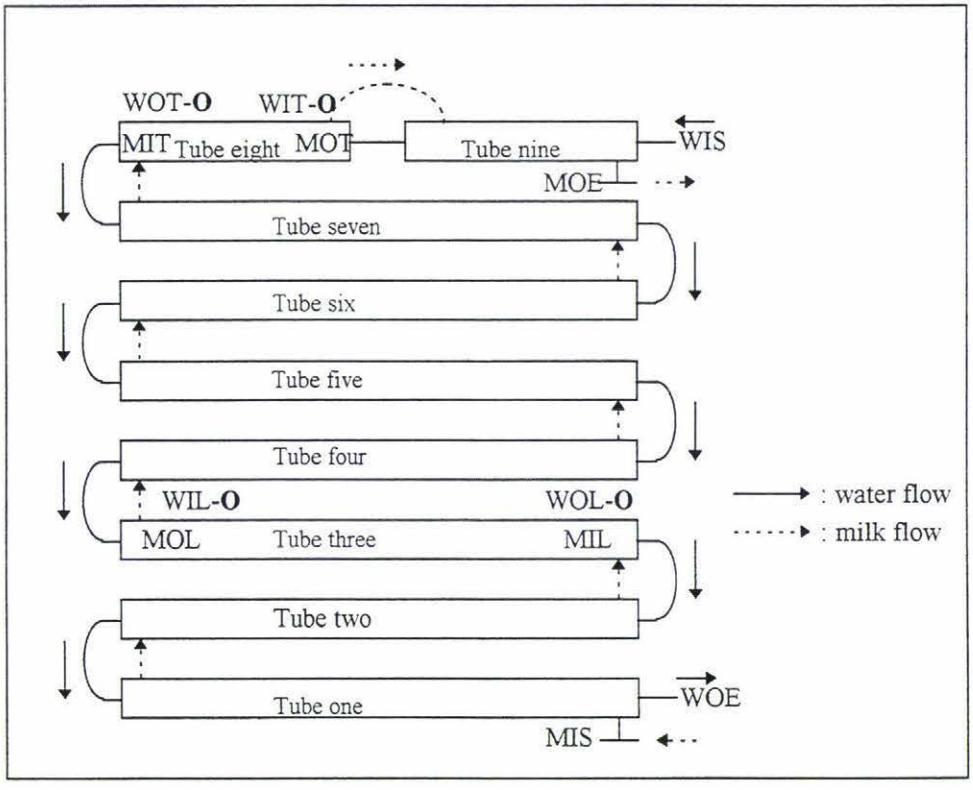


Fig. 3.6 Locations of thermocouples on the counter-current flow heat exchanger

3.4 Method of Damaging the Milk Fat Globule Membrane

As described in Section *2.6.2*, many industrial operational factors can damage the MFGM. They affect the MFGM in different ways. In this project, the MFGM was damaged by mechanical stress during pumping, caused by cavitation.

3.4.1 Cavitation

Cavitation is a phenomenon prevalent when pump sizes are inadequate for required duties. It occurs when a centrifugal pump operates at too high flow rates, causing low pressures to develop at the impeller eye. Essentially the pressure near the pump impeller becomes so low that the liquid vaporises. Tiny vapour bubbles are formed. When these migrate to the pump exit, where the pressure increases again to high levels, they collapse, tiny jets of fluid rushing into the space they occupied. The impact of these high velocity jets is very strong. The formation and collapse of vapour bubbles is called cavitation. Stresses of up to 10,000 kPa can develop during this process. Stresses are so high that they can damage even the steel of the impeller (Fig. 3.7). It is not surprising that they can damage the milk fat globule membrane.

Cavitation can be avoided by maintaining a high inlet pressure at the suction side of the pump. This is achieved by minimising the pressure loss in the suction line (large pipe diameter, short line, few valves, few bends, etc.), maintaining a high liquid level above the pump inlet, and having the liquid temperature as low as possible (Bylund, 1995).

At higher temperatures, fat globules have a lower solid to liquid fat ratio and are easily damaged by mechanical forces. The milk inlet temperature during fouling experiments was 4°C. In order to avoid the influence of temperature fluctuations, the cavitation treatment was carried out on cool milk (~ 4°C) directly from the chill room.

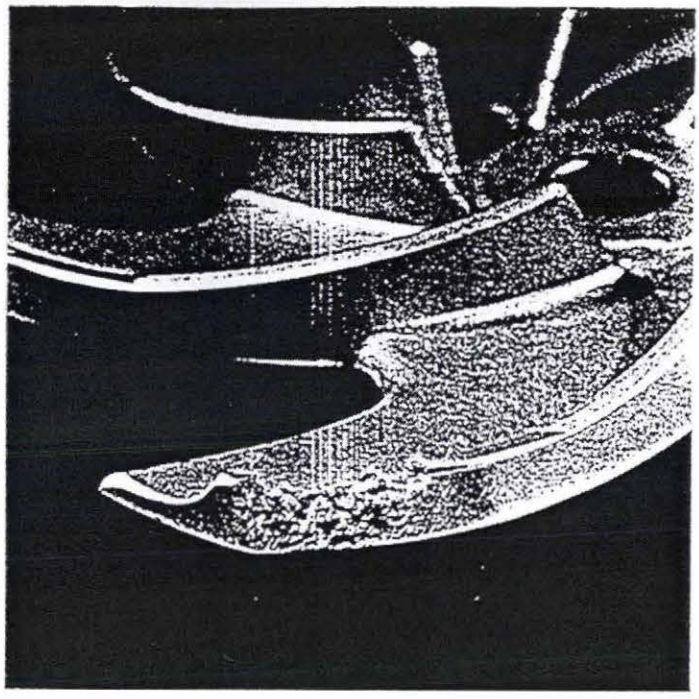


Fig. 3.7: Damage of pump impeller blade owing to extremely high mechanical stress caused by cavitation (Adapted from Rudzik, L., 1987)

3.4.2 Cavitation Rig

The cavitation rig consisted of a large centrifugal pump (Fristam, Type: FP712KF, 1.1kw, 2800 rpm, $\cos\varphi = 0.82$, made in Brazil) with two valves and a pressure gauge on its suction line (Figs. 3.8 and 3.9). A flowmeter (order number: 30FT25-AA1AA11A21B, EMC Industrial Instrumentation, New Zealand) was used to measure milk flow rate. Cavitation was achieved by throttling the valves so as to achieve a high vacuum at the pump inlet. The rig was first rinsed and tested with tap water. The suction side pressure achieved was around -93 kPa gauge with tap water, and -97 kPa to -100 kPa gauge with milk. This was reasonable, because the saturated pressure of water is higher than that of milk, at a given temperature. Since the vacuum at the eye of impeller would have been higher than that have been at the pump inlet,

when the gauge reading was equal to the vapour pressure of milk, the pump would have surely cavitated.

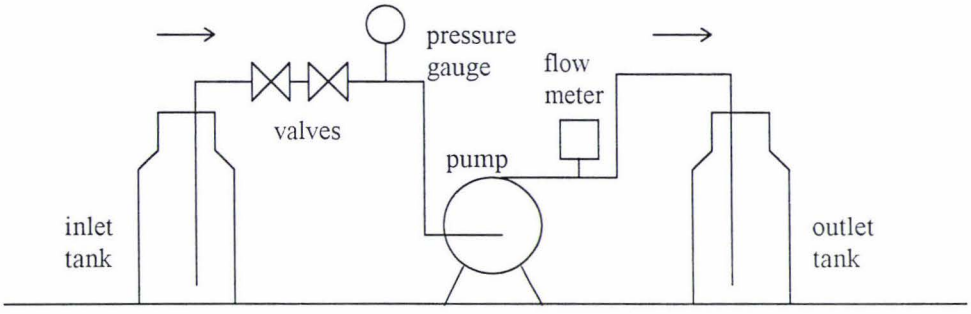


Fig. 3.8: Cavitation rig

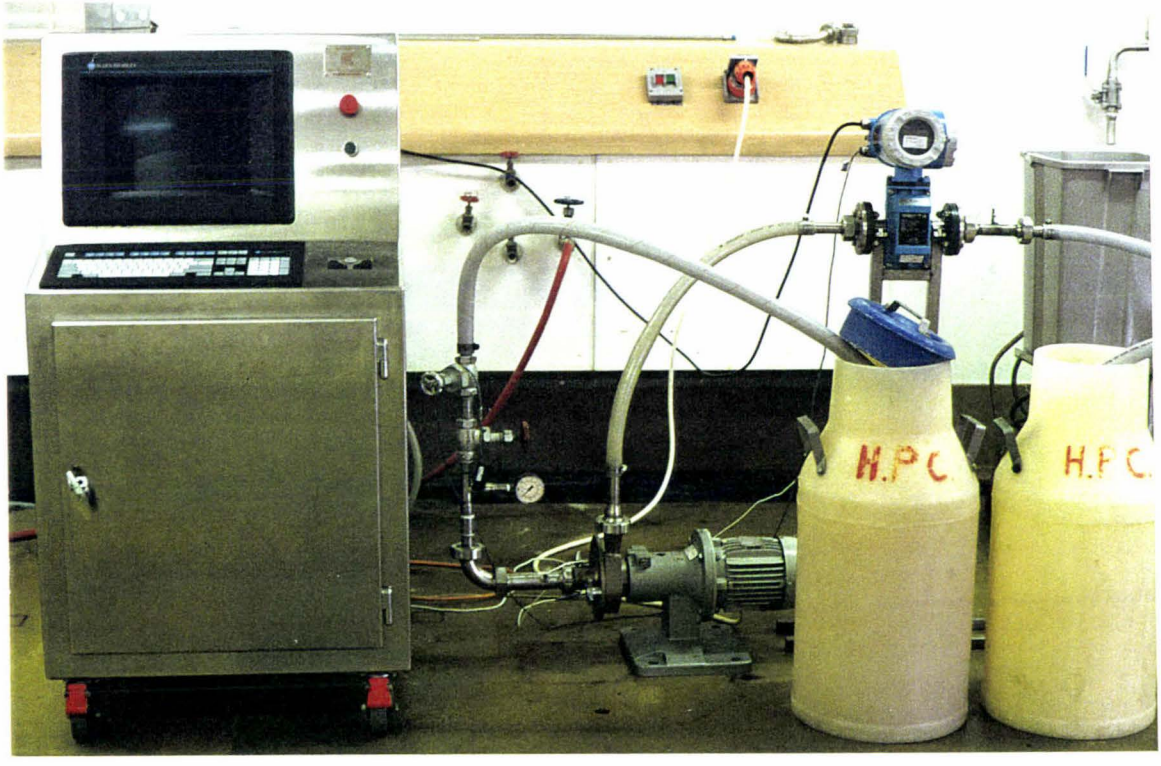


Fig. 3.9 Photograph of the cavitating rig

3.5 Methods of Measuring Milk Fat Globule Membrane Damage

In the strictest sense, proof of damage to the MFGM can be achieved by :

(a) Direct microscopic observation of the membrane. This would require higher magnification than was used in this work and would be expensive and time consuming.

(b) Biochemical analysis of the increase in phospholipids, as well as other components of the MFGM, in the milk serum.

These methods are too complicated for routine analysis, and were unsuitable for the present work within the time available. However, other indirect parameters can be used to indicate the extent of MFGM damage.

It was proposed to determine MFGM damage by four complementary techniques: an extraction based method for FF, a lipolysable FF test (FFA content), fat globule particle size distribution by Malvern Mastersizer (Type E, Malvern Instruments) and microscopic observation using a confocal laser scanning microscope (Leica TCS NT, Leica Microscopy and Scientific Instruments Group, made in Switzerland). Details are given in the following.

3.5.1 Confocal Laser Scanning Microscopy

Fig. 3.10 shows the computerised Leica confocal laser scanning microscope. This microscope achieves an x-/y- resolution of 0.2 μm and z-resolution of 0.6 μm.

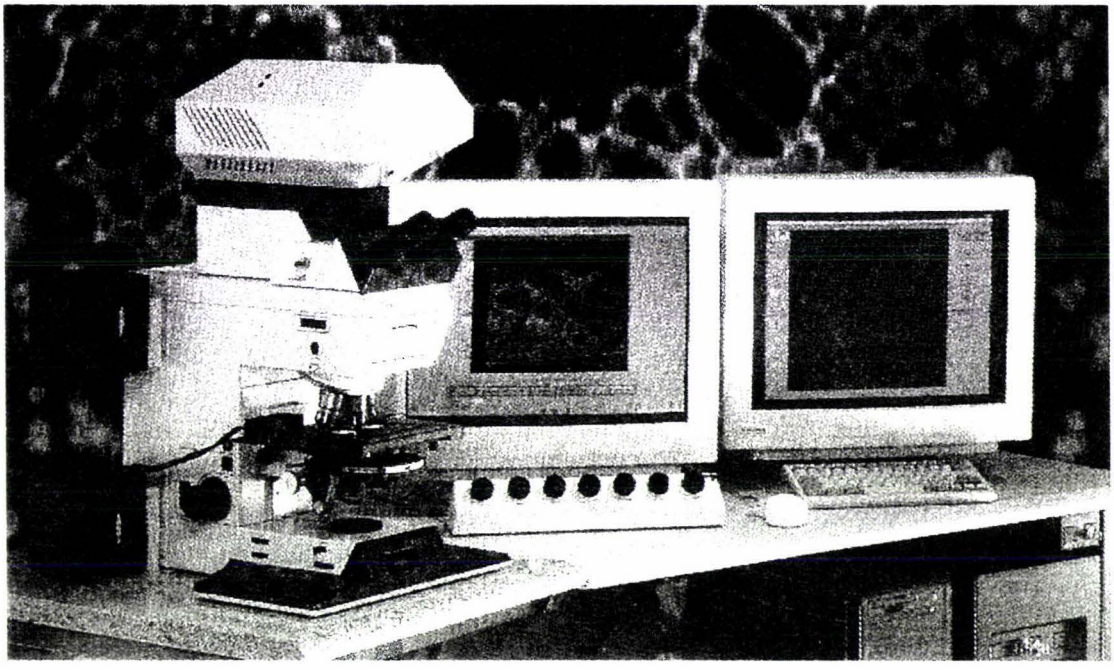


Fig. 3.10: Picture of Leica confocal scanning laser microscope

1% Nile Blue solution was used to stain fat globules, and 1% Fast Green solution was used to stain proteins in the experiments. A kind of cavity microscope slide, designed for liquid samples, was used for milk samples. The smaller the depth of the cavity, the better the avoidance of milkfat creaming effects. The slide was prepared with a well-mixed milk sample just before the measurement. The apparent diameter of a fat globule as seen by the microscope depended on the scanning depth within the globule itself. Also, some creaming of fat globules may have occurred in the slide cavity. Some consistency in globule imaging was, hopefully, attained by always scanning the central horizontal plane of the slide cavity. An oil lens with 63 times magnification was used in this study.

Dried fouling layers were also scanned using this microscope. A lens giving 10 times magnification was selected for an overall structural study.

3.5.2 Free Fat (FF) Content

In principle, fat globules "free" from the protection of their natural membranes should be separable from those with such protection. Then, one can expect more "free fat" to be recoverable with more MFGM damage. The free fat test was based on the New Zealand standard method published by the Dairy Division in 1979 (Appendix 1). It is an extraction method. Some modifications were made to this method in the present research. These are discussed in Section *3.5.4.*

In comparing centrifugal and extraction methods, it was noted that the centrifugal method is suitable only after a prolonged period of milk storage, which allows enough time for free fat to move through damaged membranes to the outside medium. The extraction method gives an early indication of damage (Fink and Kessler,1986). Thus, an extraction method was chosen to estimate MFGM damage in this work.

3.5.3 Lipolysable Free Fat (LFF) Content, Measured as Free Fatty Acid (FFA) Content

Lipase acts on fat globules without natural membrane protection, causing the release of free fatty acids. Lipolysable free fat is defined as the fraction of milk fat that can

be hydrolysed by lipase (natural or external) to release FFA, as a consequence of poor milk handling. The amount of free fatty acids produced depends mainly on factors such as incubation temperature, time and lipase activity. Miller and Puhan (1986b) suggested that the results of a lipolysable fat test could constitute an indicator for fat damage in raw milk. When the sample is incubated under controlled conditions, the induced FFA content should indicate the extent of MFGM damage as well.

The induced free fatty acid content was measured by the New Zealand standard method published by the Dairy Division in 1980 (Appendix 2). The FFA are extracted with a mixed solvent (diethyl ether : petroleum ether = 2 : 1) and titrated against potassium hydroxide (KOH). This extraction method for FFA measurement gives a high recovery of the lower molecular weight fatty acids, ranging from 63% for butyric acid to 97% for stearic acid. Thus, this method is sensitive and can estimate most of the fatty acids present (Perrin and Perrin, 1958; Joerin and Bowering, 1973). It is suitable as a routine method for small numbers of samples.

3.5.4 Modifications of Free Fat Test and Free Fatty Acid Test

Modifications of free fat test

As mentioned above, the free fat content was measured by an extraction method (see Appendix 1).

The procedure required the sample to be tempered at 40°C (step 5, Appendix 1). During the initial stages of the experimental work, which started in July, gelation

occurred after shaking the mojonnier tube filled with petroleum ether and the milk sample. Since it was winter time, the temperature difference between the ether and the sample was larger than in summer, which might have lead to easier gelation. Another factor which was considered was the extent of inversion; inverting the tube 10 times within 10 seconds as stated in the standard method (step 9, Appendix 1), seemed too vigorous, and might have precipitated gelation. Thus, two modifications of the free fat test were made as follows.

1. Put the mojonnier tube with ether and sample into a 40°C water bath for 30 seconds before shaking to minimise the temperature difference between ether and sample (step 8, Appendix 1). Of course, the stopper should be held in place while tempering in the water bath. After this treatment, invert the tube as gently as possible to mix the solvent and sample thoroughly. The shaking time was changed to 40 seconds (step 9, Appendix 1).

2. Unhomogenised milk creams rapidly. This causes the fat content to vary significantly with depth of sampling. In order to attain a representative sample but avoid the effect of extra free fat caused by shaking, inversion of the sample container 10 times before sampling was set as a standard step (step 4, Appendix 1).

Modifications of FFA test

Generally, the unmodified extraction method for FFA test described in Section ***3.5.3*** gave a satisfactory result with unpasteurised raw milk. Since pasteurised milk was used in this study, most of the natural lipase would have been destroyed by the

pasteurisation process. Thus there probably was insufficient natural lipase available to hydrolyse fully the free fat generated. External lipase was therefore added to the sample to determine the amount of lipolysable free fat present. Pancreatic lipase was found suitable for the present research.

In the Trials 2 and 3, pancreatic lipase was added to the pasteurised milk sample (100ml) to give a concentration of 10 ml/litre of milk. Then, the samples were incubated at 30°C in a warm room for approximately 24 hours. With these modification, the FFA measurement showed significant differences between untreated milk and milk passed through the cavitating pump.

The concentration of pancreatic lipase used in Trial 1 (5ml/litre) was half of that in the other two trials, and the incubation time was 18 hours. With such treatment, the concentration of FFA released was thought a little low, and thus a higher concentration and longer incubation time were used in latter trials (as described above). Also, because of the constraints of gaining access to the Mastersizer, the confocal microscope or the facilities in the pilot plant, the last runs of Trials 1 and 2 was carried out one week after the first two runs. Thus their FF and LFF contents would have been useless for comparing MFGM damage. Trial three was successfully carried out within three days, and the FF and LFF contents were measured immediately.

The modified FFA test is called here the lipolysable free fat (LFF) test.

The results obtained by applying the FFA test to an aged pasteurised milk sample without added lipase, incubated at 30°C for 48 hours after cavitation treatment, are shown in Table 3.3. The milk was stored at 4°C for 6 days before cavitation treatment. The FFA contents obtained were quite high.

Table 3.3: FFA content without added lipase, milk samples incubated at 30°C for 48 hours after storage at 4°C for 6 days followed by cavitation treatment

Pasteurised milk		FFA content mmol/litre)
Untreated	1	6.62
	2	6.40
	3	6.51
	average	6.51
One cavitation pass	1	7.61
	2	7.37
	3	7.49
	average	7.49
Two cavitation passes	1	7.78
	2	7.88
	3	7.83
	average	7.83

Different results were obtained when an external lipase was added to sample prior to incubation. Two kinds of lipase were tested: pancreatic lipase and calf lipase.

Pancreatic lipase (SIGMA Chemical Co.) was commercially available in powder form. It was dissolved in distilled water and stored at 4°C before being added to a fresh pasteurised milk sample (100ml) to give a concentration of 10ml/litre of milk (which is about 5 times the concentration of the natural lipase in milk). Then, the samples were incubated at 30°C for about 24 hours. The FFA content of untreated samples obtained in this method were up to 1 mmol/litre (Table 3.4) and the difference

between untreated and treated samples were larger than in the case of aged pasteurised milk no added lipase (Table 3.3).

Calf lipase supplied by the New Zealand Rennet Company was also trialled in this study. It was produced from calf tongue secretions and came in liquid form. The calf lipase was diluted and added at a concentration of 0.11 LFU/ml to the fresh pasteurised the milk sample (100ml), where LFU/ml stands for lipase fore-stomach units/ml. The conversion of units LFU/ml for calf lipase and ml/litre for pancreatic lipase was not studied here. The samples were at 30°C for about 24 hours. After incubation, the milk samples had a very sour smell. Compared with samples using pancreatic lipase, the released FFA concentrations were much higher, but the difference between treated and untreated samples were very small (Table 3.4). It seems that the calf lipase was very active and acted even on those fat globules without MFGM damage. In other words, the calf lipase was not selective.

Table 3.4: Comparison of pancreatic lipase and calf lipase

Pasteurised milk sample		FFA content (mmol/litre)	
		Pancreatic lipase	Calf lipase
untreated	1	1.16	10.10
	2	1.16	10.77
	3	1.13	10.61
	average	1.15	10.49
One cavitation pass	1	3.13	10.40
	2	3.24	10.53
	3	3.11	10.67
	average	3.16	10.53

These results indicated that it was reasonable to add external lipase to study MFGM damage in milk samples in which insufficient active natural lipase was present to turn

all lipolysable free fat into FFA. This method was more efficient and sensitive than simply incubating samples for a long time (48 hours). Of the two lipases tested in present study, pancreatic lipase was found to be suitable while calf lipase was considered to be non-selective. However, the present study was essentially exploratory; other kinds of lipase need to be tested, and an optimal lipase concentration determined. The incubation time is another issue which needs further attention; the shorter the time the more efficient the test will be.

The technical characteristics of the lipases used in the present study are given in Appendices 3 and 4.

3.6 Composition Analyses of Liquid Milk Samples and Fouling Deposits

The following chemical analyses was carried out on liquid milk samples or fouling deposits or both. All analyses were done in triplicate. The methods described in this section are given in Appendices 5 - 8.

3.6.1 Moisture Test

A known weight of fouling deposit was dried in a 102°C air oven to constant weight, and cooled in a desiccator at room temperature, and weighed. The detailed procedure is given in Appendix 5.

3.6.2 Ash Test

A known weight of fouling deposit was charred over a bunsen burner and ashed in a muffle furnace at 550°C for 4-5 hours. The white ash was cooled in a desiccator at room temperature and weighed. The detailed procedure is given in Appendix 6.

3.6.3 Total Fat Content

The total fat contents of liquid milk samples and fouling deposits were measured by the Mojonnier method. The detailed procedure is given in Appendix 7.

3.6.4 Total Protein Content

Total protein contents of liquid milk samples and fouling deposits were measured using the Kjeldahl method and a total nitrogen to protein conversion factor of 6.38. The detailed procedure is given in Appendix 8.

3.7 Sampling Method

Generally, a sample had to be representative, and microbiological and chemical contamination had to be avoided. The sample had to be protected against any change during the period between sampling and analysis.

Liquid milk was thoroughly mixed by agitation before random sampling. Clean, dry and sterile plastic bottles were used to store the samples. The samples were labelled, cooled immediately and maintained at about 4°C. Three 100ml milk samples were collected in each run. One for the free fat test, one for the lipolysable free fat test and the last for microscopy and particle size analysis.

Fouling deposit from the heating surfaces of 7 tubes (not the 8th tube at the milk outlet end of the heat exchanger) was collected and dried at room temperature. The dry deposit was milled to a powder before any chemical analysis. The mill used was Type A10, 20,000 rpm, 180W, manufactured by Janke & KunKei GmbH & Co. KG. Since the deposits were always very fatty, it was difficult to mill the deposits into fine powders. The particles obtained were still fairly large.

Part of the fouling layer (that on the last two 0.5 meter tubes of the heat exchanger) was left attached to the steel heating tube (inner tube) and dried at room temperature to keep its structure undamaged for microstructural analysis later. These samples were cut with a very sharp blade to protect the structure and glued on to microscope slides with Araldite.

3.8 Fouling trials

Three formal trials were conducted during this research. Each trial included three individual experiments, two with a different number of passes through the cavitating pump and one control run with untreated milk. On average, each trial consumed

nearly 400 litres of pasteurised milk. A different batch of milk was used for each trial. Each experiment included cavitation treatment to damage the MFGM, passing the milk through the fouling rig, measurements of MFGM damage and of fouling deposit structure, and chemical analyses. While the fouling runs themselves took only three days, the analyses were time-consuming and each trial took three weeks to complete.

Only one fouling run could be performed each day. The stability of milk components such as the fat globules, the MFGM and proteins change even under chilled storage. In order to minimise the effects of storage time, the fouling experiments with milk subjected to different numbers of passes through the cavitating pump were randomised among the three trials (Table 3.5).

All experiments were conducted under similar operating conditions. In the fouling rig, the Reynolds number of the milk ranged from 220 to 310, the milk inlet temperature from 3°C to 6°C (the cavitating pumping increased milk temperature), and the initial milk outlet temperature from 90°C to 94°C. The suction side pressure in the cavitating rig varied from -97 kPa to -100 kPa. Table 3.5 summarises key characteristics of the three trials.

A small agitator in the milk tank (Fig. 3.1) was operated at 125 rpm during each fouling run. Thermocouples measuring milk inlet and outlet temperatures were pulled out several times during the experiments to clean fouling deposits on the probes. A stopper replaced the probes temporally to avoid milk leakage and air inclusion.

The rig was rinsed for 4 minutes with distilled water at the same flow rate as for milk at the end of each run, shut down and dismantled to collect the fouling deposit. Finally, the rig was cleaned with 1% caustic solution and rinsed with water to make it ready for another run.

A detailed chronology of experimental work is given in Appendix 9.

Table 3.5: Descriptions of three trials performed

Trial	Runs	Date	Pumping treatment		Milk				Hot water		Analytical tests
			Cavitation passes	Cavitating pressure	Amount	Flow rate	Re	Temp. increase	Flow rate	Temp. drop	
1	1.1	25/09/97	0	*	170L	36L/hr	226	4°C - 90°C	3.6L/min	98°C - 80°C	F,FF, LFF,M,C
	1.2	26/09/97	1	-100kPa	120L	39L/hr	247	6°C - 91°C	3.6L/min	99°C - 82°C	F,FF, LFF,M,C
	1.3	1/10/97	2	-97kPa	90L	36L/hr	226	4°C - 94°C	3.6L/min	100°C - 85°C	F,M,C
2	2.1	15/10/97	1	-97kPa	160L	48L/hr	303	4°C - 90°C	3.6L/min	98°C - 81°C	F,P,FF,LFF,M,C
	2.2	16/10/97	0	*	160L	48L/hr	303	3°C - 93°C	3.6L/min	98°C - 79°C	F,P,FF,LFF,M,C
	2.3	22/10/97	4	-97kPa	70L	48L/hr	303	4°C - 93°C	3.6L/min	98°C - 81°C	F,P,M,C
3	3.1	29/10/97	5	-100kPa	70L	48L/hr	303	5°C - 93°C	3.6L/min	99°C - 83°C	F,P,FF,LFF,M,C
	3.2	30/10/97	3	-100kPa	90L	48L/hr	303	5°C - 93°C	3.6L/min	98°C - 81°C	F,P,FF,LFF,M,C
	3.3	31/10/97	0	*	210L	48L/hr	303	3°C - 93°C	3.6L/min	98°C - 80°C	F,P,FF,LFF,M,C

Note: Pasterised, unhomogenised full cream milk was used in the experiments. The milk was stored in a 4°C chill room before any process or analysis. A combination of the following tests was carried out in each run.

F: total fat test (Mojonnier method)
FF: free fat test (extraction method)
LFF: lipolysable free fat (induced free fatty acid content) test (extraction method)
P: total protein test (Kjeldahl method)
M: paticle size analysis by Mastersizer
C: micro-appearance by confocal laser scanning microscopy
*: no cavitation treatment for this run

Chapter *4* Results and Discussion

4.1 Properties of Raw Materials

The total fat contents, the total protein contents and the pH values of the three batches of pasteurised milk used are given in Table 4.1.

4.2 Result of Measurements of Milk Fat Globule Membrane Damage

Free fat (FF) and lipolysable free fat (LFF) contents

The free fat and free fatty acid (lipolysable free fat) contents of milk samples measured in each trial are shown in Table 4.2. Although what is actually measured in a free fat test is debatable, the results presented here revealed significant differences between untreated and treated milk and exhibited a trend with increasing degree of cavitation treatment.

The induced free fatty acid (FFA) content in the lipolysable free fat test increased with the number of passes through the cavitating pump, a result expected on the basis of the assumption that an increase in cavitation treatment causes an increase in MFGM damage.

Table 4.1: Some composition data for pasteurised whole milk

Contents		Trial one	Trial two	Trial three
Total Fat (fat/milk,%)	1	4.34	4.20	4.33
	2	4.33	4.22	4.39
	3	4.30	4.16	4.38
	average	4.32	4.19	4.37
Total Protein (Pr./milk, %)	1	NA	3.15	3.13
	2	NA	3.17	3.17
	3	NA	3.19	3.17
	average	NA	3.17	3.16
pH Values		6.56 at 21°C	6.64 at 21.5°C	6.60 at 24°C

Table 4.2: The chemical analysis results for the milk samples

Contents		Trial one			Trial two			trial three		
		Run 1.1	Run 1.2	Run 1.3	Run 2.2	Run 2.1	Run 2.3	Run 3.3	Run 3.2	Run 3.1
		w/o cavitation	one pass	two passes	w/o cavitation	one pass	four passes	w/o cavitation	three passes	five passes
FFA (mmol/l)	1	0.85	1.02	NA	1.16	3.13	NA	1.40	4.64	6.08
	2	0.87	0.95	NA	1.16	3.24	NA	1.49	4.83	6.32
	3	0.83	0.97	NA	1.13	3.11	NA	1.59	4.70	6.02
	average	0.85	0.98	NA	1.15	3.16	NA	1.49	4.72	6.14
FF (FF/fat,%)	1	0.46	0.79	NA	1.62	1.62	NA	1.65	3.94	5.56
	2	0.51	0.67	NA	1.46	1.53	NA	1.42	3.07	5.72
	3	0.53	0.72	NA	1.21	1.86	NA	1.37	3.84	6.11
	average	0.50	0.73	NA	1.43	1.67	NA	1.48	3.62	5.80

Note: the concentration of pancreatic lipase in trial one was half of that in latter trials, incubation time was 18 hours (explaination in Chapter 3),while in other two trials was 24 hours, and NA means the tests were not carried out, so results were not available for that run

Particle size distribution

Figs. 4.1, 4.2 and 4.3 show the milk particle size distribution curves for the three trials . The particle size distribution curves tended to shift to the right (towards larger particle sizes) when the MFGM was extensively damaged by cavitation. A completely new peak was found after extensive cavitation treatment (five passes) in Trial three (Fig. 4.3). But only very small differences could be detected when the MFGM was damaged slightly. Thus the extent of the MFGM damage could not be expressed quantitatively in terms of average particle sizes.

Compared with the particle size distribution curve for the control sample, sometimes the percentage of small particles in the milk increased with cavitation while the percentage of very large particles showed little change (Trial one, Fig. 4.1). At other times (Trial two, Fig.4.2), the percentage of small particles decreased, while the percentage of large particles increased. One possible interpretation is that the cavitating pump broke the fat globules into smaller ones initially, and these smaller fat globules then coalesced to form larger particles later. Thus the length of time between sampling and analysis would have had an effect. The difference between the control sample and the five pass sample in Trial three (Fig. 4.3) was substantial.

Confocal laser scanning micrographs

Figs. 4.4 to 4.12 show micrographs of the fat globules in samples from the three trials. Looking at Fig. 4.4, 4.8, 4.12 (all untreated milk), 4.5, 4.7 (both one cavtiation pass), 4.6 (two passes), 4.4 (three passes), 4.9 (four passes) and 4.10 (five passes) in that order, there is clear evidence the particle size increased with the extent of cavitation treatment.

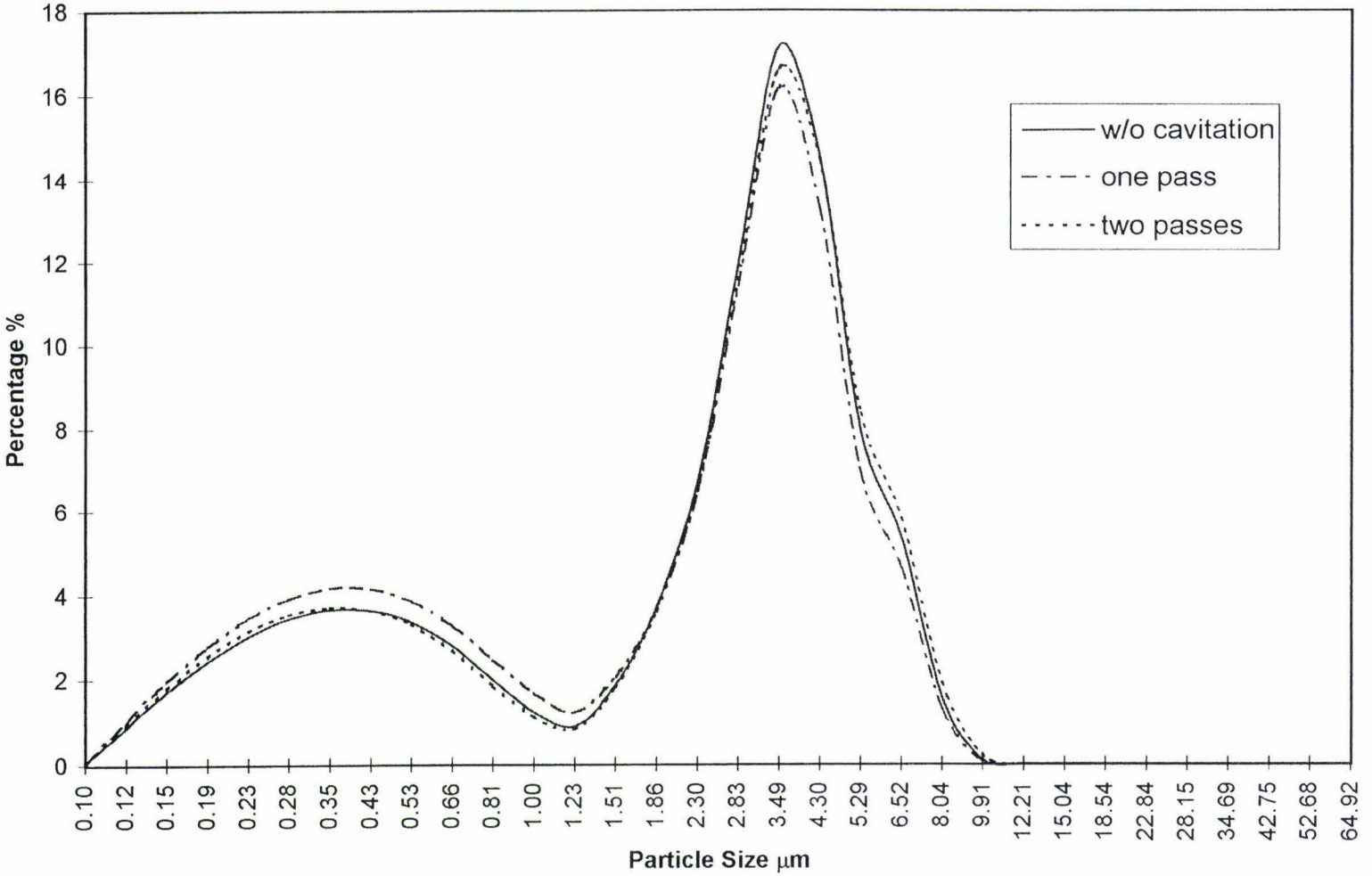


Fig.4.1: Fat Particle Size Distribution - trial one

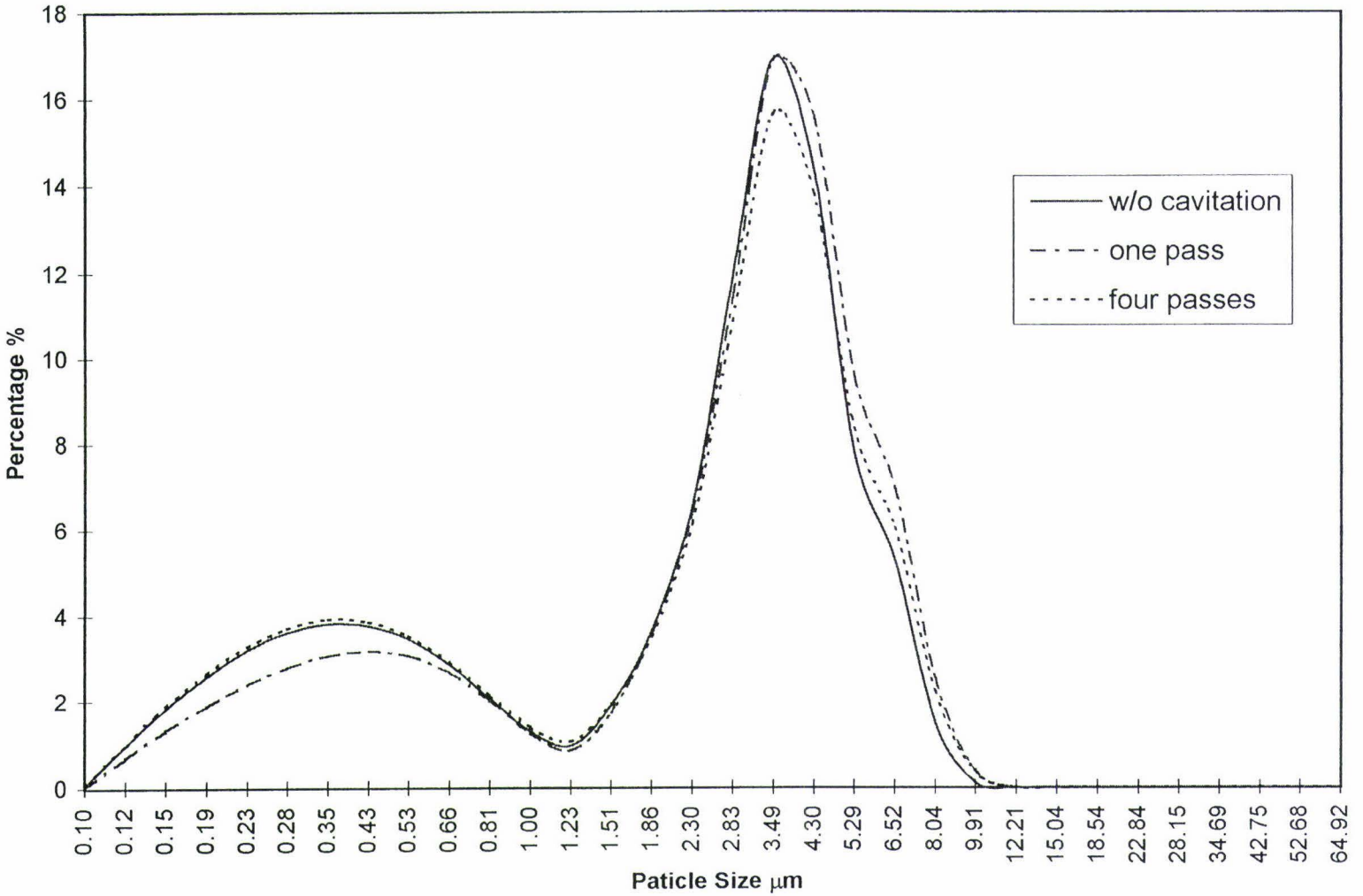


Fig. 4.2: Fat Particle Size Distribution - trial two

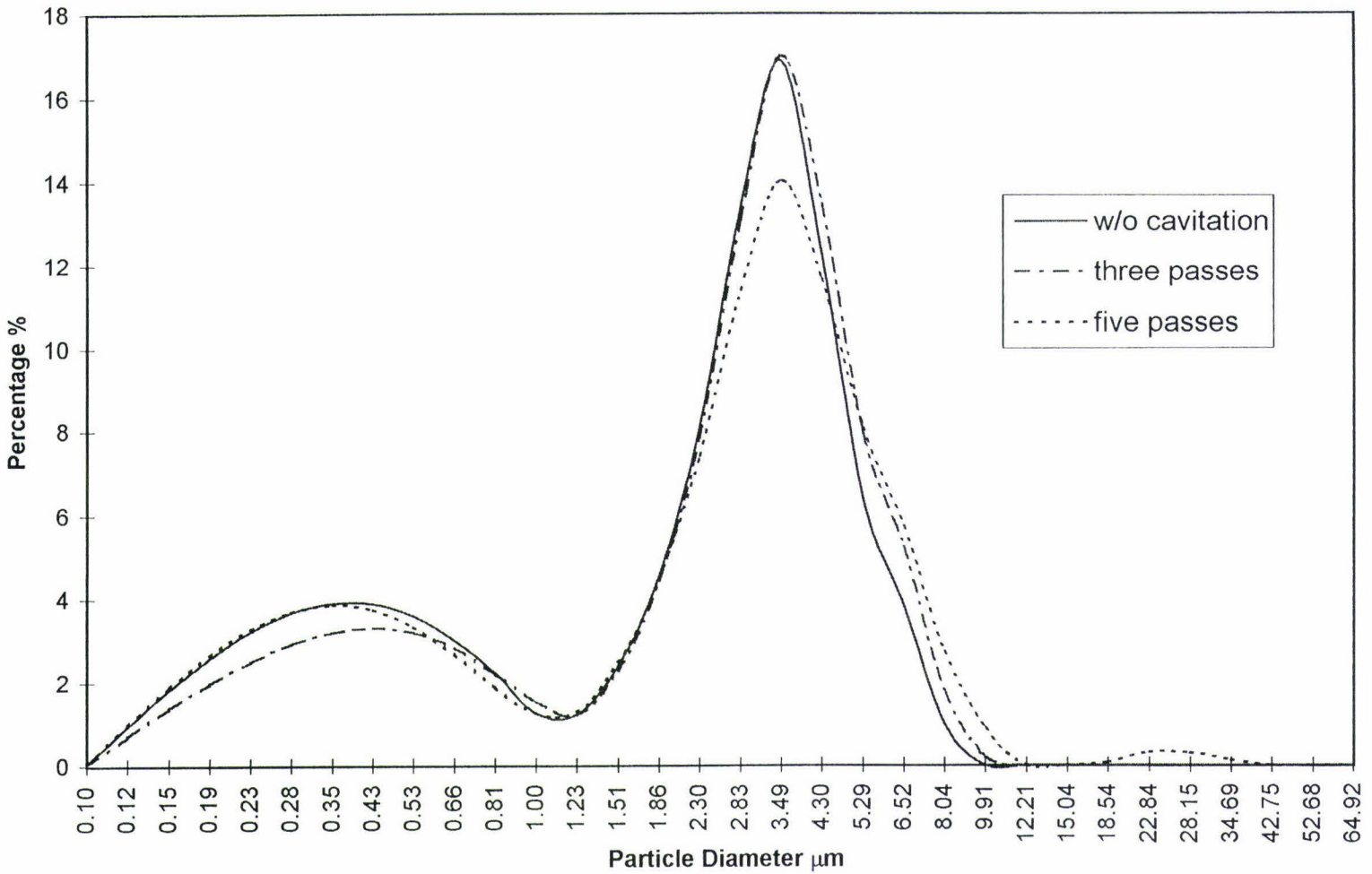


Fig. 4.3: Fat Particle Size Distribution - trial three

This could have been due to globules with damaged MFGMs coalescing more readily. The larger globules were non-spherical.

The micrograph Fig. 4.13 shows both fat and protein (protein is red and fat is green). It can be seen that the proteins, which are thought to be casein micelles, covered the fat globules. But the coverage was obviously not complete.

Some of the microscopy was not performed on the same day as the fouling experiment because of difficulties in gaining access to the microscope.

The effect of cavitation on MFGM damage

There was an increase in both FF and LFF with the number of passes through a cavitating pump. However, the results shown in Table 4.2 indicate that the following.

1. The FF and LFF content of the raw milk supplied varied between trials: the quality of the raw milk was variable. This makes quantitative comparisons between runs difficult.

2. For Trial three, the last trial, in which there was greater experience with analytical and experimental methods, the trends in FF and LFF, with number of passes through a cavitating pump (Fig. 4.14), agree quite well.

3. However, the quantitative agreement is not so good when Trials one and two are included, even though the general trends in both cases are the same. This

is because there was adjustment of the amount of external lipase added, and of the incubation time used, between Trial one and Trials two and three. Because of time constraints, it was not possible to perform further trials.

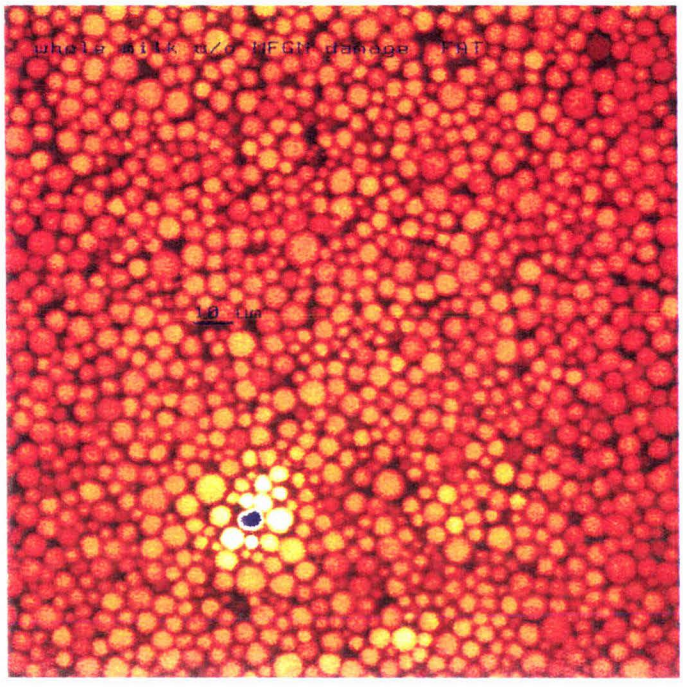


Fig. 4.4 Micrograph (63×) of fat globules in untreated milk. Trial one (run 1.1)

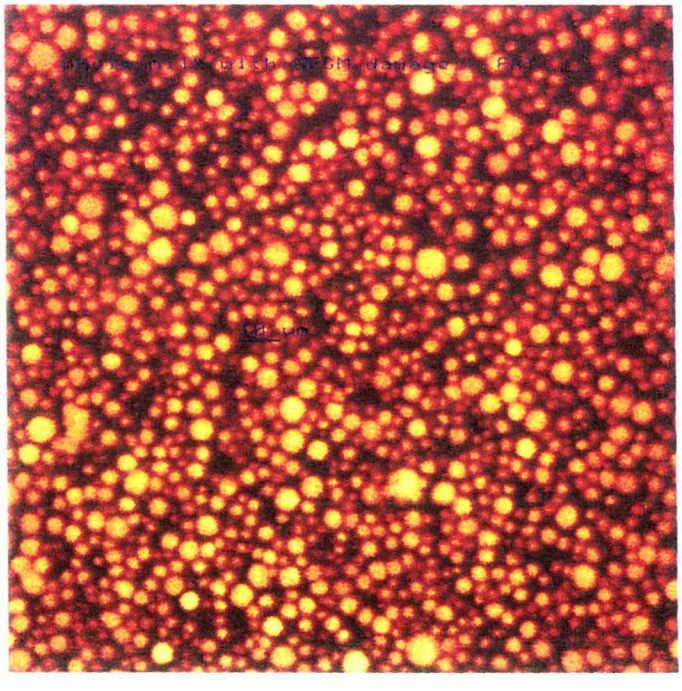


Fig. 4.5 Micrograph (63×) of fat globules in milk after one pass through the cavitating pump. Trial one (run 1.2)

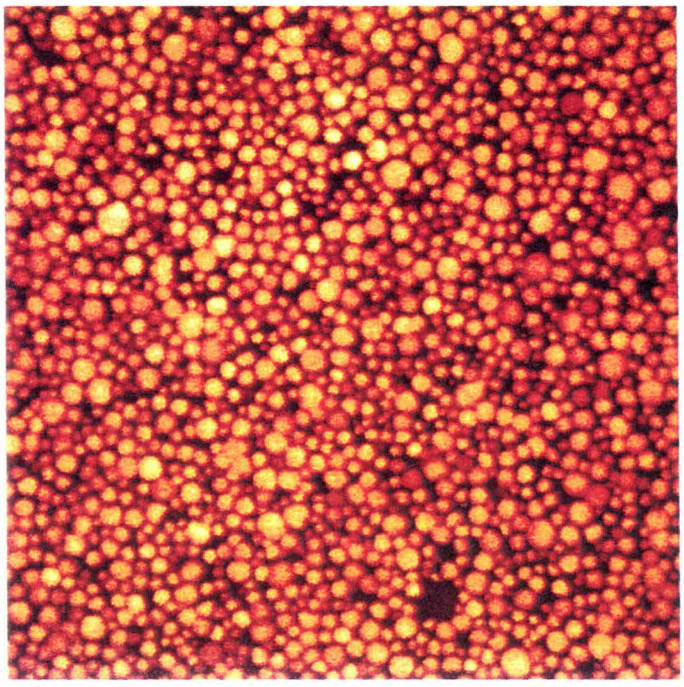


Fig. 4.6 Micrograph (63×) of fat globules in milk after two passes through the cavitating pump. Trial one (run 1.3)

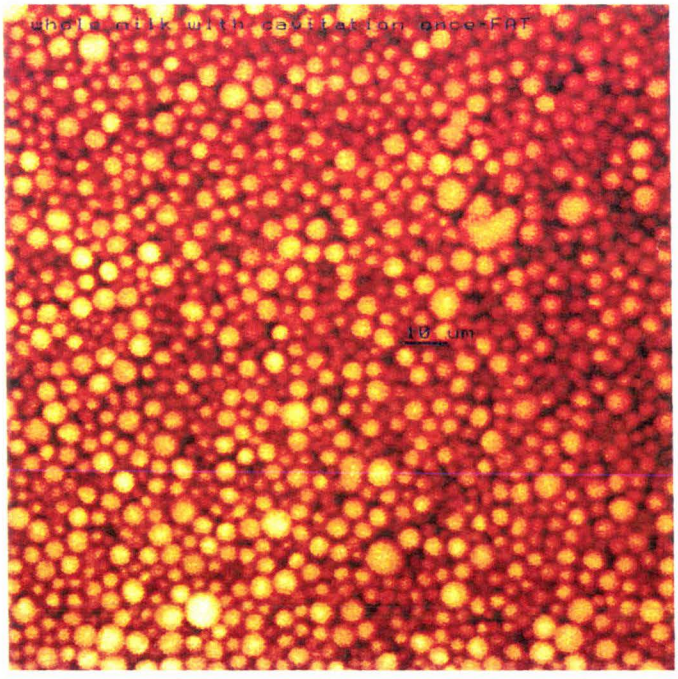


Fig. 4.7 Micrograph (63×) of fat globules in milk after one pass through the cavitating pump. Trial two (run 2.1)

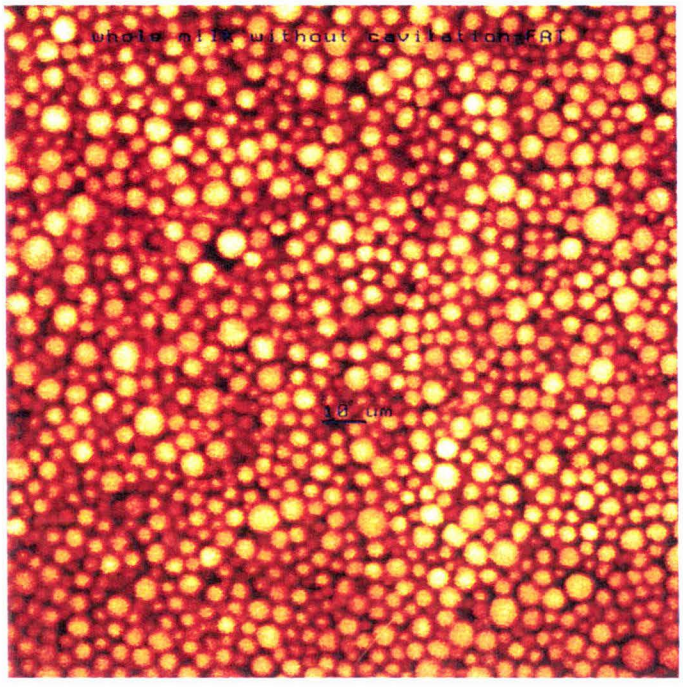


Fig. 4.8 Micrograph (63×) of fat globules in untreated milk. Trial two (run 2.2)

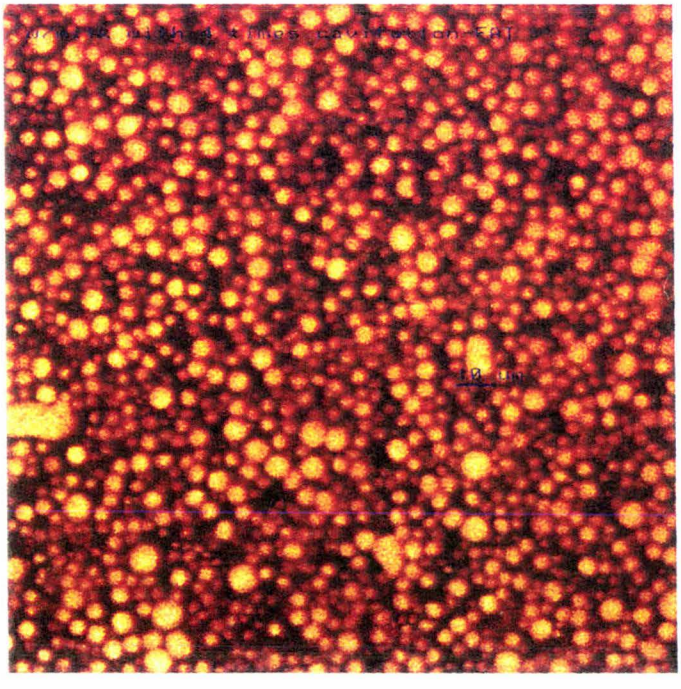


Fig. 4.9 Micrograph (63×) of fat globules in milk after four passes through the cavitating pump. Trial two (run 2.3)

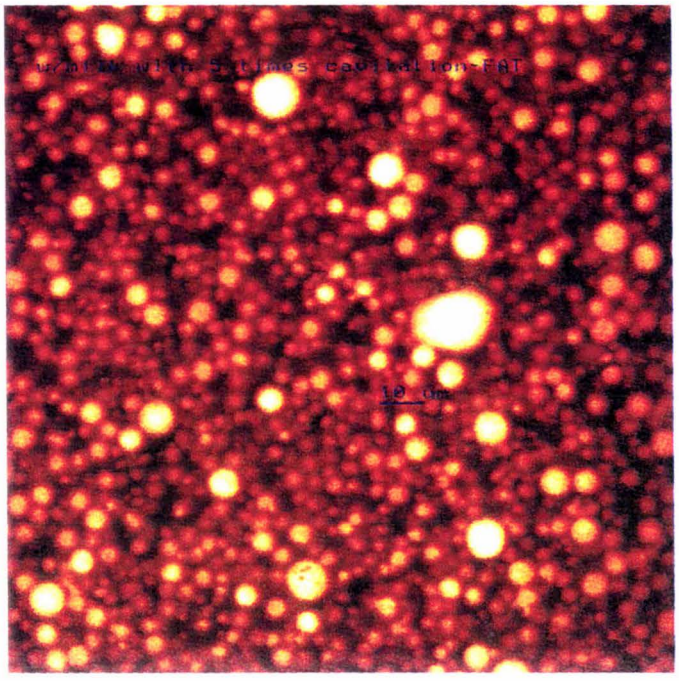


Fig. 4.10 Micrograph (63×) of fat globules in milk after five passes through the cavitating pump. Trial three (run 3.1)

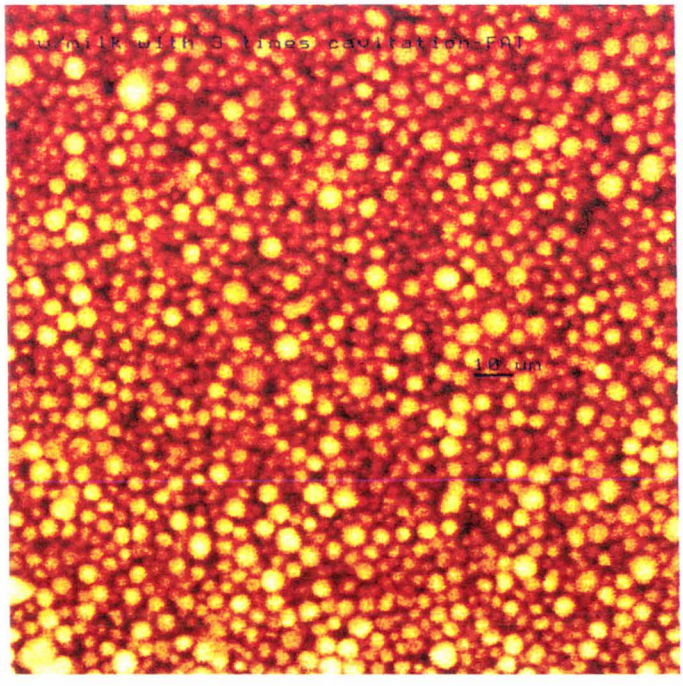


Fig. 4.11 Micrograph (63×) of fat globules in milk after three passes through the cavitating pump. Trial three (run 3.2)

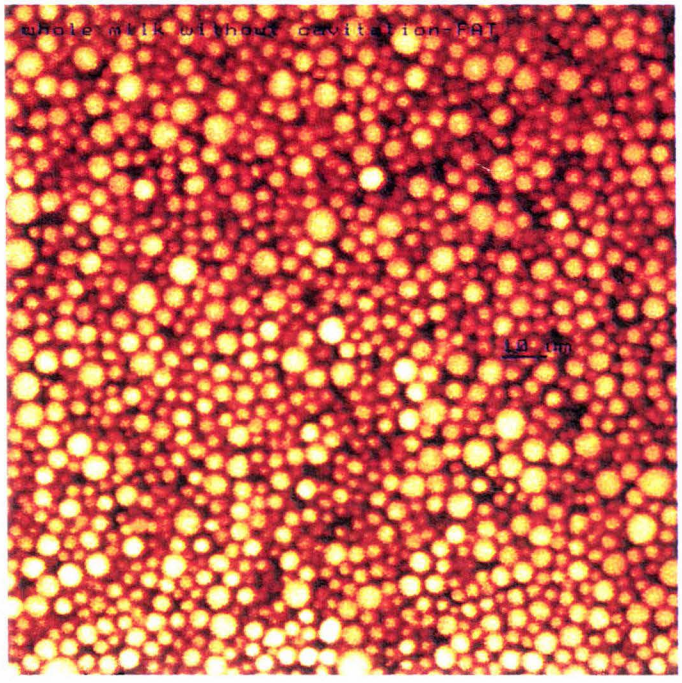


Fig. 4.12 Micrograph (63×) of fat globules in untreated milk. Trial three (run 3.3)

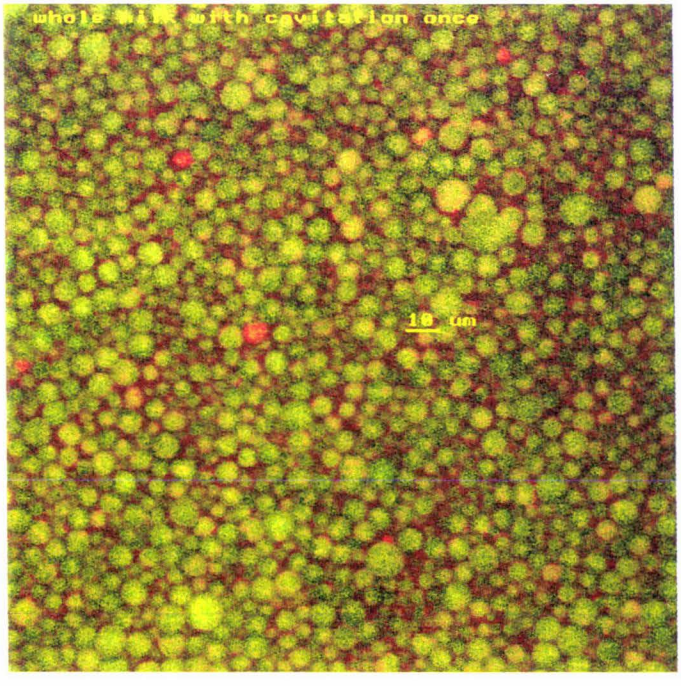


Fig. 4.13 Micrograph (63×) of fat and protein (protein in red and fat in green) in milk after one pass through the cavitating pump. Trial two (run 2.1)

The results suggest that cavitation generates strong mechanical stresses that damage the MFGM. Parameters such as free fat (FF) and free fatty acid (lipolysable free fat) content are not direct measurements of MFGM damage but can be used as indicators. In this work, the extent of cavitation was investigated by varying the number of passes through a single cavitating pump. Another method would be to vary the degree of cavitation by controlling the inlet pressure, for a set number of passes.

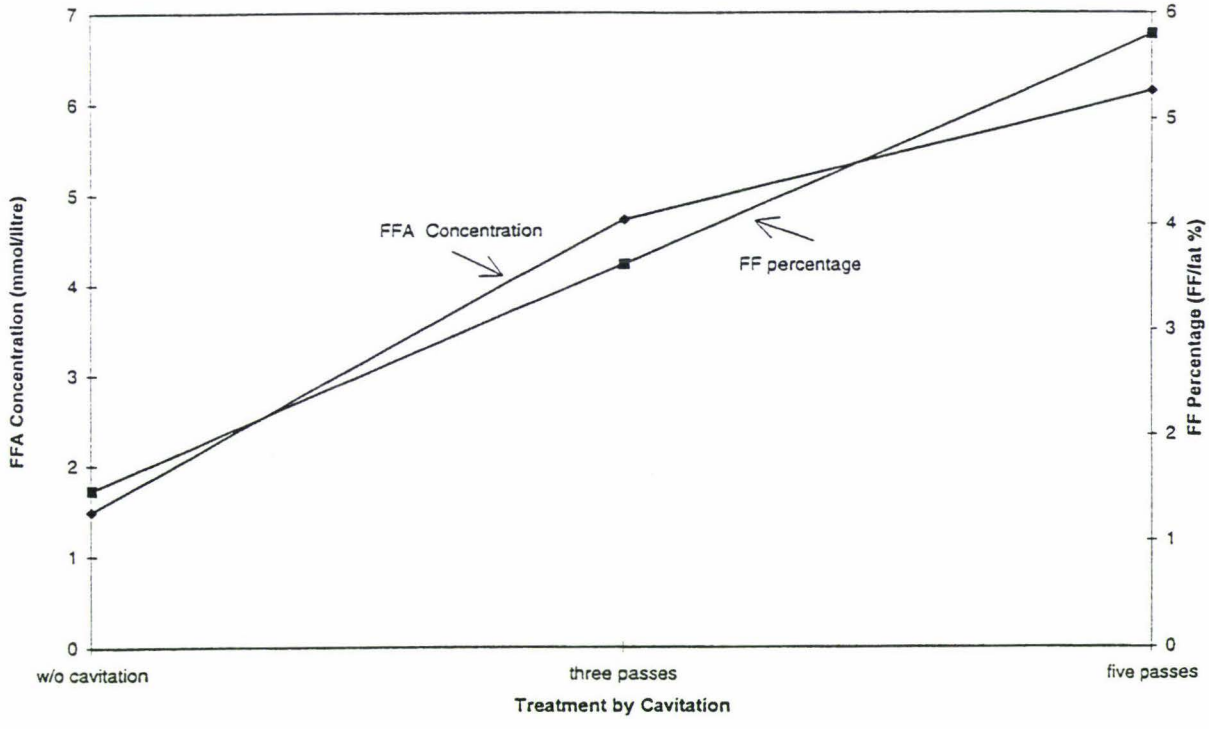


Fig. 4.14: FF and FFA contents versus the number of passes through the cavitating pump - Trial three

The particle size distributions obtained with Mastersizer and the micrographs obtained with the confocal laser scanning microscope were very helpful in recognising MFGM damage. Particle size distributions, however, showed clear changes only upon extensive MFGM damage (Trial three, Fig. 4.3). The largest particle detected in trial three was up

to ten times larger than unaffected fat globules, even though less than 6% of the fat was affected by cavitation (Table 4.2).

The micrographs were easier to analyse than the particle size distributions, because confocal laser scanning microscopy shows damage clearly and directly. The micrographs give a visual confirmation of Mastersizer analysis. It may be possible to quantify MFGM damage using the digital information represented by the micrographs by quantifying the sizes and shapes of fat globules using specially designed software.

Confocal laser scanning microscopy and Mastersizer analysis can complement the FF and lipolysable FF tests, but the latter are more readily performed in industry.

4.3 Extent of Fouling

4.3.1 Observations of Fouling Layers

1. Fouling occurred in all sections of the fouling rig heat exchanger. There was a deposit even on the first tube near the milk inlet. This occurrence was unusual because in the literature fouling is linked with protein denaturation, which occurs at temperatures beginning at around 70°C; the milk inlet temperature range was 4°C - 6°C.

   Since the highest milk outlet temperature was below 100°C, the fouling layer studied here belongs to Type A (Burton, 1968).

2. The most fouling deposit was found on the hotter tubes where milk temperatures were above 70°C.

3. The fouling layer in the low temperature region (below 70°C) was loose and porous, while that in the high temperature region (70°C - 95°C) was relatively hard and solid. The fouling layer from the hottest surface had brown dots (Fig. 4.15), which were attributed to the Maillard reaction or to local burning of the deposit.

Fig. 4.15: Brown Dots on the fouling layer from the hottest section (Trial 1, Run 1.1)

4. The fouling layer in a given section was not uniform. The upper and lower side layers of the inner tube had deposits of different structures (Fig. 4.16). The bottom side had a looser and more porous deposit than the top; this difference was particular clear on the low temperature tubes closest to the inlet. The

fouling layer was more uniform at higher milk temperatures closer to the milk outlet.

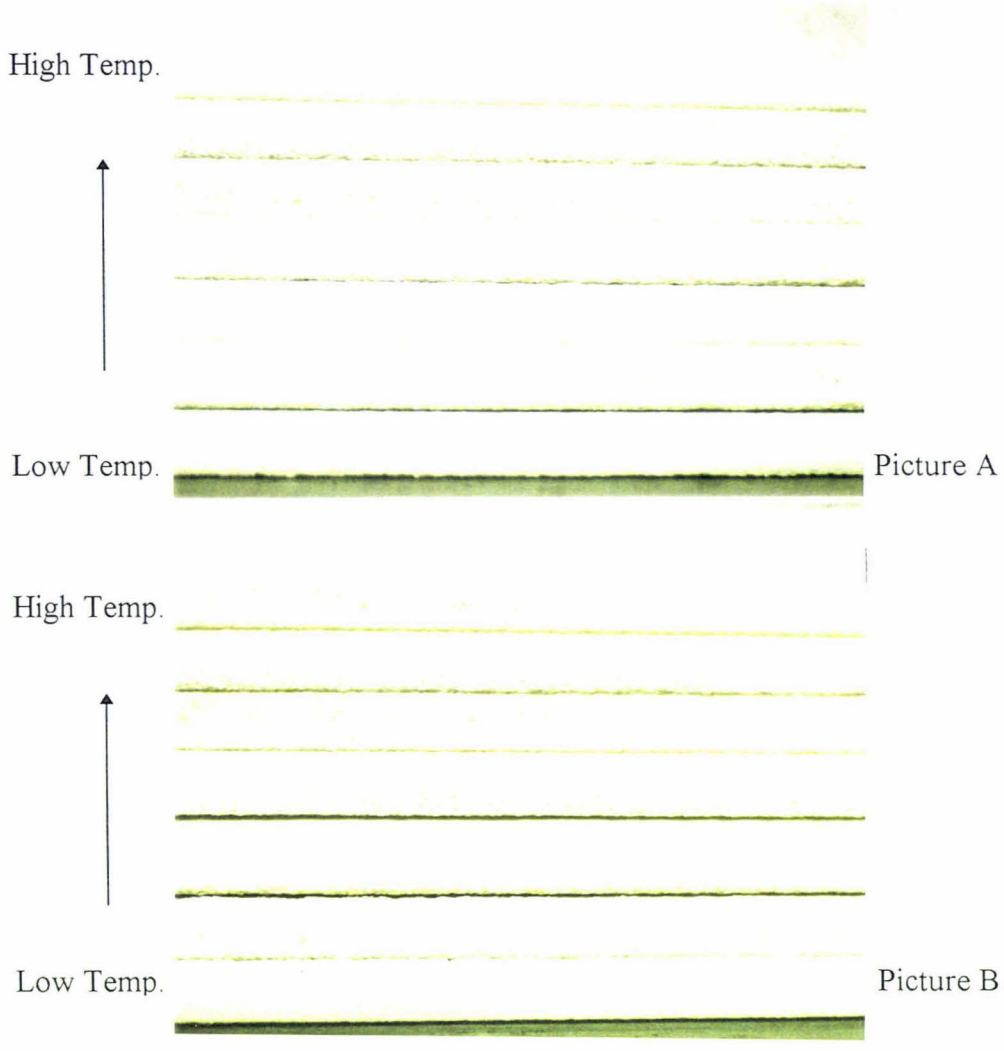


Fig. 4.16 Fresh fouling layers on the upper and lower sides of the inner stainless steel tubes of the fouling rig heat exchanger (Picture A: fouling layer on the upper side; Picture B: fouling layers on the lower side)

5. The fresh fouling layer from cavitation treated milk was found to be more porous and seemed thicker than that of untreated milk. The deposit was mud-like and attached to the steel surface loosely. The fresh fouling layer of untreated milk was skin-like with a more compact structure. However, after

drying at room temperature, the dry fouling layer from treated milk was thinner than that of untreated milk.

6. Generally, the total amount of fouling deposit was found to increase with the volume of milk used in the experimental run. No estimate was made of a quantitative relationship between these two factors.

7. Almost all fouling deposits were found on the heating surface (the outer surface of the inner tube). Hardly any deposits were found on the unheated surface (the inner surface of the outer tube).

4.3.2 Fouling Rate

8. It was not possible to increase the inlet temperature of the heating medium (hot water) during a run (owing to the 100°C boiling point limitation). Therefore, the outlet milk temperature was allowed to drop with time as fouling occurred. The rate of decrease of this temperature dropped to a low value once the milk outlet temperature had dropped to 70°C, indicating that rapid fouling required a milk temperature of ≥70°C. Fig. 4.17 shows an example of temperature-time curves for milk at different locations. Further curves can be found in Appendix 10.

The rate of milk temperature decrease at a given location caused by fouling showed a positive relationship with the number of cavitation passes. The time for a temperature decrease from, say, 90°C to 70°C was longest for untreated

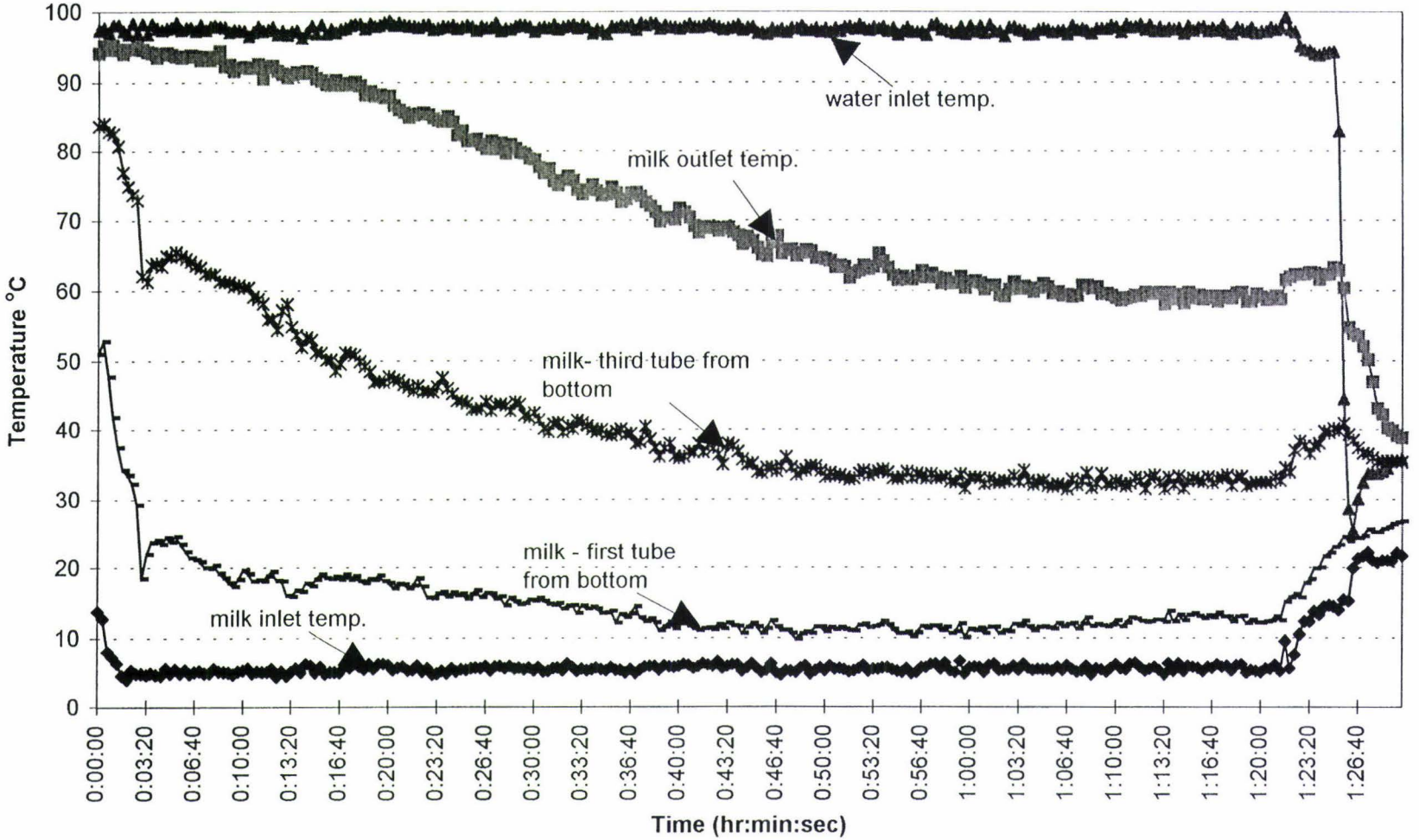


Fig. 4.17 Temperature Drop by Fouling of Pasteurised Whole Milk with 5 Passes by Cavitating Pump (Trial 3, Run 3.1)

milk, somewhat shorter for slightly damaged milk, and shortest for highly damaged milk.

9. Figs. 4.18 - 4.20 show plots of normalised overall resistance to heat transfer ($R_F U_o$) versus time for trial one to three. This resistance was calculated as follows.

Sample calculation:

For Trial two, Run 2.1,

Q' = 48 litre milk hr^{-1}

$t_m = 91°C$

$T_m = 4°C$

$t_w = 72°C$

$T_w = 98°C$

$\rho_{milk} = 1.035\ kgm^{-3}$

$\mu_{milk} = 1.6 \times 10^{-3}$ Pas

$c_{p,\,milk} = 3.852\ kJkg^{-1}K^{-1}$

$$S' = \pi/4 (D_i^2 - d_o^2) = 3.11 \times 10^{-4}\ m^2 \qquad (4.1)$$

$$d_e = D_i - d_o = 10.9\ mm \qquad (4.2)$$

$$V' = Q'/(1000 \times 3600 S') = 0.043\ ms^{-1} \qquad (4.3)$$

$$Re = \rho_{milk} V' d_e / \mu_{milk} = 303 \qquad (4.4)$$

$\Delta T_1 = T_w - t_m = 7°C$

$\Delta T_2 = t_w - T_m = 68°C$

$$\Delta T_m = \frac{\Delta T_1 - \Delta T_2}{\ln(\Delta T_1 / \Delta T_2)} = 27°C \quad (4.5)$$

$$Q_1 = c_{p,\,milk} \times m \times (t_m - T_m) = 4625\ W \quad (4.6)$$

$$A = \pi d_o L = 0.319\ m^2 \quad (4.7)$$

$$Q_1 = Q_2 = UA\Delta Tm, \quad (4.8)$$

$1/U = A\Delta Tm/Q_1 = 1.862\times10^{-3}\ m^2KW^{-1}$

Since the overall heat transfer coefficient decreased with fouling formation, the fouling resistance $R_F = \frac{1}{U} - \frac{1}{U_o} = \frac{1}{U_o}(\frac{U_o}{U} - 1)$, where U_o is the initial overall heat transfer coefficient. The fouling resistance normalised with the initial overall heat transfer coefficient, $R_F U_o$, was therefore calculated as $(\frac{U_o}{U} - 1)$ at various times during a run. U_o was calculated using the means of the four terminal temperatures that existed in the heat exchanger during the first five minutes of a run. (Each temperature value used was the mean of the values recorded at 20 seconds intervals during the first five minutes).

The use of the initial overall heat transfer coefficient as a normalising parameter is not perfect. As fouling developed, the R_F as calculated above would have included not only the resistance of the fouling layer itself, but also any change in the milk side resistance (which might be called $1/h_m$, the water side resistance being $1/h_w$). h_m would have tended to increase with time during a run because as the fouling layer became thicker, the cross-sectional area for milk flow would

have become smaller and thus milk velocity higher. Other changes in h_m (either positive or negative) would have been caused by factors such as time dependent changes in milk composition in the heat exchanger which themselves might have been caused by the varying fouling rates during a run.

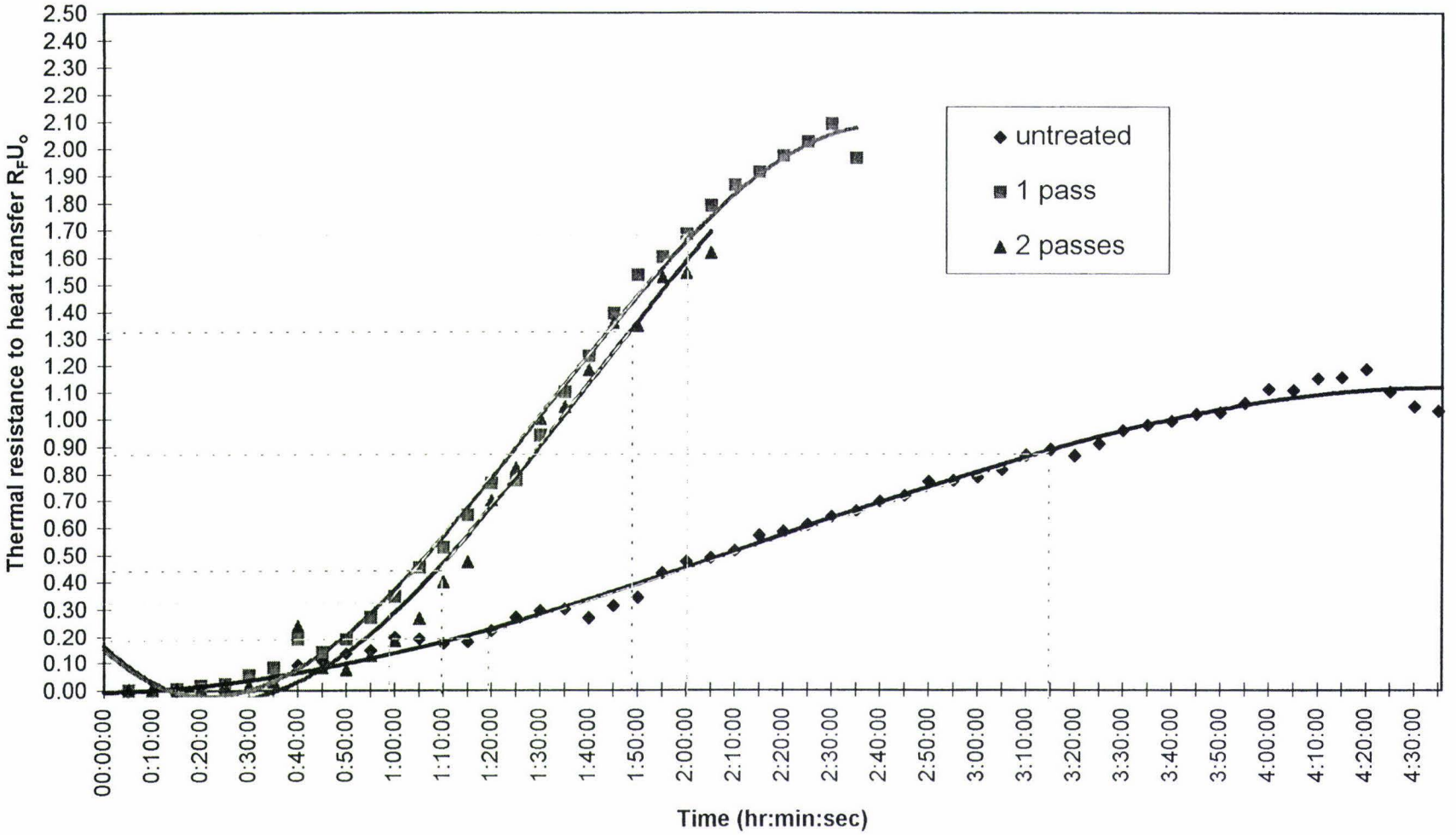


Fig. 4.18 The thermal resistance to heat transfer versus time --- Trial one

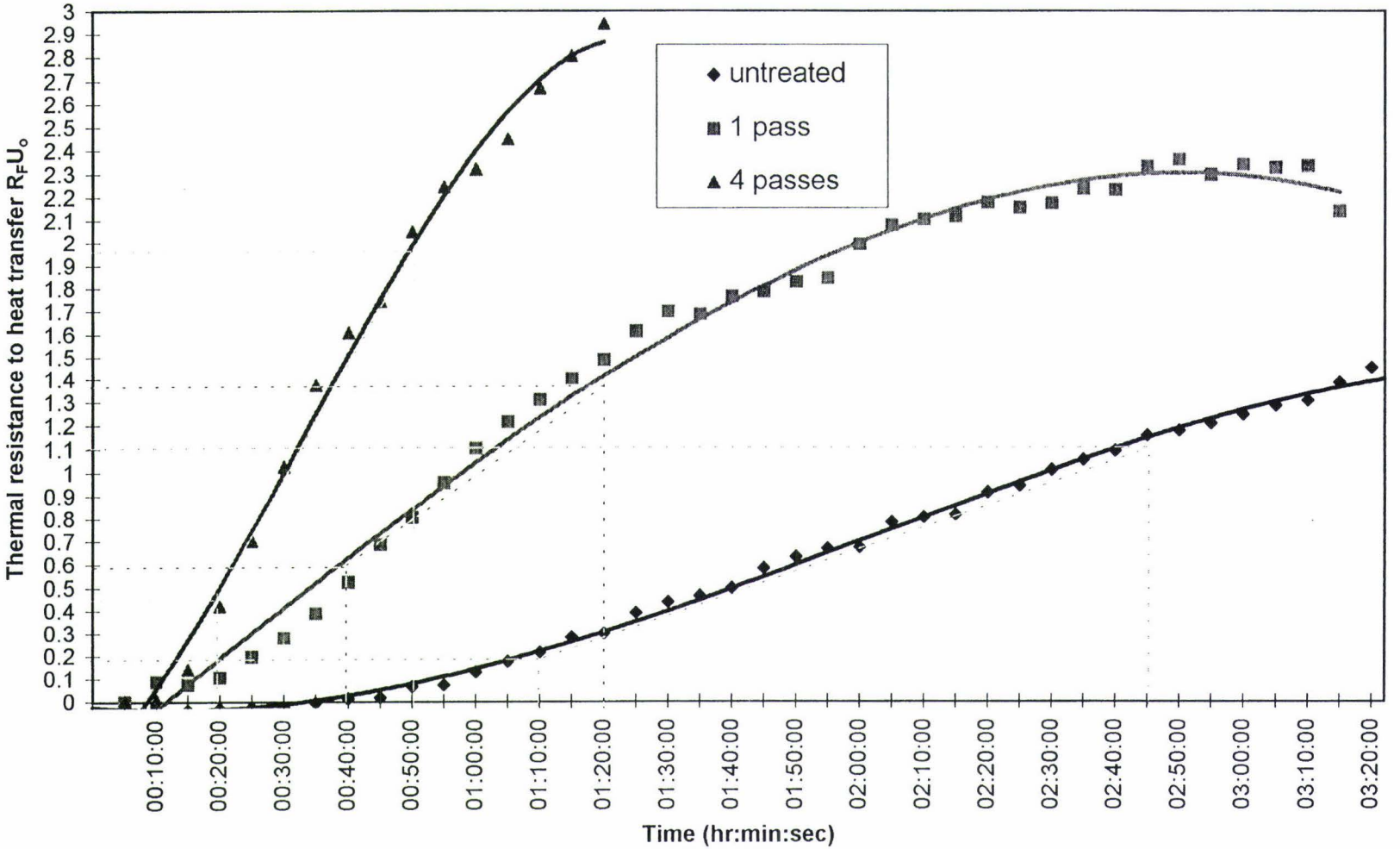


Fig. 4.19 The thermal resistance to heat transfer versus time --- Trial two

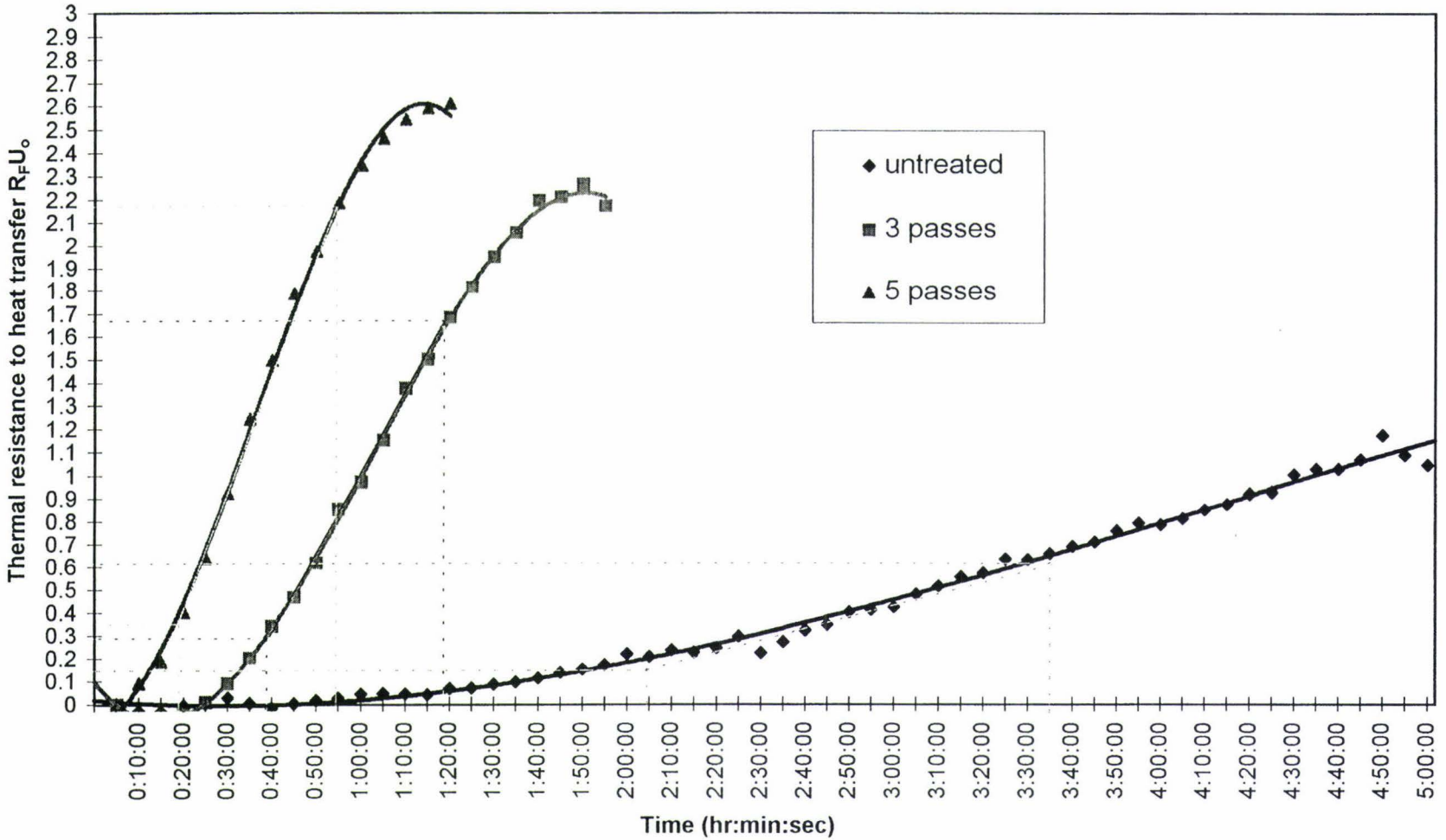


Fig. 4.20 The thermal resistance to heat transfer versus time ---Trial three

Interpretation of the results shown in Figs. 4.18 - 4.20 must be made with these uncertainties in mind.

In optimally designed experimental equipment, the thickness of the fouling layer that develops may be large in absolute terms, but must be small compared to the hydraulic diameter of the flow system.

4.3.3 Experimental Constraints

10. During the trials, the flow rates of milk and hot water could be kept only within certain ranges, and not at exact values for all experiments. This was also the case for the inlet and outlet temperatures of the milk and hot water. The experimental rig was controlled manually. Automatic control would have given more accurate results.

11. At the end of each run, distilled water at the same flow rate as the milk was used to rinse the heat exchanger and remove loose adhering material for four minutes. At the end of the rinse, although the distilled water was almost clear, there was some turbidity. The heat exchanger was then carefully disassembled, and the fouling layer observed. The relationship between the extent of fouling and the degree of cavitation damage to the milk could not be detected by eye.

4.3.4 Performance of the Fouling Rig

The heat balance for each run is shown in Table 4.3, where Q_m and Q_w are the energy required to heat up the milk and the energy lost by the hot water respectively in the heat exchanger. The heat balance was calculated from the temperatures and flow rates of milk and water at one hour into the run. Generally, the heat balance was acceptable; except for the first two runs (runs 1.1 and 1.2), the percentage differences were within ±20%. Since there was no insulation installed for runs 1.1 and 1.2, energy losses were larger. Once the heat exchanger had been insulated with plastic foam, the heat balance was better. Obviously, insulation was important for efficient heat transfer.

Table 4.3: Performance of the fouling rig heat exchanger

	Trial one			Trial two			Trial three		
	Run 1.1	Run 1.2	Run 1.3	Run 2.1	Run 2.2	Run 2.3	Run 3.1	Run 3.2	Run 3.3
T_m (°C)	4.09	4.8	5.2	5.65	3.26	4.38	5.78	5.1	3.48
t_m (°C)	86.48	85.53	91.21	73.44	90.49	62.48	60.34	77.43	92.56
T_w (°C)	96.75	97.16	99.93	98.45	97.66	97.19	98.43	98.67	97.5
t_w (°C)	75.79	78.42	84.28	80.79	76.11	83.05	85.17	80.82	78.91
ΔT (°C)	31.61	33.58	31.89	45.56	28.29	53.72	58.55	44.61	26.7
$U(WK^{-1}m^{-2})$	325.73	325.477	337.053	247.927	513.777	180.21	155.27	270.164	555.918
$Q'_{water}(Ls^{-1})$	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Q_m (W)	3284.5	3486.5	3428.8	3602.9	4636.8	3088	2900	3844.5	4734.9
Q_w (W)	5289.5	4729.2	3949.4	4456.7	5438.4	3568.4	3346.3	4504.6	4691.4
% difference	37.9	26.3	13.2	19.2	14.7	13.5	13.3	14.7	-0.93

Note: The equations used for above calculations were: $Q_m = c_{p,\ milk}\ m_{milk}\ (t_m - T_m)$, and $Q_w = c_{p,\ water}\ m_w\ (T_w - t_w)$. %difference = $\frac{(Q_w - Q_m)}{Q_w} \times 100\%$. $c_{p,\ water} = 4.206\ kJkg^{-1}K^{-1}$, $\rho_{water} = 1000\ Kg\ m^{-3}$.

4.4 Fouling Deposit Analyses

4.4.1 Chemical Analysis

The chemical analyses of the fouling deposit from each run included total fat, total protein, ash content and moisture (Appendix11). Table 4.4 shows the average compositions of fouling deposits on a dry weight basis. Rinsing, described above, may have swept away some clumped fat. Also, the dried fouling deposit was very oily, and was hard to mill into a fine powder. When measuring total fat content using the Mojonnier method, there were always some big particles left. These might have contained some unextracted fat. Thus, the accuracy of total fat measurements may have been significantly affected. The sum of the fat, protein, moisture and ash contents of each dried sample was not 100%; the fouling layer might have contained other components such as lactose, for which measurements were not made.

Table 4.4: Compositions of fouling layers

Trials	Run No.	Treatment	Amount of Milk	FAT	PROTEIN	ASH	MOISTURE	SUM
Trial 1	1.1	untreated milk	170 litres	53.37%	31.20%	4.92%	5.34%	94.83%
	1.2	one pass	120 litres	49.13%	32.20%	4.54%	6.65%	92.53%
	1.3	two passes	90 litres	48.27%	30.21%	4.22%	5.88%	88.58%
Trial 2	2.1	untreated milk	160 litres	49.36%	32.13%	4.88%	6.16%	92.54%
	2.2	one pass	160 litres	48.55%	32.84%	4.47%	4.86%	90.72%
	2.3	four passes	70 litres	45.55%	32.42%	4.31%	9.85%	92.12%
Trial 3	3.1	untreated milk	210 litres	47.58%	33.26%	5.39%	8.08%	94.31%
	3.2	three passes	90 litres	51.62%	32.26%	4.49%	8.77%	97.15%
	3.3	five passes	70 litres	49.15%	32.79%	4.13%	10.29%	96.36%

Only small amounts of free fat were released in the milk by cavitation treatment (Table 4.2). Therefore it is perhaps not surprising that large differences in fouling layer fat

content between runs were not found. It is postulated that quite small increases in free fat could have enhanced significantly the formation of large protein-fat aggregates and thus caused more rapid and extensive fouling. No significant differences in composition among the fouling layers from the runs carried out could be found that correlated with the extent of cavitation treatment (Table 4.4).

The fat contents of the fouling layers were very high (more than 45% fat in dry deposits) compared with results reported in the literature (normally 4-8%) (Table 4.4). Similar results have been found by other workers at Massey University (Truong, personal communication). It is not clear why the fat content in milk fouling layers is much higher in New Zealand than in countries in Europe. This phenomenon could be linked to different milk compositions and/or to different milk handling practices. Further studies are needed.

The protein contents of the fouling layers was rather invariant (Table 4.4), but were lower than values of 50-70% commonly reported in the literature (Chapter *2*). The ash contents were also relatively low in this study. These results were a natural consequence of the high fat contents. However, the ash content seems to have increased with the amount of milk used in the run (Table 4.4).

4.4.2 Observation of the Fouling Layer by Confocal Laser Scanning Microscopy

The structure of the fouling layer was intended to be studied by confocal laser scanning microscopy. After several attempts, it was concluded that confocal laser scanning microscopy was not entirely suitable. Only low magnification (10 times) can give a picture of the entire porous structure. Fig. 4.21 is micrograph showing fat particles and proteins in a fouling layer. It indicates that large fat particles (in red) were attached to the stainless steel surface and may have constituted a structural framework for the fouling layer. Proteins (in green) were adsorbed on the fat particles. How the fat combined with proteins to form a porous fouling layer cannot be seen in the micrograph. Higher magnification achieved with an oil immersion lens was not suitable. Scanning electron microscopy (SEM) might be a better technique for fouling structure study.

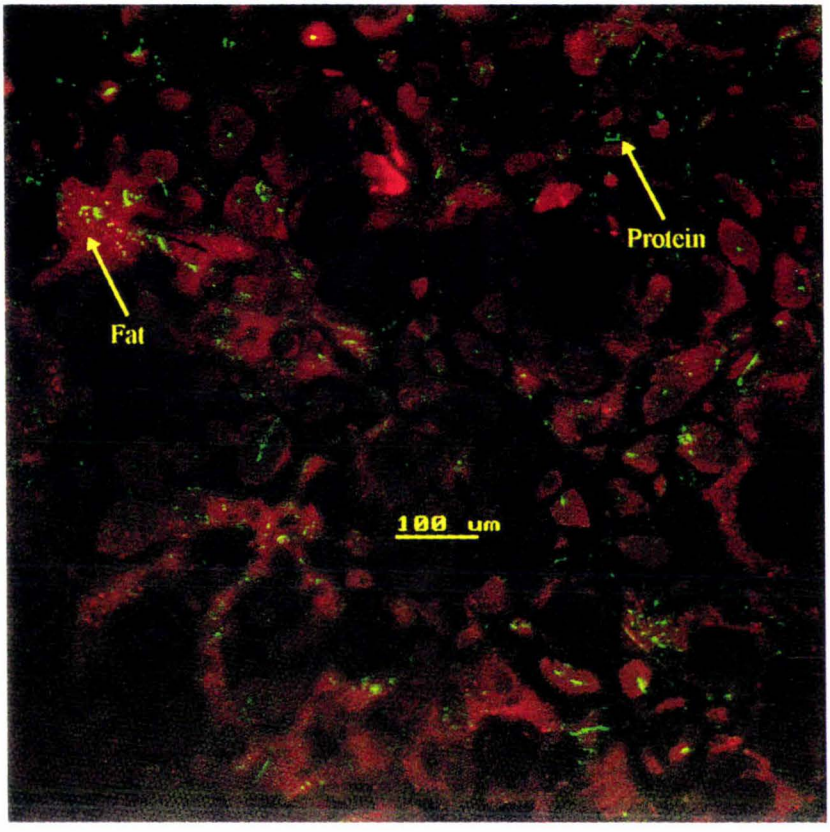


Fig. 4.21: Confocal laser scanning micrograph (10×) shows fat particles (in red) and proteins (in green) in a dried fouling layer. Trial one (run 1.2)

4.5 The Effect of Milk Fat Globule Membrane Damage on Fouling

To try and establish a relationship between MFGM damage and fouling rate, the R_FU_o - time curves of Figs. 4.18 - 4.20 were put together in the same plot (Fig. 4.22). Although Fig. 4.22 shows that milk fouls faster with more extensive MFGM damage, the results are blurred by large differences between the three batches of milk used; even the curves for the control samples (untreated milk) do not agree well.

This problem was reduced by further processing the data in Fig. 4.22, in the following way. For every run, a fouling rate (R_FU_o/time (min^{-1})) was calculated by determining the slope of the middle (close to linear) portion of the R_FU_o - time curve (Fig. 4.22). Two

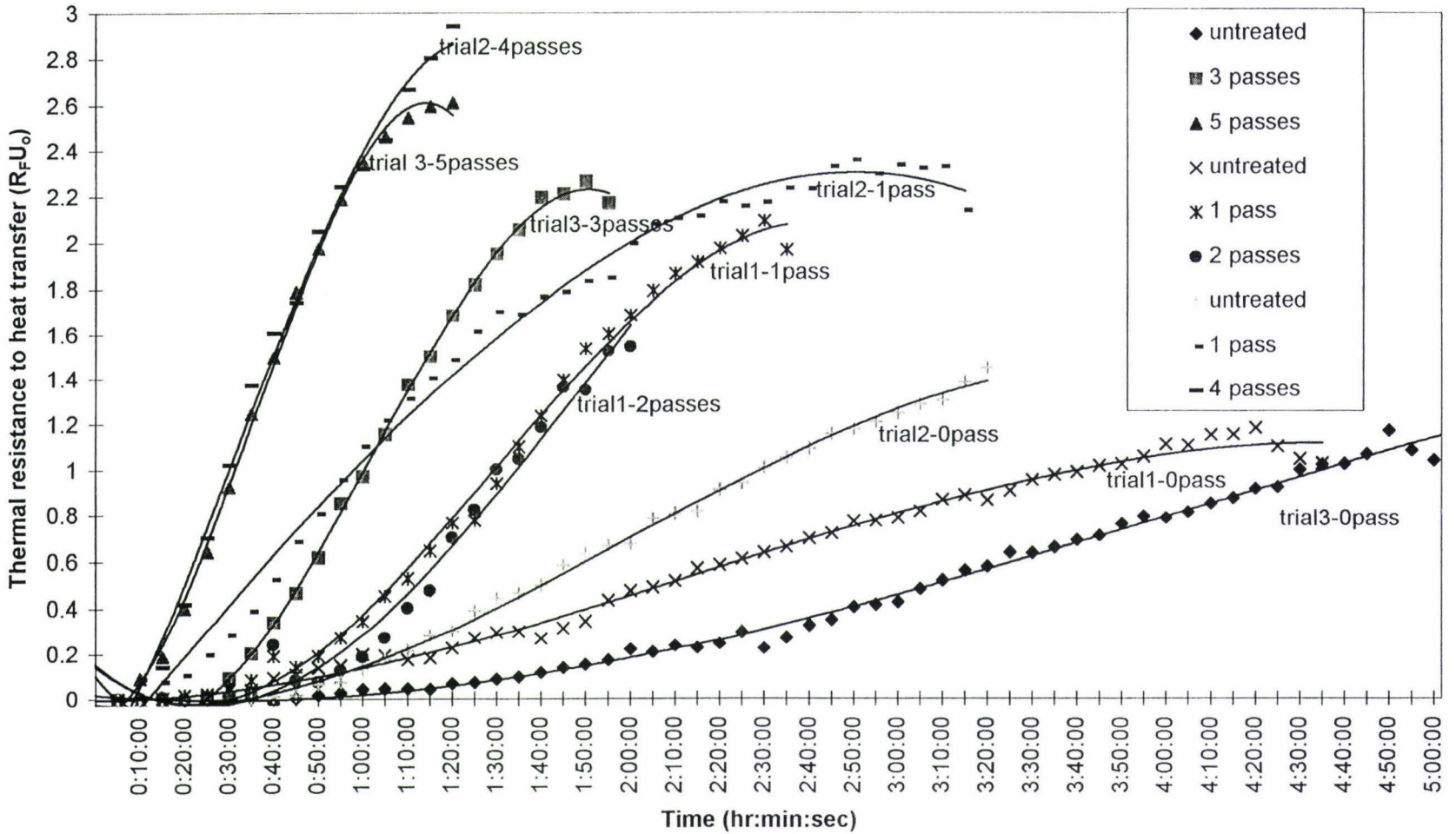


Fig. 4.22 : The thermal resistance to heat transfer versus time --- Trials 1 - 3

points on the curve were selected (as indicated in Figs. 4.18 - 4.20) and the slope calculated on the basis of the assumption that the curve was perfectly linear between these two points.

For each trial, the fouling rate for each run was normalised using the fouling rate for untreated milk in that trial as a divisor. The results are presented in Table 4.5 and Fig. 4.23.

While there is a significant amount of scatter in Fig. 4.23, the plot demonstrates clearly a distinct positive relationship between normalised fouling rate and the extent of cavitation treatment. More severe cavitation treatment resulted in significantly faster fouling, and this is assumed to have been due to more severe MFGM damage. A comparison of Figs. 4.14 and 4.23 strongly suggests this assumption.

The following comments may be made on the basis of the results presented. The results presented in Tables 4.2 and 4.5, and Fig. 4.23, indicate that an increase in free fat of the order of 290% (Trial 3, Table 4.2) caused an increase in fouling rate of up to 800%.

In absolute terms, only a small percentage of the total fat (less than 6% in Trial 3) has to be converted to free fat by damage to cause substantial and rapid fouling.

Table 4.5: Fouling rates and normalised fouling rates for all runs

Trial	Run	Number of passes	Fouling rate (min^{-1})	Normalised fouling rate
1	1.1	0	0.0061	1
	1.2	1	0.0228	3.738
	1.3	2	0.0218	3.574
2	2.1	0	0.0099	1
	2.2	1	0.02	2.020
	2.3	4	0.0507	5.121
3	3.1	0	0.005	1
	3.2	3	0.0345	6.9
	3.3	5	0.0509	10.18

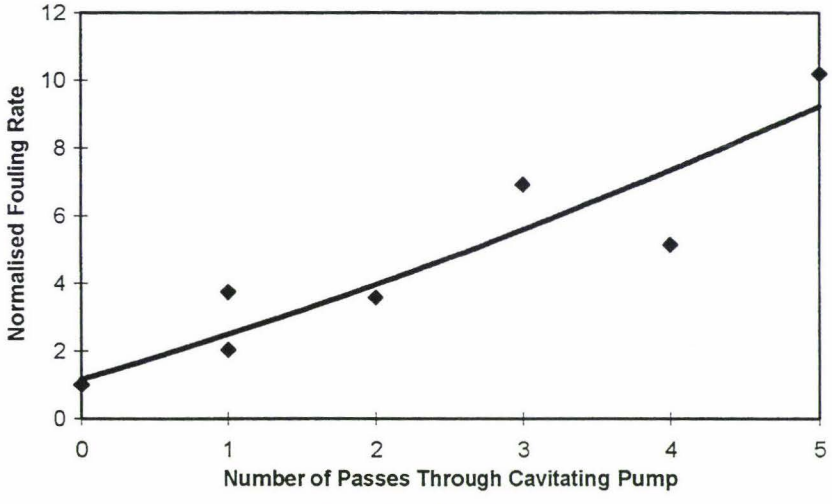


Fig. 4.23: Normalised Fouling Rate versus Number of Passes Through Cavitating Pump

Since cavitation is only one possible mechanism by which damage is done to milk that will affect production efficiency and product quality in the dairy industry, it is reasonable to propose that milk handling is a very important issue.

It is expected that air incorporation in the milk prior to cavitation would result in enhanced damage to the MFGM, and also affect the state of milk proteins by surface denaturation. Study of these phenomena was not part of the present work but will be

carried out as a continuation of this research. It is likely to demonstrate even more serious effects of poor handling on the processability of milk.

The effects of cavitation alone on milk components other than the MFGM were not studied here. For example, mechanical treatment such as cavitation (in the absence of air) might change the characteristics of milk proteins in such a way as to decrease the stability of the proteins to heat.

The Reynolds number range in this study was 220-310. Compared with milk flow rates in industry, where the Reynolds number can range up to 10,000, the milk flow rates in this study were very low. In addition, the fouling results span a range of milk temperatures. The results obtained characterise fouling formation in the temperature range that existed over the whole length of the heat exchanger rather than the fouling behaviour at a given location and a given temperature. The literature reports that the rate of fouling changes with temperature. Thus the increases in fouling rate observed in this study are underestimates, because the mean milk temperature decreased with accumulation of fouling deposit. For continuing work, better experimental facilities need to be built that enable the milk temperature to be kept constant.

4.6 Mechanism of Fouling --- Hypothesis

The low Reynolds number in this fouling rig possibly made milk fat globule creaming or migration easier than usual. Creamed fat might have had tendency to migrate towards

and adhere loosely to the steel surface. (Yamamoto, et al., (1986) have demonstrated migration of milk fat globules during flow of milk in capillary tubes at low flow rate.)

Fat globules with damaged membranes tend to coalesce and form larger globules. Large coalesced fat globules might migrate to and adhere more easily to the stainless steel surface than original undamaged globules. These globules could be coated with denatured proteins and could act as anchor points in the protein matrix. Thus, whole milk would be expected to foul faster with an increasing extent of MFGM damage, because of the greater rate of migration of large coalesced fat globules. The more and larger coalesced or clumped fat particles that existed, the higher the fouling rate might be expected to be. Even though only a small percentage of the total fat was damaged in this study, the disproportionately large increase in fouling rate that resulted could have been due to the fact that all the damaged fat ended up in the fouling layer, which itself represents only a small proportion of total milk solids. The low milk flow rates used could have accentuated this trend by facilitating fat migration to the heating surface. Confocal laser scanning micrographs did show evidence of large fat globules in the fouling layer (Fig. 4.21). However, more work must be carried out before this overall hypothesis can be proved (or otherwise).

Chapter 5 Conclusions and Recommendations

5.1 Conclusions

Generally, the present research was an exploratory study of the effects of MFGM damage on milk fouling. It yielded a significant positive relationship between MFGM damage and fouling rate. It showed that when only a small amount of fat was damaged (to give less than 6% free fat as a percentage of total fat) during handling (deliberate cavitation here), fouling rate increased significantly.

Within this study, two other principal objectives were achieved. Firstly, it was found that the MFGM can be damaged in a controlled, repeatable way by deliberately causing a milk pump to cavitate. Secondly, it was found that although the proposed methods of measuring MFGM damage need to be further developed, they do successfully indicate the extent of MFGM damage. Both the free fat and lipolysable free fat contents of whole milk increase with the extent of MFGM damage. Particle size distribution measurement and confocal laser scanning microscopy can complement the FF and lipolysable FF tests. In the lipolysable free fat test, external pancreatic lipase was added to attack lipid lacking the protection of the natural MFGM. The high fat percentage found in the fouling layers suggests that the fat does not always play merely a minor role in fouling deposition. More work needs to be carried out for a better understanding of whole milk fouling.

5.2 Recommendations for Further Work

5.2.1 Lipolysable Free Fat Test

1. According to Olivecrona & Bengtsson-Olivecrona (1991), an activator protein, apolipoprotein CII (apo-CII), is required for an optimal activity of lipase. It is supplied as an integral part of the substrate lipoproteins, chylomicra and very-low-density lipoproteins, and its interaction with lipase presumably takes place with, or after, the binding of the lipoprotein particle to the enzyme. Some results obtained by Olivecrona & Bengtsson-Olivecrona are shown in Fig. 5.1. They show that under the conditions they used, no lipolysis occurred unless the activator was present, and that the effect of the activator and the effect of mechanical treatment by sonification were synergistic.

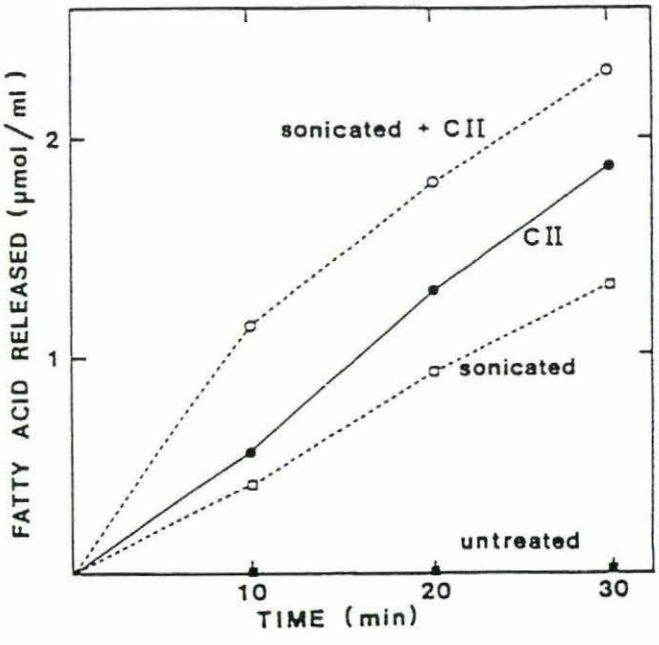


Fig. 5.1: Effect of activator in milk lipolysis
(Adapted from Olivecrona and Bengtsson-Olivecrona, 1986)

In addition, Jellema (1975) found that adding 2% bovine blood serum activated whole milk lipolysis. He concluded that bovine blood serum contained an activator or activators for the lipolytic system in milk, possibly α-lipoproteins or high-density lipoproteins.

These reported results suggest that the lipolysable free fat test developed in the present study might be made better (e.g. more rapid) by the use of a suitable activator system.

2. Miller & Puhan (1986) found that pH influenced the extent of lipolysis in milk., but that the effect was small relative to the effect of damaging mechanical treatment (Fig. 5.2). However, proper optimisation and control of milk pH in the lipolysable free fat test might well improve the test, and should be investigated.

3. Other factors pertiment to the lipolysable free fat test, such as added lipase type, lipase concentration and incubation time, need to be studied and optimised to solidify the method.

4. The modified free fat and lipolysable free fat tests used in this study, after further modification and improvement, should be tested in the field to investigate their sensitivity for detecting fat damage incurred as milk moves from the cow, through treatment and processing, to the final product.

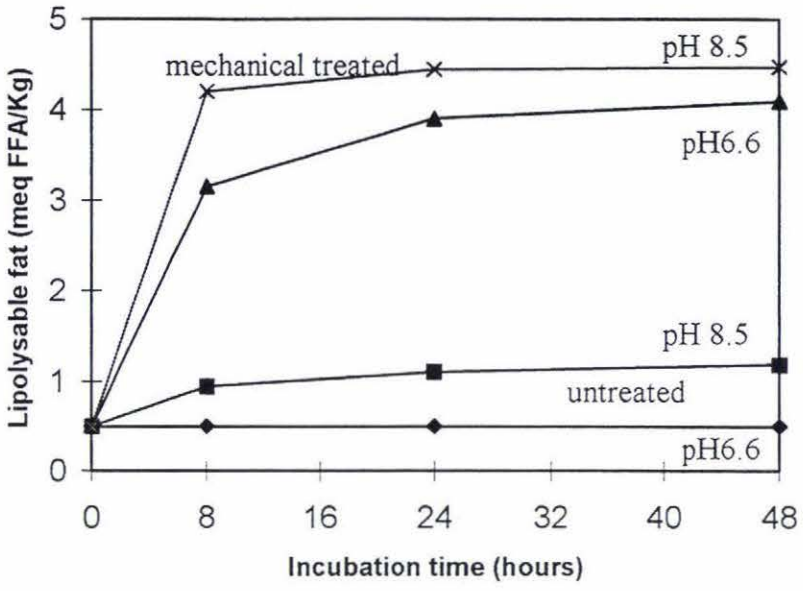


Fig. 5.2: Effect of pH on the amount of lipolysable fat in untreated and mechanically treated raw milk (Adapted from Miller and Puhan, 1986)

5.2.2 Fouling Rig

1. In order to ensure stable heat transfer in the fouling rig, the heat exchanger could be arranged vertically, so as to give axial symmetry in milk temperature, and a fouling layer that was uniform around the circumference of the heated tube at any point.

2. In order to study fouling at a specific temperature (for example, fouling in a holding tube), the milk outlet temperature should be kept constant; improved experimental facilities are needed to achieve this.

5.2.3 Fouling Layer

1. The high fat percentage in the fouling layers studied in the present work is an interesting topic for further study. Many workers have found low fat contents in fouling deposits, but our results are very different. No data appears to have been published on the fat contents of fouling layers formed during commercial scale dairy processing.

2. It is recommended that the structure of the type of fouling layer found in this work be explored using scanning electron microscopy (SEM). An understanding of how fat globules and proteins aggregates to form the fouling layer will be vital to the elucidation of how fouling actually takes place.

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Appendix 1

Modified Free Fat Test for Whole Milk

Free fat is ether extracted from the sample under standard conditions, the solvent evaporated and the residue weighed.

Apparatus:

1. Balance, weighing to 4 decimal places
2. Extraction flasks, Mojonnier with non-porous stoppers resistant to solvents
3. Centrifuge, Mojonnier type
4. Tongs
5. Flasks, 150ml, fat bottom wide necked
6. Boiling chips
7. Water bath, constant level, boiling
8. Oven, thermostatically controlled at 102±1°C, with fan

Reagents:

Petroleum ether, boiling range 40-60°C, AR or redistilled, filtered before use.

Procedure:

1. Heat the flasks containing some boiling chips in the oven for 30 minutes, one flask for each determination plus one for the blank will be required. At all times handle the flasks with the tongs.
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)
4*. Well mix the sample by inverting the container 10 times.
5. Temper the sample at 40°C for 10 minutes.
6. Accurately weigh about 20g of the well mixed sample into a Mojonnier flask (w_2).
7. Add 50 ml petroleum ether.
8*. Stopper the flask and temper the ether and sample at 40°C for 30 seconds.
9*. Mix the sample and ether by inverting 10 times in 40 seconds. (Stopper the flask and mix by inverting 10 times in 10 seconds.)
10. Centrifuge for 5 minutes.
11. Carefully pour the ether layer into the pre-weighed flask from step 3.
12. Carefully evaporate the ether on a water bath.
    CAUTION: Use an adequately ventilated cupboard.
13. When evaporation is complete, wipe the outside of the flask with a clean dry towel.
14. Heat the flask in oven for 1 hour.
15. Cover and cool the flask as in step 2 for 1 hour.
16. Reweigh the flasks (w_3 and blank as w_b).

Calculation and Report:

$$\% \text{ free fat in sample} = \frac{w_3 - w_1 - w_b}{w_2} \times 100$$

Repeatability:

Duplicate determinations should agree within 0.5%.

Note:

1. Carry out the test, except for procedure step 1, 5, and 8, at approximately 20°C.
2. Petroleum ether has an extremely low flash point, and being denser than air it can "creep" along bench tops to be ignited by remote heat sources.
3. The symbol * refers to the modifications to the Dairy Division Standard Method (1979). The words in parentheses are those in the standard method.

Appendix 2

Lipolysable Free Fat Test

Apparatus:
1. Burette, 1 ml with 0.02 ml graduations
2. 30°C warm room

Reagents:
Pancreatic lipase (see Appendix 4) solution, 50 mg/100 ml.

Procedure:
1. Pipette 1 ml pancreatic lipase solution into 100 ml milk sample
2. Mix the sample with glass rod gently
3. Put the sample in a warm room at 30°C and incubate for 24 hours
4. Measure the free fatty acid content by the following method

Free Fatty Acid Content
(Dairy Division Standard Method, 1980)

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

Apparatus:
1. Extraction flasks (Mojonnier tube)
2. Centrifuge, Mojonnier type
3. Burette, 10 ml with 0.05 ml graduations
4. Erlenmeyer flasks, 100ml

Reagents:
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 (B.P. 40/60°C)
4. Alpha-naphtholphthalein indicator, 1% solution in ethanol
5. Neutralised ethanol
6. Neutralised methanol
   To neutralise reagent 5 to 6, add 0.5 ml of reagent 4 to 100 ml of the alcohol, neutralise with alcoholic potassium hydroxide to the first sign of a greenish tinge in the yellow solution.
7. Alcoholic potassium hydroxide, 0.01M in ethanol, standardised frequently against benzoic acid dissolved in the mixed solvent.

Procedure:

1. Pipette 10.0 ml of well mixed sample at 20±2°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 ml 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 and Report:

Free fatty acid content as % oleic acid = $1.35 \times 0.0284 \times (T-B)$

where T = sample titration figure
B = Blank titration figure

Report to the nearest 0.05 mmols/litre or 0.001% as oleic.

Appendix 3

The Technical Characteristics of Calf Lipase

Supplied by New Zealand Rennet Company.

The lipase is an aqueous extract of the Epiglottis (Tongue Root) of calves, supplied as a water miscible liquid. The liquid is produced by a process which removes insoluble material and gives a high degree of bacteriological sterilisation. It is a red, slightly cloudy non-settling liquid which is completely water miscible.

It is at 225 LFU/ml (lipase fore-stomach units/ml). One LFU releases 1.25 μmoles of butyric acid per minute at 42°C and at pH 6.20 from tributyrin as substrate under the conditions defined by the Food Chemical Codex (FCCIII, p493, 1981).

It is recommended that the product be stored and transported under refrigeration (less than 8°C) and kept away from direct sunlight. Containers should be kept closed. For long term storage frozen storage is preferred. At the recommended storage conditions, the liquid loses activity at a rate of less than 1% per month. Higher storage temperature lead to much increased rates of activity loss.

Appendix 4

The Technical Characteristics of Pancreatic Lipase

Supplied by SIGMA chemical Co.

The product is Type II, Crude; from Porcine Pancreas.

It contains approximately 25% protein. One unit will hydrolyse 1.0 microequivalent of fatty acid from a triglyceride in one hour at the indicated pH and incubation time. 7.5 g protein (Biuret) 43 units/mg protein (using triacetin at pH 7.4). 190 units/mg protein (using olive oil at pH 7.7).

Caution: Desiccate, Store at 2 - 8°C. Avoid contact and inhalation.

Appendix 5

Moisture Test

Moisture is normally considered to be the material lost by a foodstuff on heating at a temperature around that of boiling water or by allowing the sample to stand over a dehydrating agent or by some similar form of measurement. It is generally considered to be water but is actually the total volatile matter lost or drained off under these particular conditions. The residue remaining is termed the total solids.

Apparatus:

1. Aluminium moisture dishes, diameter 55 mm, height 40 mm, provided with well fitting slipover covers. Previously dry at 108±5°C and keep in a desiccator at room temperature.
2. Desiccator, air-tight, containing dry silica gel or phosphorus pentoxide.
3. Air oven capable of being accurately maintained at 108±5°C and provided with openings for ventilation. Thermometer should pass into the oven in such a way that the tip of the bulb is level with the top of moisture dishes and is not directly exposed in currents of escaping water vapour.
4. Note: Always use metal tongs when handling metal dishes and lids. Never use hands, as moisture and oils from hands can cause a significant error in the measurements.

Procedure:

1. Accurately weigh a dry, cooled aluminium moisture dish and lid. Add approximately 2g of sample to the dish, replace the lid and quickly reweigh.
2. Place the dish, lid, and contents in the air oven at the prescribed temperature for 3 hours. Remove the lid from the dish and place the dish inside the lid to avoid confusion later. Before removing from the oven, cover the dish with the lid and transfer rapidly to the desiccator.
3. Once cool, weigh accurately.
4. Return to oven and repeat steps 2 and 3. Repeat until the weight in step 3 is constant.

Calculation:

$$\% \text{ total moisture and volatile matter} = \frac{(A - B) \times 100}{A}$$

where A = original weight of sample (g)
B = final dry weight of sample (g)

Appendix 6

Ash Content

The ash of a food is the inorganic residue remaining after the organic matter has been burnt away. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food as there may be losses due to volatilisation or some interaction between constituents. The ash can be regarded as a general measure of quality and often is a useful criterion in identifying the food or as an index of the presence of an adulterant.

Procedure:

1. Heat a silica or platinum dish (or crucible) for 60 minutes in the muffle furnace at 525-550°C. Cool in a desiccator for at least 60 minutes.
2. Using forceps, remove a cooled crucible and accurately weigh.
3. Accurately weigh about 1-2 g of sample into the crucible spreading the sample uniformly in the crucible before weighing.
4. Char over a Bunsen burner, taking care that sample does not ignite.
5. Place the dish in muffle furnace at 525-550°C and ash for 5 hours.
6. Remove the dish from the muffle furnace, cool thoroughly and weigh.

Calculation:

Ash content is the final weigh of residue remaining in the crucible.

Appendix 7

Mojonnier Method for Crude Fat Content

Apparatus:

1. Water bath
2. Beaker, 50ml
3. Mojonnier fat extraction apparatus
4. Steam bath
5. Drying oven
6. Centrifuge, Mojonnier type

Reagents:

1. Ethyl alcohol (95%)
2. Diethyl ether, free from residue on evaporation
3. Petroleum ether, boiling point below 60°C
4. 35% w/w ammonium hydroxide
5. 2% phenolphthalein

Procedure:

1. Weigh accurately sufficient sample to give between 0.3 and 0.7g of extracted fat into a dry Mojonnier tube (e.g. 10ml milk; 1-2 g milk powder). Make up to 10 ml if necessary with water, and shake to dissolve or blend.
2. Add 2 ml ammonium hydroxide and mix well in the lower bulb. Place in 60°C water bath for about 5 minutes and swirl occasionally. Cool. Add 2-4 drops phenolphthalein.
3. Add 10 ml of ethyl alcohol and mix by allowing the liquid to flow backwards and forwards between the two bulbs; avoid bringing the liquid too near the neck of the tube. The complete extraction of the fat is dependent on satisfactory mixing at each stage.
4. Add 25 ml of diethyl ether, close the tube with the stopper and shake gently for about one minute.
5. Remove the stopper and add 25 ml petroleum ether, using the first few ml to rinse the stopper and the neck of the tube, allowing the rinsing to run into the tube.
6. Replace the stopper, again wetted with water, and rock carefully for 30 seconds.
7. Centrifuge Mojonnier flask for 2 minutes at 600 RPM
8. Examine the tube to see if the interface of the liquid is in line with the upper junction of the neck of the tube. If it is below this, it should be raised by the addition of a little water run down the side of the tube.
9. Remove the cork and carefully decant as much as possible of the organic solvent layer into a preweighed short-necked flask by gradually bringing the cylindrical bulb of the tube into a horizontal position.
10. Add 5 ml ethyl alcohol and mix. This helps prevent emulsions forming and is in accord with the AOAC method.
11. Repeat the extraction using 15 ml of diethyl ether and 15 ml of petroleum ether (step 4 to 9). Add second extract into the same flask as used in step 9.

12. Distil carefully the solvents from the flask using a rotary evaporator and dry the flask in the oven at 100°C for 90 minutes, taking precautions to remove all traces of solvent vapour, prior to placing in the oven.
13. Allow the flask to cool to room temperature. Do not use a desiccator.
14. Weigh the flask, and record the fat content of the sample.
15. At the same time as the above procedure is carried out, make a blank determination with 10 ml of water in place of the sample. Use a similar extraction apparatus, the same reagents and the same technique throughout. Correct the apparent weight of fat for the change, if any, in the weight of the flask used for the blank determination.

Calculation:

Crude fat content is the final weight of residue remaining in the flask, expressed as a percentage of the weight of the original sample.

Appendix 8

Kjeldahl Method

The quantitative determination of total organic nitrogen in foods is often desired for estimating the total protein content but the method will include other nitrogenous compounds present usually in minor proportions. The crude protein is calculated by multiplying the total nitrogen by an empirical factor (for dairy product, 6.38). It should be borne in mind, however, that such a figure for protein often includes some non-protein nitrogenous compounds and also that nitrogen in certain forms (e.g. nitrates, nitrites and nitroso compounds) is not estimated in the Kjeldahl process.

Apparatus:
Kjeltec 1026 system (Tecator Sweden)

Reagents:
1. Concentrated H_2SO_4
2. Kjeltabs
3. 4% boric acid solution
4. 250 ml conical flask
5. 0.1 M HCl

Digestion:
1. Accurately weigh about 0.5 g sample into the digestion tube.
2. Add two kjeltabs (each containing 3.5g K_2SO_4 and 0.0035g Se) and then 15 ml concentrated H_2SO_4.
3. Carry on a blank digestion at the same time (no sample, but all other reagents). Set up block digestor unit and digest samples at 420°C for 40 minutes or until clear.
4. Remove the tubes carefully from the heating unit, leaving the exhaust manifold in place and water aspirator about half on. Allow to cool until the tops of the tubes are cool to touch.
5. Add approximately 70 ml hot distilled water to each tube and shake gently to mix. Ensure all solids have been dissolved.

Distillation and titration:
1. Add 25 ml 4% boric acid solution to 250 ml conical flask.
2. The distilling unit has been prepared for distillation and is set on "automatic"
3. Connect the digestion tube with the first sample to be distilled in position.
4. Place the receiver flask and boric acid solution on the platform and raise it to its upper position. To avoid contamination don't touch the glass outlet tube with your fingers. Hold it by its plastic tubing.
5. Close the safety door. The distillation automatically starts.
6. When the distillation is complete, the machine will "beep" several times. Remove the digestion tube and the receiver flask.

7. Titrate the sample with 0.1M HCl to grey-mauve end point.

Calculation:

$$\%N = \frac{(A \times B) \times 14 \times 100}{1000 \times C}$$

$$\%\text{Protein} = \%N \times 6.38$$

where A = mls HCl used
B = exact molarity (normality) of HCl
C = weight of original sample taken (g)

Appendix 9

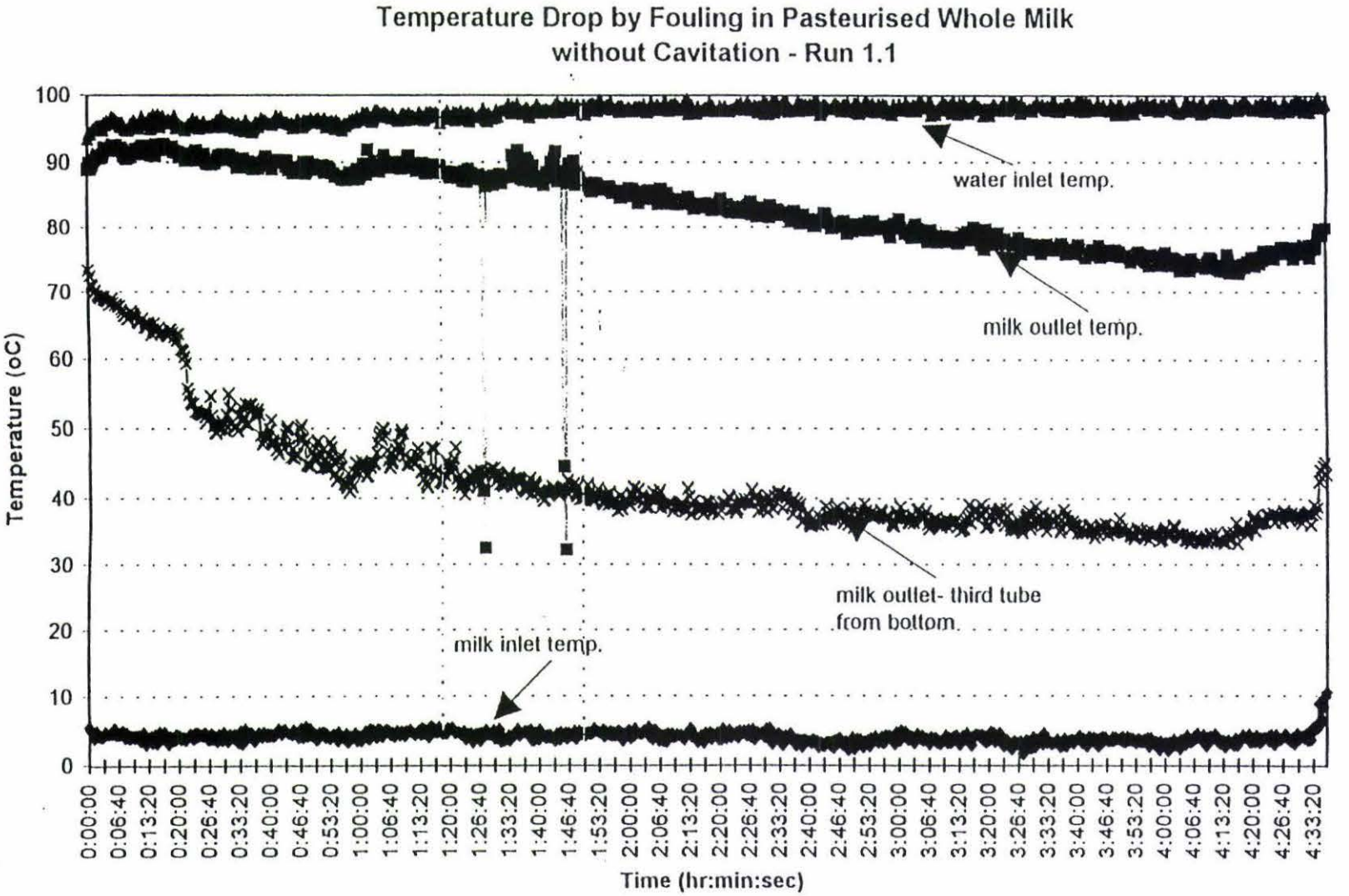
Chronology of Experimental Work

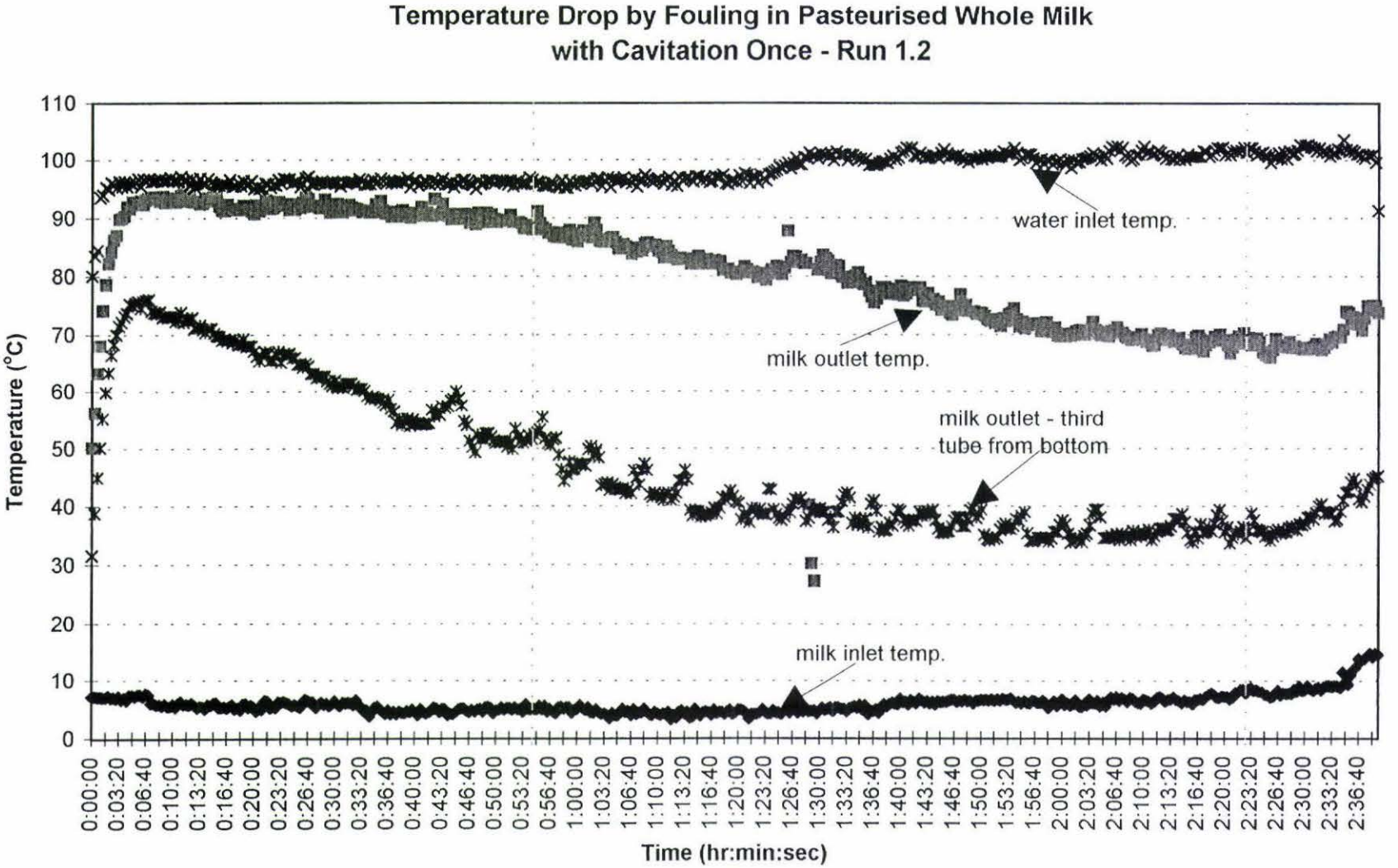
		Procedure
Date	**Steps**	**Description**
23/09/97		Prepared the lipase solution for lipolysable free fat test, stored at 4°C
24/09/97		Set up and cleaned the rig
25/09/97	1	400 litre pasteurised milk arrived (10 litre/bag X 40 bags)
	2	Fouling test of untreated milk
	3	Sampling 3X100ml
	4	Crude fat test and pH of the whole milk
	5	Particle size distribution by Mastersizer
	6	Collected fouling layer and cleaned the rig
26/09/97	1	One pass through the cavitating pump
	2	Fouling test of treated milk
	3	Sampling 3X100ml
	4	Particle size distribution by Mastersizer
	5	Micrographs by confocal laser scanning microscopy
	6	Collected fouling layer and cleaned the rig
28/09/97	1	Prepared the chemicals for lipolysable free fat test
	2	Added lipase to the samples and incubated the samples at 30°C for 18 hours
29/09/97	1	Lipolysable free fat test (FFA content)
	2	Free fat test
1/10/97	1	Two passes through the cavitating pump
	2	Fouling test of treated milk
	3	Particle size distribution by Mastersizer
2/10/97		Micrographs by confocal laser scanning microscopy
6/10/97		Milled the fouling deposit
7/10/97	1	Crude fat test of fouling deposit
	2	Moisture test of fouling deposit

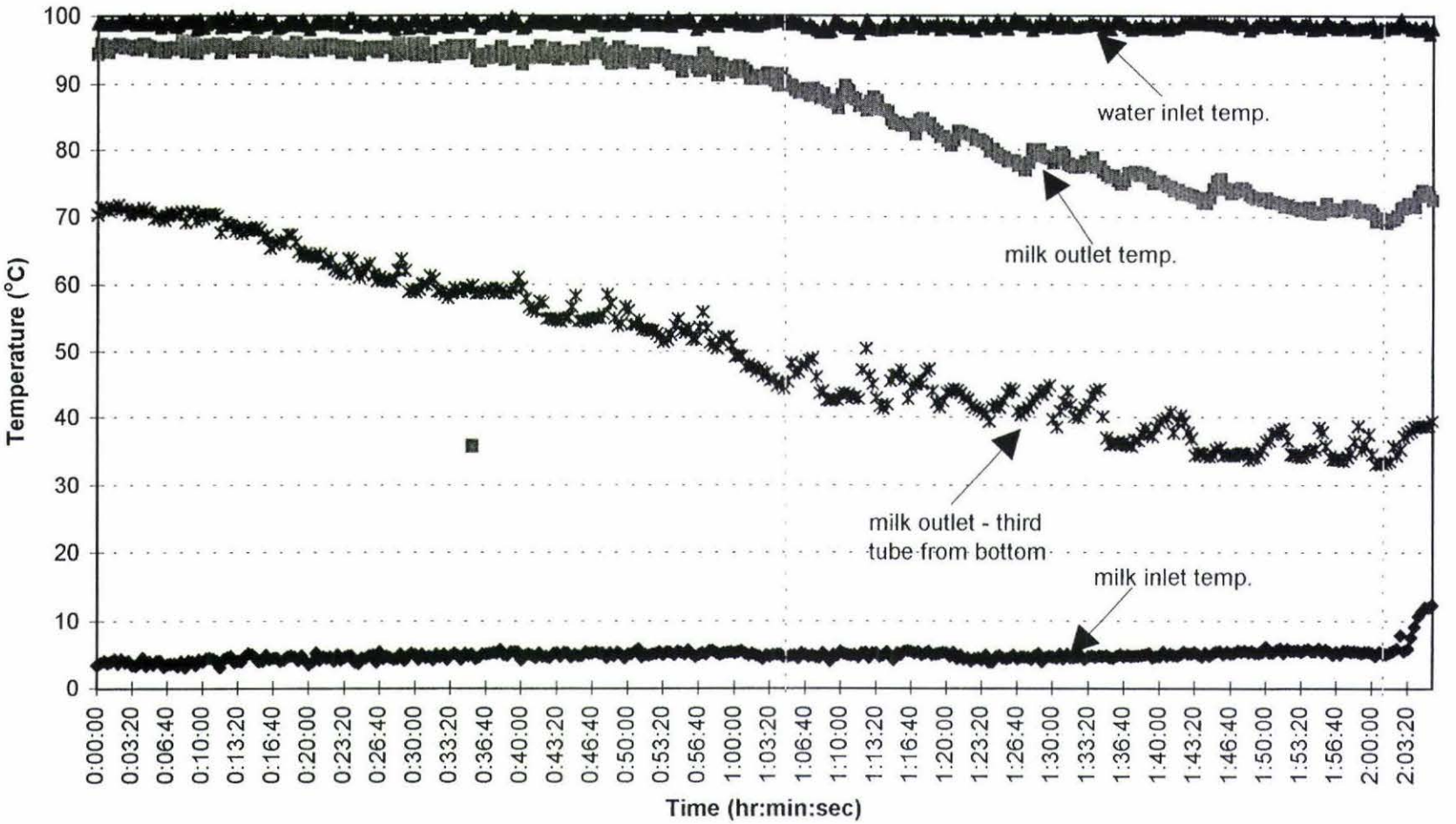
8/10/97		Crude protein test of fouling deposit
9/10/97	1	Crude fat test of fouling deposit
	2	Moisture test of fouling deposit
10/10/97	1	Crude protein test of fouling deposit
	2	Ash test
13/10/97		Prepare the chemicals for lipolysable free fat test
14/10/97	1	400 litre pasteurised milk arrived (10 litre/bag X 40 bags)
	2	Crude fat test and pH of the whole milk
15/10/97	1	One pass through the cavitating pump
	2	Fouling test of treated milk
	3	Particle size distribution by Mastersizer
	4	Micrographs by confocal laser scanning microscopy
	5	Collected fouling layer and cleaned the rig
16/10/97	1	Fouling test of untreated milk
	2	Micrographs by confocal laser scanning microscopy
	3	Collected fouling layer and cleaned the rig
17/10/97	1	Free fat test
	2	Prepared the lipase solution for lipolysable free fat test, stored at 4°C
19/10/97	1	Added lipase to the samples and incubated the samples at 30°C for 23 hours
	2	Moisture test of fouling deposit
20/10/97		Lipolysable free fat test (FFA content)
21/10/97		*Free fat test
22/10/97	1	Four passes through the cavitating pump
	2	Fouling test of treated milk
	3	Particle size distribution by Mastersizer
	4	Collected fouling layer and cleaned the rig
23/10/97	1	Crude fat test of fouling deposit
	2	Moisture test of fouling deposit
24/10/97	1	Crude protein test of liquid milk and fouling deposit
	2	Micrographs by confocal laser scanning microscopy
28/10/97	1	Prepared the lipase solution for lipolysable free fat test, stored at 4°C
	2	Crude fat test and pH of the whole milk
	3	Crude protein test of the whole milk

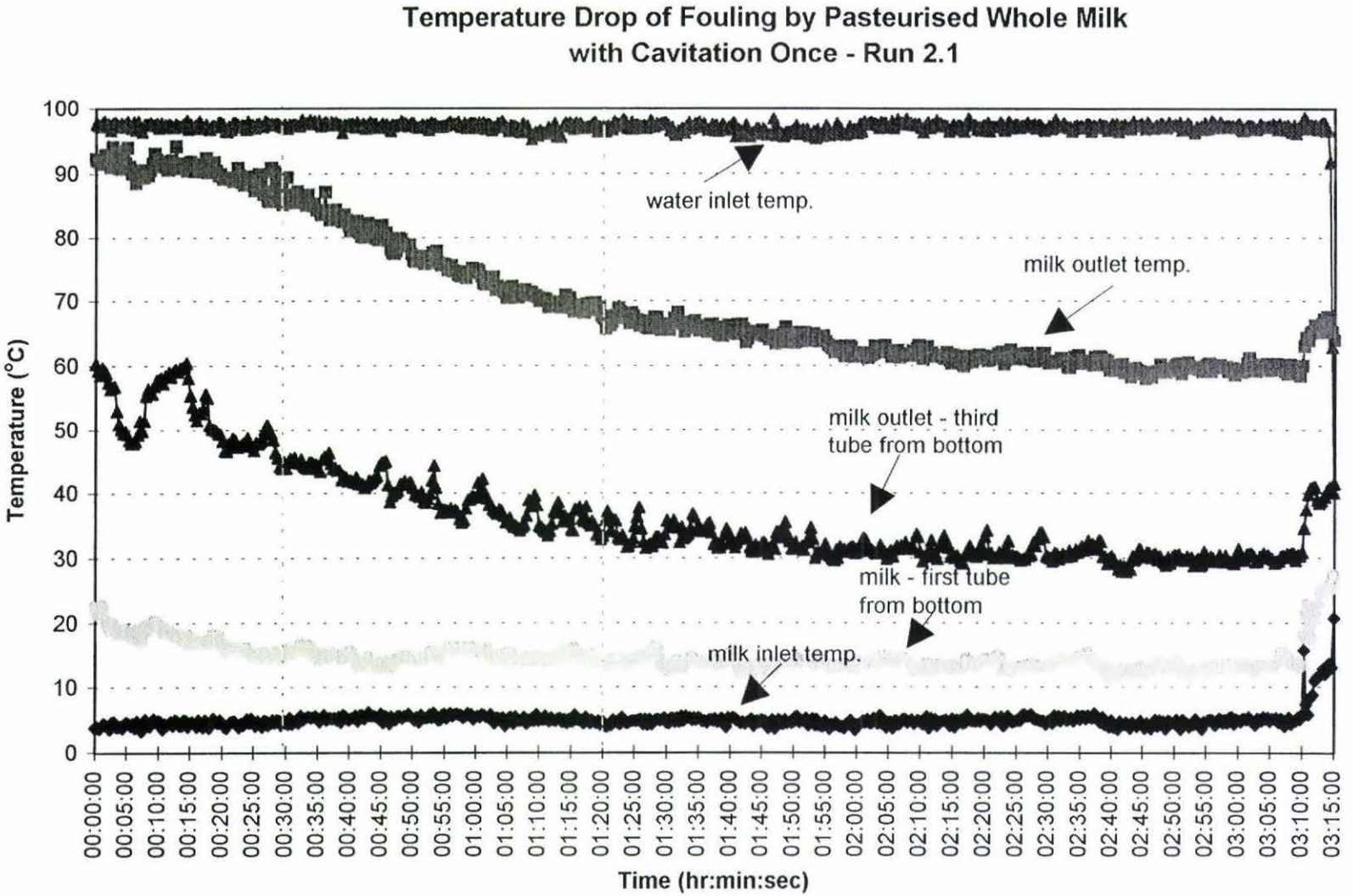
29/10/97	1	Five passes of cavitating pump
	2	Fouling test of treated milk
	3	Particle size distribution by Mastersizer
	4	Micrographs by confocal laser scanning microscopy
	5	Collected fouling layer and cleaned the rig
30/10/97	1	Three passed of cavitating pump
	2	Fouling test of treated milk
	3	Particle size distribution by Mastersizer
	4	Micrographs by confocal laser scanning microscopy
	5	Collected fouling layer and cleaned the rig
31/10/97	1	Fouling test of untreated milk
	2	Free fat test
	3	Collected fouling layer and cleaned the rig
2/11/97	1	Added lipase to the samples and incubated the samples at 30°C for 23 hours
	2	Prepared chemicals for lipolysable free fat test
3/11/97	1	Lipolysable free fat test (FFA content)
	2	Ash test
5/11/97	1	Crude protein content of fouling deposit
	2	Ash test
6/11/97		Moisture test of fouling deposit
7/11/97		Crude fat content of fouling deposit
8/11/97		Moisture test of fouling deposit
10/11/97	1	Crude protein content of fouling deposit
	2	Ash test
11/11/97		Crude fat test of fouling deposit
12/11/97		Crude fat test of fouling deposit

Appendix 10 Curves of Temperature Versus Time in the Fouling Rig Heat Exchanger

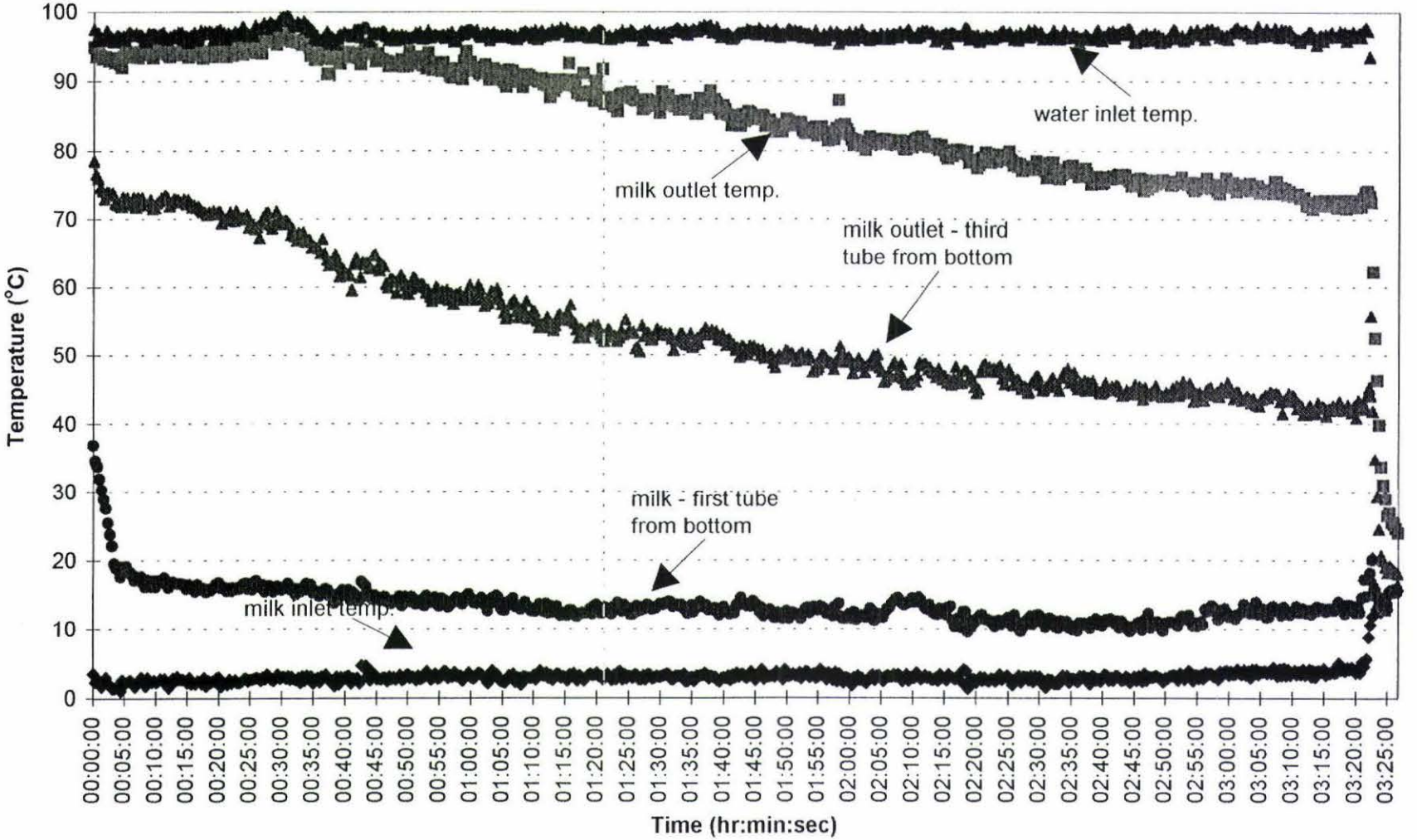



Temperature Drop by Fouling in Pasteurised Whole Milk
with Cavitation Once - Run 1.2
Temperature (°C)
110
100
90
80
70
60
50
40
30
20
10
0
water inlet temp.
milk outlet temp.
milk outlet - third
tube from bottom
milk inlet temp.
0:00:00
0:03:20
0:06:40
0:10:00
0:13:20
0:16:40
0:20:00
0:23:20
0:26:40
0:30:00
0:33:20
0:36:40
0:40:00
0:43:20
0:46:40
0:50:00
0:53:20
0:56:40
1:00:00
1:03:20
1:06:40
1:10:00
1:13:20
1:16:40
1:20:00
1:23:20
1:26:40
1:30:00
1:33:20
1:36:40
1:40:00
1:43:20
1:46:40
1:50:00
1:53:20
1:56:40
2:00:00
2:03:20
2:06:40
2:10:00
2:13:20
2:16:40
2:20:00
2:23:20
2:26:40
2:30:00
2:33:20
2:36:40
Time (hr:min:sec)

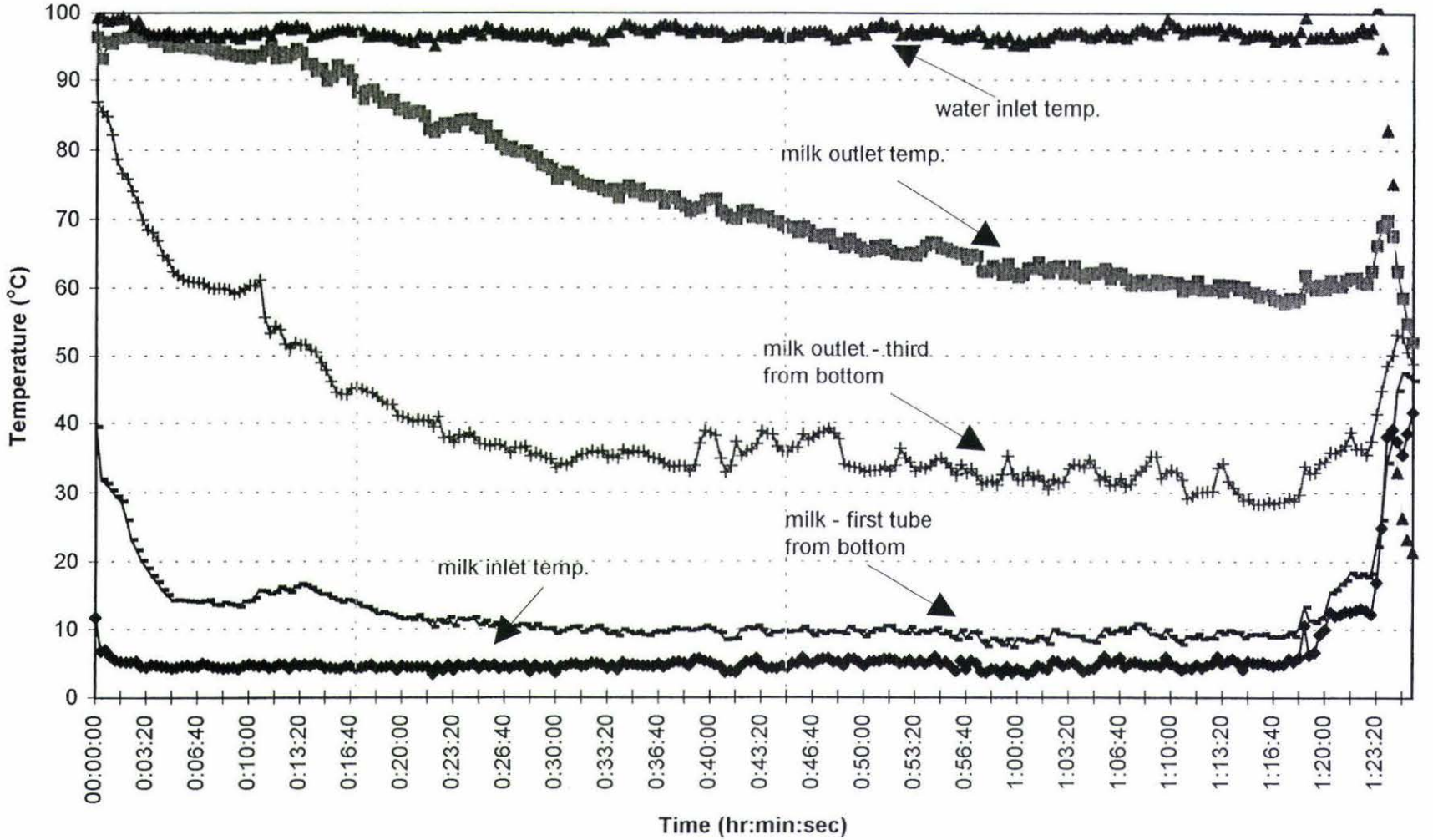

Temperature Drop by Fouling in Pasteurised Whole Milk
with Cavitation Twice - Run 1.3
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
water inlet temp.
milk outlet temp.
milk outlet - third
tube from bottom
milk inlet temp.
0:00:00
0:03:20
0:06:40
0:10:00
0:13:20
0:16:40
0:20:00
0:23:20
0:26:40
0:30:00
0:33:20
0:36:40
0:40:00
0:43:20
0:46:40
0:50:00
0:53:20
0:56:40
1:00:00
1:03:20
1:06:40
1:10:00
1:13:20
1:16:40
1:20:00
1:23:20
1:26:40
1:30:00
1:33:20
1:36:40
1:40:00
1:43:20
1:46:40
1:50:00
1:53:20
1:56:40
2:00:00
2:03:20
Time (hr:min:sec)

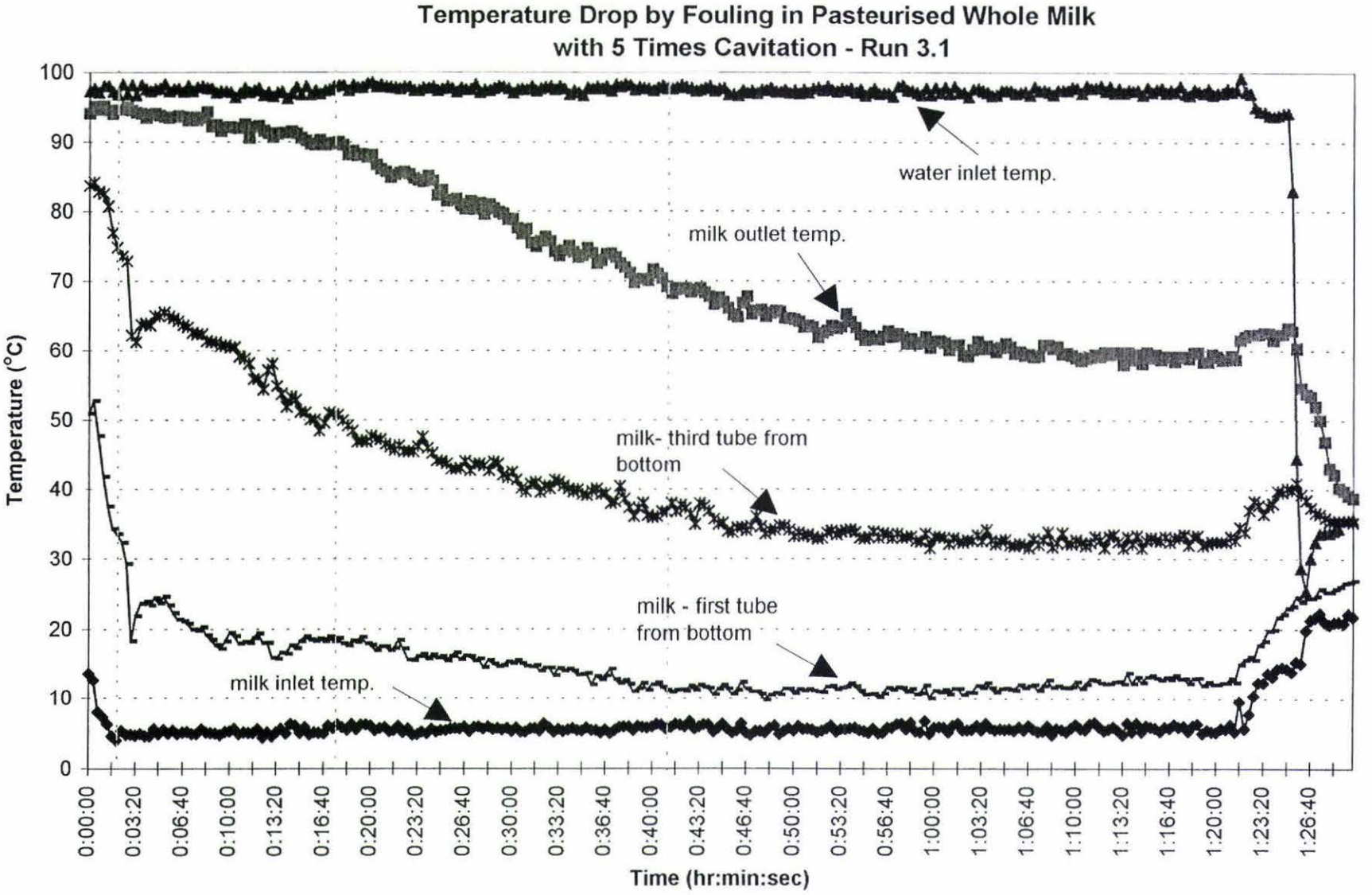

Temperature Drop of Fouling by Pasteurised Whole Milk
with Cavitation Once - Run 2.1
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
water inlet temp.
milk outlet temp.
milk outlet - third
tube from bottom
milk - first tube
from bottom
milk inlet temp.
00:00:00
00:05:00
00:10:00
00:15:00
00:20:00
00:25:00
00:30:00
00:35:00
00:40:00
00:45:00
00:50:00
00:55:00
01:00:00
01:05:00
01:10:00
01:15:00
01:20:00
01:25:00
01:30:00
01:35:00
01:40:00
01:45:00
01:50:00
01:55:00
02:00:00
02:05:00
02:10:00
02:15:00
02:20:00
02:25:00
02:30:00
02:35:00
02:40:00
02:45:00
02:50:00
02:55:00
03:00:00
03:05:00
03:10:00
03:15:00
Time (hr:min:sec)

Temperature Drop by Fouling in Pasteurised Whole Milk without Cavitation - Run 2.2



Temperature Drop by Fouling of Pasteurised Whole Milk with 4 Times Cavitation - Run 2.3




Temperature Drop by Fouling in Pasteurised Whole Milk
with 5 Times Cavitation - Run 3.1
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
water inlet temp.
milk outlet temp.
milk- third tube from bottom
milk - first tube from bottom
milk inlet temp.
0:00:00
0:03:20
0:06:40
0:10:00
0:13:20
0:16:40
0:20:00
0:23:20
0:26:40
0:30:00
0:33:20
0:36:40
0:40:00
0:43:20
0:46:40
0:50:00
0:53:20
0:56:40
1:00:00
1:03:20
1:06:40
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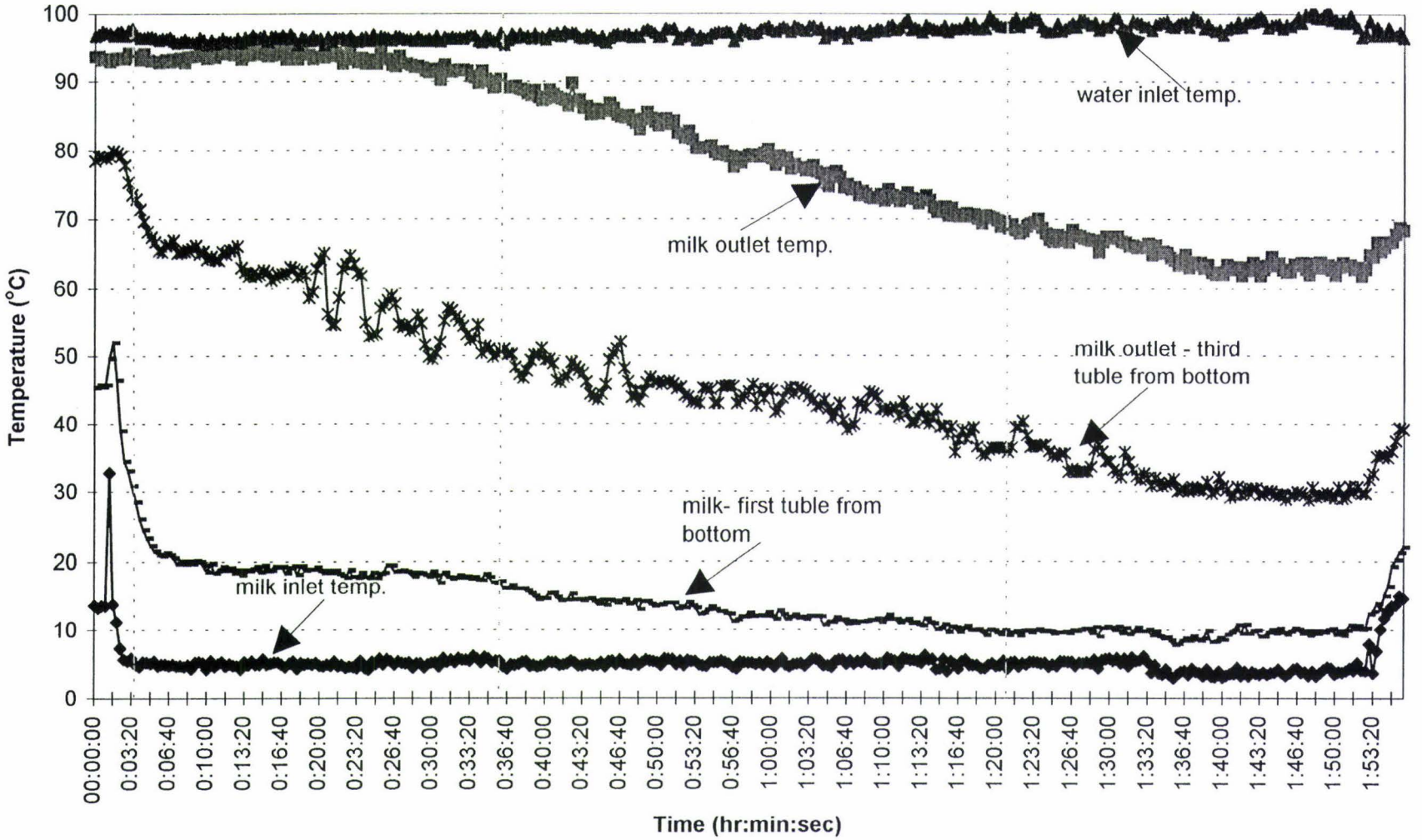
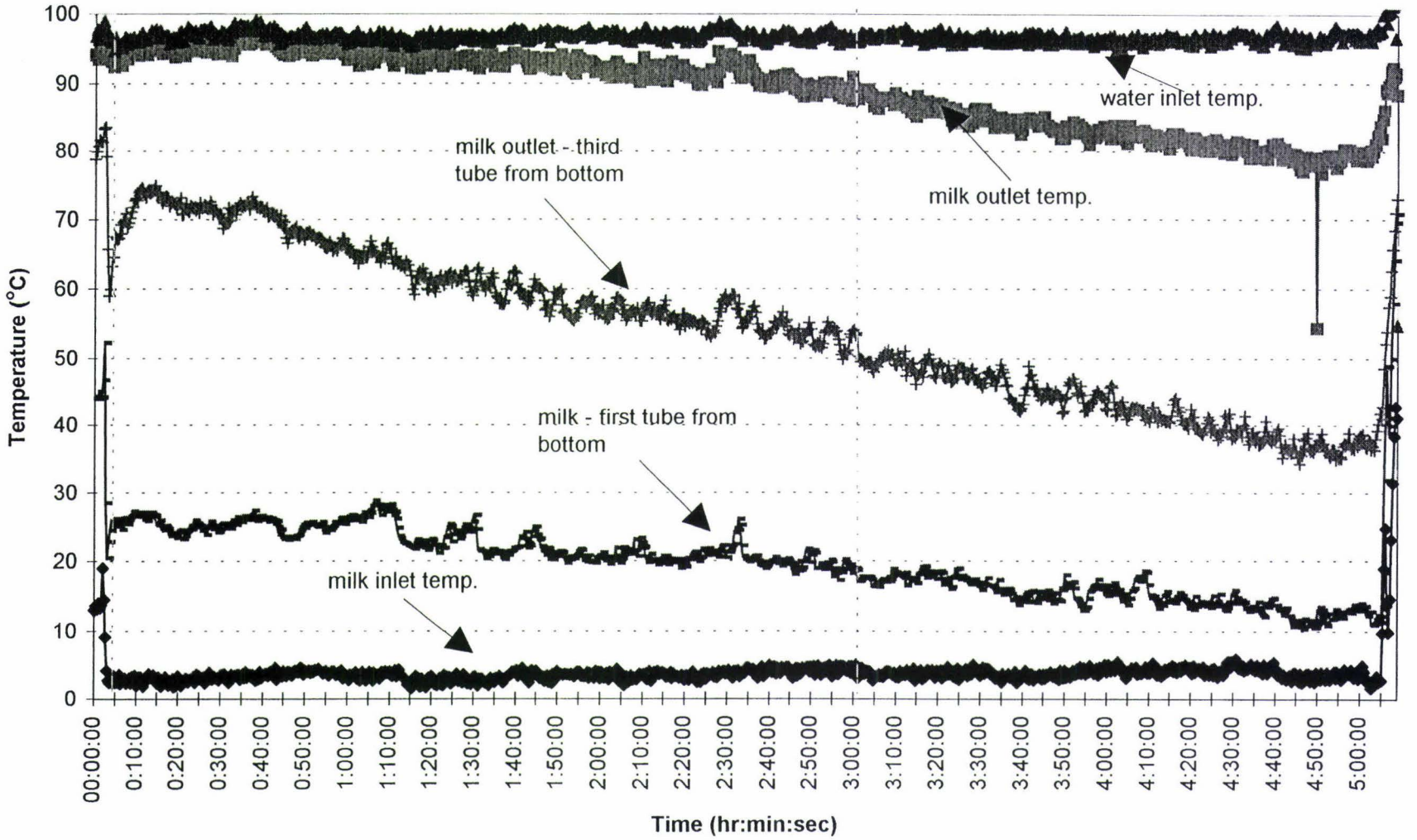
1:23:20
1:26:40
Time (hr:min:sec)


Temperature Drop by Fouling in Pasteurised Whole Milk
with 3 Times Cavitation - Run 3.2
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
water inlet temp.
milk outlet temp.
milk outlet - third
tuble from bottom
milk- first tuble from
bottom
milk inlet temp.
00:00:00
0:03:20
0:06:40
0:10:00
0:13:20
0:16:40
0:20:00
0:23:20
0:26:40
0:30:00
0:33:20
0:36:40
0:40:00
0:43:20
0:46:40
0:50:00
0:53:20
0:56:40
1:00:00
1:03:20
1:06:40
1:10:00
1:13:20
1:16:40
1:20:00
1:23:20
1:26:40
1:30:00
1:33:20
1:36:40
1:40:00
1:43:20
1:46:40
1:50:00
1:53:20
Time (hr:min:sec)

Temperature Drop by Fouling in Pasteurised Whole Milk without Cavitation - Run 3.3



Appendix 11

Results of Chemical Analyses of Fouling Deposits

Results of chemical analysis of *fouling depositt - trial one*

Run Number		Run 1.1	Run 1.2	Run 1.3
CONTENT	SAMPLE	UNDAMAGED (170L)	DAMAGED ONCE (120L)	DAMAGED TWICE (90L)
		percentage	percentage	percentage
TOTAL FAT	1	50.49%	45.60%	45.17%
	2	50.92%	45.96%	46.73%
	3	50.58%	46.66%	44.88%
	AVERAGE	50.66%	46.07%	45.59%
PROTEIN	1	29.46%	30.13%	28.45%
	2	29.35%	30.11%	28.64%
	3	30.03%	30.33%	28.52%
	AVERAGE	29.61%	30.19%	28.54%
ASH	1	4.66%	4.50%	4.01%
	2	4.63%	4.10%	3.94%
	3	4.71%	4.18%	4.02%
	AVERAGE	4.67%	4.26%	3.99%
MOISTURE	1	5.14%	6.29%	5.35%
	2	4.98%	6.21%	5.70%
	3	5.10%	6.21%	5.60%
	AVERAGE	5.07%	6.24%	5.55%

Results of chemical analysis of *fouling deposit - trial two*

Run Number		Run 2.2	Run 2.1	Run 2.3
CONTENT	SAMPLE	UNDAMAGED (160L)	DAMAGED ONCE (160L)	DAMAGED 4 TIMES(70L)
		percentage	percentage	percentage
TOTAL FAT	1	46.80%	46.00%	41.67%
	2	46.50%	47.70%	41.59%
	3	46.10%	45.20%	41.15%
	AVERAGE	46.50%	46.30%	41.47%
PROTEIN	1	30.05%	31.26%	29.32%
	2	30.11%	31.47%	29.39%
	3	30.63%	31.22%	29.82%
	AVERAGE	30.26%	31.32%	29.51%
ASH	1	4.64%	4.29%	3.94%
	2	4.60%	4.18%	3.90%
	3	4.57%	4.31%	3.92%
	AVERAGE	4.60%	4.26%	3.92%
MOISTURE	1	5.89%	4.53%	8.94%
	2	5.77%	4.62%	8.90%
	3	5.75%	4.76%	9.05%
	AVERAGE	5.80%	4.64%	8.96%

Results of chemical analysis of *fouling deposit - trial three*

Run Number		Run 3.3	Run 3.2	Run 3.1
CONTENT	SAMPLE	UNDAMAGED (210L)	DAMAGED 3 TIMES (90L)	DAMAGED 5 TIMES (70L)
		percentage	percentage	percentage
TOTAL FAT	1	43.39%	47.83%	44.20%
	2	44.17%	47.62%	44.12%
	3	44.50%	46.93%	45.37%
	AVERAGE	44.02%	47.46%	44.56%
PROTEIN	1	31.09%	29.71%	29.78%
	2	30.66%	29.56%	29.58%
	3	30.56%	29.70%	29.84%
	AVERAGE	30.77%	29.66%	29.73%
ASH	1	4.98%	4.09%	3.74%
	2	4.96%	4.19%	3.74%
	3	5.02%	4.10%	3.74%
	AVERAGE	4.99%	4.13%	3.74%
MOISTURE	1	7.50%	8.01%	9.29%
	2	7.56%	8.11%	9.41%
	3	7.38%	8.08%	9.30%
	AVERAGE	7.48%	8.07%	9.33%