NATIVE MILK FAT GLOBULE MEMBRANE DAMAGE – MEASUREMENT AND EFFECT OF MECHANICAL FACTORS IN MILK POWDER PROCESSING OPERATIONS

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"Trust in the LORD with all your heart
and lean not on your own understanding;
in all your ways acknowledge him,
and he will make your paths straight."

The Bible: Proverbs, chapter 3 verses 5-6

Abstract

The goal of this work was to measure native milk fat globule membrane (NMFGM) damage in a number of processing operations within the milk powder manufacturing process.

Analysis of the literature showed that NMFGM damage was not well understood, particularly as caused by processing operations within factories. Reliable methods of measuring NMFGM damage were not available; current methods had limited scope or were qualitative in nature. In the highly mechanised dairy industry, damage to the NMFGM can lead to serious quality and financial losses owing to consequences such as lipolysis and creaming. The aims of this work were to develop new techniques for measuring NMFGM damage, and to use these in assessing the effects of a number of operations within the milk powder process.

The majority of time was spent on developing two new tests, the selective lipolysis (SL1) test and the particle size zoning (PSZ) test. The SL1 test measures a chemical consequence of NMFGM damage, that is the production of free fatty acids (FFAs). The PSZ test measures a physical consequence of NMFGM damage, that is the change in the fat globule size distribution.

Controlled experiments were used to measure NMFGM damage in process operations including pumping, agitation, preheating and evaporation. For these operations, variables such as shear, time, temperature, air inclusion and cavitation were investigated. Surveys of two industrial milk powder plants were also conducted.

The results showed that the SL1 and PSZ tests were reproducible, sensitive enough to detect NMFGM damage in a number of process operations, and, together, could give a reasonably comprehensive picture of NMFGM damage. The results of pumping and agitation experiments were consistent with previous research, but were more comprehensive. The effects on measured NMFGM damage of the presence of separated fat in foam or as churned fat have hardly been described by previous workers. Results for the effects of preheating and evaporation on NMFGM damage are new, and challenged the findings of previous research.

The need to improve the flexibility and practicality of the SL1 and PSZ tests, so they can be used as widely as possible to gain a comprehensive picture of NMFGM damage across many dairy processes, was identified. Studies should be made to connect the results of the particle size zoning and selective lipolysis tests with product quality and process efficiency data from industrial sites.

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List of Abbreviations

Abbreviation	Definition
IFA	incubation fatty acids
OFA	original fatty acids
ADV	acid degree value
CIP	clean(ed)-in-place
CL	calf lipase
DSI	direct steam injection
FAPF	fatty acids from protected fat
FAUPF	fatty acids from unprotected fat
FF	free fat
FFA(s)	free fatty acid(s)
GLC	gas-liquid chromatography
KOH	potassium hydroxide
LPL	lipoprotein lipase
P.R.	primary region
PF	protected fat
PHE	plate heat exchanger
PLC	programmable logic controller
PPL	pig pancreatic lipase
PSD(s)	particle size distribution(s)
SL1 test	selective lipolysis test – version 1
SL2 test	selective lipolysis test – version 2
SLFA	selective lipolysis fatty acids
UHT	ultra high temperature
UPF	unprotected fat

Chapter 1
Introduction

Virtually all of the fat in milk is found in the form of spherical globules, the disperse phase of an emulsion in milk plasma. Each globule is surrounded by a native milk fat globule membrane (NMFGM) composed of mostly surface-active lipids and proteins. The NMFGM constitutes approximately 2% of fat globule mass and therefore less than 1% of the total solids in milk but has an essential role in the stability of the emulsion.

The NMFGM acts as a barrier between the core fat and the milk plasma. This barrier resists such phenomena as:

- Linkages between, or coalescence of, fat globules, and consequent changes in milk fat globule size distribution.
- Enzymes that attack milk fat to convert them into free fatty acids (FFAs).

The composition of the NMFGM is very different from the two phases it separates – globular fat and milk plasma (Mulder & Walstra, 1974). Most globular fat is in the form of triacylglycerols. NMFGM fat consists mostly of phospholipids. Milk plasma includes proteins in the form of casein micelles, colloidally dispersed serum (whey) proteins and enzyme, but the NMFGM is largely unique. The NMFGM protein has an amino acid pattern similar to that of the apical cell-membrane of the lactating cell (Keenan et al., 1970).

The unique composition of the NMFGM makes it a significant component. Its function however, makes it a critical component! If the NMFGM is breached (i.e. if NMFGM damage has occurred) the above mentioned phenomena result, and the quality of the milk is reduced. McPherson & Kitchen (1983) asserted that "Thus many current problems of the dairy industry are directly related to an understanding of this unique membrane system."

The NMFGM is easily damaged during handling operations such as milking, storage at the farm and transportation. Even the simple process of chilling the milk can lead to changes in the NMFGM such as loss of phospholipids (Anderson et al., 1972) and

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alterations to the protein composition (Thompson et al., 1961). Breaches of the NMFGM can lead to physical changes such as disruption of fat globules, with subsequent difficulties in separation into cream and skim milk, and chemical changes such as lipolysis, with consequent formation of off-flavours. Any uncovered fat globule surface resulting from damage to the NMFGM is almost instantly covered by serum proteins and casein micelles (Walstra et al., 1999). It is therefore useful to differentiate between the NMFGM, which consists mainly of phospholipids and proteins derived from the apical membranes of secretory cells of the cow, and the milk fat globule membrane found in processed milk, which also contains serum proteins and casein micelles.

In New Zealand, around 13 billion litres of milk are processed each season at 29 manufacturing sites (http://www.fonterra.com/content/aboutfonterra/factsandfigures/default.jsp, March 2004). This equates to an average of over 2 million litres processed at each site in New Zealand every day of the season. This high throughput, coupled with the tendency for milk to lose its quality rapidly over time, means that even small delays in production can result in significant financial loss to the industry.

The New Zealand dairy industry has put considerable effort into making itself as cost-effective, and its products as attractive to customers, as possible, including efforts to:

- Maximise milk quality entering factories.
- Reduce microbial contamination in factories.
- Minimise product losses through the reprocessing of waste streams and fine particulates.
- Maximise the sizes of factories and therefore reduce capital costs.
- Minimise transportation costs.

This has made the New Zealand dairy industry highly mechanised.

Evidence of this mechanisation can be seen in many parts of the New Zealand dairy industry. On farms, it is typified by the universal use of pipeline milking machines, and storage of milk in large silos, often of up to 28,000 litres in the case of farms with large herds. Milk tankers transport the milk from the farm and can carry up to 27,000 litres at one time (http://meadowfresh.net.nz/about_milk/from_moo_to_you.php, 2004). However, in some

parts of the country, the milk is transported very long distances by train, from intermediate storage sites to the factories. New Zealand dairy factories are among the largest in the world, many having multiple storage silos in excess of 250,000 litres individual capacity, with considerable use of pumps to transport the milk around the factory.

With the push for mechanisation and larger factories, potentially significant financial losses may be created through loss of product quality resulting from rough handling of the milk. While the handling of raw milk on dairy farms has been studied to some extent, very little attention has been paid to raw and pasteurised milk in factory processing steps following farm handling. Such processes include, in addition to pasteurisation, storage, pumping, separation, and thermal processes such as preheating and evaporation.

Various authors have highlighted the potentially harmful effects of poor milk handling, during process operations, that damages the milk fat. Such effects include fouling of heat exchangers, poor cream separation and flavour defects. These effects result in loss of product and poor milk and cream quality. While no formal cost analysis has been applied to these effects, it is clear they do cost money, and given the size of the New Zealand dairy industry must be taken seriously. Damage to the NMFGM in process operations within factories was the focus of the present work.

The work began with the aim of using existing NMFGM damage measurement techniques to assess NMFGM damage in milk powder manufacturing processes, because milk powders constitute over 30% of all New Zealand dairy products. (http://www.fonterra.com/content/aboutfonterra/factsandfigures/default.jsp, 2004), and very little research has been conducted on the impact of milk powder manufacturing operations on the milk powders. It was hypothesised that incremental increases in NMFGM damage in individual operations within the milk powder manufacturing process would contribute to an overall loss of milk powder quality.

However, it became clear over time that the NMFGM damage measurement tests based on the measurement of lipolysis, which were initially chosen, were not reliable enough

to allow a valuable survey of NMFGM damage in some situations. Hence, much of the early work could only highlight the weaknesses of the existing tests.

Therefore, the first objective of this project was to develop a technique for the measurement of NMFGM damage.

A second objective was to identify and analyse the factors that affect damage to the NMFGM using the measurement techniques devised.

The final objective was to make case studies of the damage occurring in a number of key operations or equipment found in milk powder plants where the factors identified in reaching the second objective could be found.

Therefore, this thesis is an account of an ongoing journey that began with concepts on NMFGM damage that ultimately had to be redeveloped before the original aim of the work could be pursued.

Chapter 2
Literature Review

2.1 Introduction

In order to understand how native milk fat globule membrane (NMFGM) damage affects process operations five basic topic areas must be reviewed:

- Measurement of NMFGM damage
- Factors affecting NMFGM damage
- Process operations that cause NMFGM damage in model experiments
- NMFGM damage in industrial applications
- The impact of NMFGM damage on the final products of dairy manufacturing processes, especially milk, cream and milk powders.

In each section the wide range of terms used in the literature will be reorganised and simplified to clarify the terminology and give a clear distinction between work directly relevant to NMFGM damage and work in related areas but of less relevance. A full coverage of NMFGM chemistry has not been attempted, particularly as this topic is not directly relevant to NMFGM damage that is caused by mechanical factors. Excellent reviews are available on the origin (Mather & Keenan, 1998) composition and structure of the NMFGM (Mulder & Walstra, 1974; McPherson & Kitchen, 1983; Walstra et al., 1984).

Damage to the NMFGM may be caused by mechanical means, such as agitation, or chemical means, such as attack by lipolytic enzymes, made up of lipases and esterases (Chen et al., 2003). The activity of lipolytic enzymes either endogenous (e.g. Downey, 1975; Driessen & Stadhouders, 1975; Anderson, 1983; Sundheim, 1988; Girardet et al., 1993) or microbial (e.g. Suhren, 1989; Swarna et al., 1997) has been studied extensively. Methods of detection for lipolytic activity in final milk products (e.g. Antonelli et al., 2002) are important since many lipolytic enzymes originating from psychrotrophic bacteria are known to be heat resistant (e.g. Kishonti, 1975; Adhikari & Singhal, 1992; Choi et al., 1994). An excellent review on protease and lipase activity

has been published by Chen et al. (2003). The book by McKellar (1989) remains a useful introduction to enzymes of psychrotrophs.

Lipolysis, the attack of fat by lipolytic enzymes, has serious consequences to milk flavour and formed the topic of three reviews (Deeth & Fitz-Gerald, 1976, 1995; Fleming, 1979). The techniques for measuring free fatty acids (FFAs) that result from lipolysis have received a large amount of attention with several good reviews (Kuzdzal-Savoie, 1980; Anderson et al., 1991; Joshi & Thakar, 1994; Collomb & Spahni, 1995).

This review covers specifically damage to the NMFGM by mechanical factors.

2.2   Measurement of NMFGM damage

It is necessary to develop reliable measurements of NMFGM damage before the causes of damage, changes in process efficiency and consequences to product quality from NMFGM damage can be analysed. This task is made more difficult because of the lack of a clear definition of NMFGM damage. It has not been clearly established whether the stability of the milk emulsion requires actual removal, whether partial or complete, of the NMFGM or can result from the removal of only specific NMFGM components from the fat globule surface. For the purposes of this review, damage to the NMFGM is defined as change(s) that leave fat globules unprotected against attack from endogenous milk lipolytic enzymes, in particular lipoprotein lipase (LPL). LPL is known to attack only fat not protected by the NMFGM but does not attack the membrane itself. In that sense it can be said to be substrate selective. Damage to the NMFGM would allow chemical changes to the milk system such as lipolysis and interactions between the fat globules and proteins, in particular casein and to some lesser extent serum proteins, as well as physical changes such as increases in fat globule size through the process of coalescence or decrease through disruption.

In order to analyse the state of the present literature effectively, the author proposes that two concepts must be differentiated. Firstly, the detection of NMFGM damage through any of its consequences such as production of FFAs, lipolysis and changes to particle size distribution (PSD) and secondly, the quantitative measurement of that damage,

which requires more rigorous protocols for the complete recovery of all the NMFGM damage. It will be apparent in the following sections of the review that the vast majority of work in this area can claim successful detection of NMFGM damage but that a reliable method for the quantitative measurement of NMFGM damage is still lacking. Unfortunately, authors do not often differentiate between these two concepts.

Measurement of NMFGM damage will be split into three parts:

- Firstly, direct measurement of the NMFGM, which requires an understanding of the complex composition of the NMFGM.

- Secondly, measurement of the chemical consequences of damage to the NMFGM, including FFA tests and sensory evaluations.

- Thirdly, measurement of the physical consequences of NMFGM damage, including the so-called "free fat" (FF) tests and the use of particle size measurement and microscopy.

2.2.1   Direct measurement of NMFGM damage.

In order to measure directly NMFGM damage, the composition of the NMFGM must be known. This review is not intended to examine NMFGM composition in depth, partly as it is a subject requiring an entire literature review on its own, but also because the work on composition does not look at NMFGM damage in much depth. The NMFGM, which originates from the apical membrane of the secretory cells of the mammary gland of the cow, consists of a complex mixture of proteins, phospholipids, glycoproteins, triacylglycerols, cholesterol, enzymes and other minor components (McPherson & Kitchen, 1983). A qualitative understanding of NMFGM composition has been established and ranges of the major NMFGM components are shown in Table 2.1 (e.g. Brunner, 1974; McPherson & Kitchen, 1983). Proportions of many of the components vary depending on dairying factors, for example the stage of lactation of the cow, type of feed and breed of cow (Brunner, 1974) and experimental factors, for example the method of extraction of the NMFGM for analysis.

Table 2.1: Quantitative description of NMFGM composition. Based on data from Brunner (1974) and McPherson & Kitchen (1983).

Component	Average (%)	Range (%)
Phospholipids	20	2-42
Neutral Lipids	38	12-61
Protein	42	23-63

The most direct measurement of NMFGM damage would involve visual observation, for example, by electron microscopy. However, the logistical problems of this task are daunting and there is no record of any such attempt. An alternative technique is to try and identify components that are present only in the NMFGM and detect their presence in the milk serum once the NMFGM has been damaged.

Alkaline phosphatase is an enzyme found almost exclusively in the NMFGM (Stannard, 1975). Stannard reasoned that when the NMFGM is damaged this enzyme would move into the skim milk phase. However, the measurement of this enzyme in the skim milk phase correlated poorly with measurements of free fat and lipolysis (Stannard, 1975; Deeth & Fitz-Gerald, 1978). It therefore appears that the movement of alkaline phosphatase from the NMFGM into the skim milk phase involves more than one mechanism.

Thus to date no reliable direct measurement of NMFGM damage exists and much more work has been based on the chemical and physical consequences of NMFGM damage.

2.2.2 Consequences of NMFGM damage

The consequences of NMFGM damage will be grouped into three categories: chemical, physical and sensory.

Chemical Consequences

In this review a chemical consequence of NMFGM damage is where damage leads to the conversion of triacylglycerols inside a fat globule into free fatty acids (FFAs), i.e. lipolysis, as described below.

If the NMFGM is removed from a fat globule the fat is more susceptible to attack from lipolytic enzymes. Surface-active components from milk plasma (especially casein micelles) adsorb to the fat globule surface (Walstra et al., 1984) and lipolytic enzymes, often associated with the casein micelles can more easily adsorb onto the fat globule surface. Lipolytic enzymes attack acylglycerol molecules, in particular triacylglycerols, by removing individual fatty acid chains. Removal of some of the proteins from the NMFGM also promotes attachment of lipolytic enzymes to the fat globules (e.g. Sundheim & Bengtsson-Olivecrona, 1987a).

Physical Consequences

In this review physical consequences of NMFGM damage are defined as those effects that alter the structure but not the composition of the milk.

Walstra et al. (1999) have summarised the changes in particle size distribution (PSD) upon NMFGM damage with a useful diagram, reproduced in Figure 2.1. When damage to the NMFGM is substantial, the original fat globules are reduced in size in a process they called disruption. This nomenclature is widely accepted. For example, Deeth & Fitz-Gerald (1978) used it to describe the splitting or dispersion of fat globules. Mulder & Walstra (1974) used that term to describe the effect of homogenisation of whole milk. When damage to the NMFGM is moderate, Walstra et al. (1984) reported that the size of fat globules actually increases. The level of shear applied to the milk fat and the ratio of solid and liquid fat affect this phenomenon. At temperatures when all the milk fat has become liquid the original fat globules tend to coalesce into larger ones upon NMFGM damage. At lower temperatures, when only part of the fat has been liquefied, the combination of fat globules can flocculate into irregular clusters such as those shown in Figure 2.2 (Fang, 1998).

The coalescence of damaged fat globules can be accentuated by centrifugation to the point that a layer of fat can be gathered above the milk emulsion. This is known by some authors as "free fat" (FF) (e.g. Halter et al., 1978).

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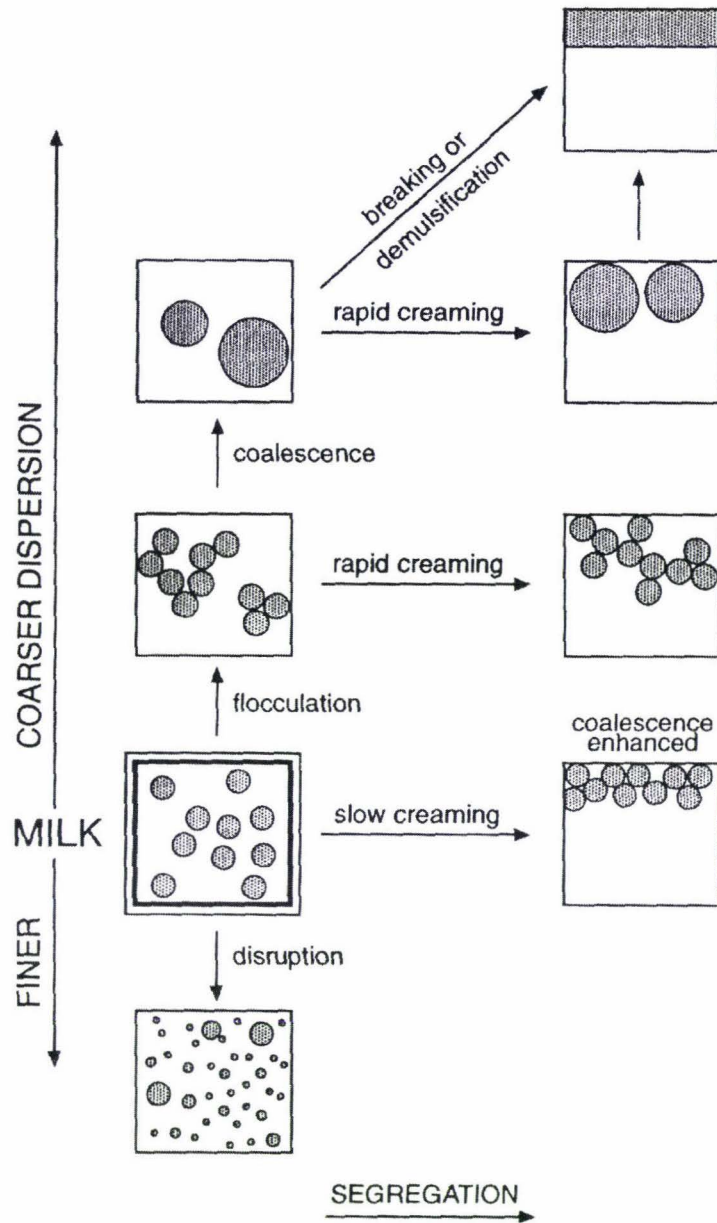
Figure 2.1: Generalised description of emulsion stability in milk; highly schematic. After Walstra et al. (1999).
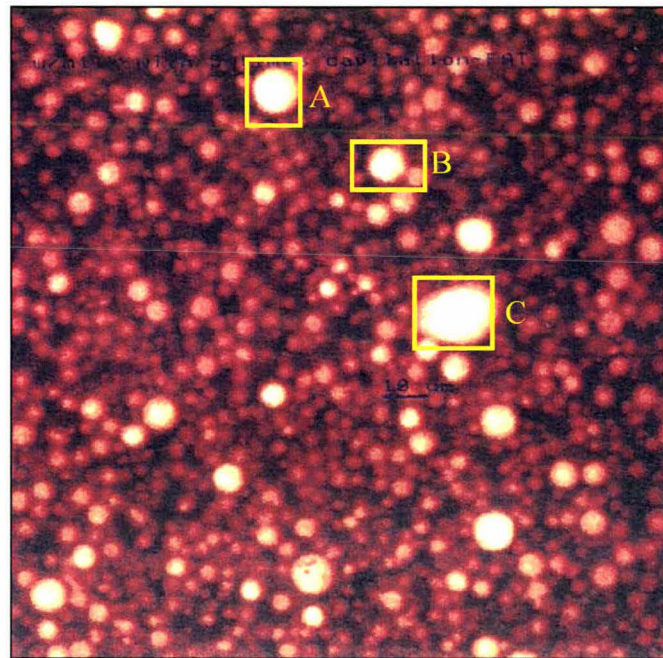
Figure 2.2: Confocal microscope slide showing coalescence (A) and flocculation (B, C) of milk fat globules after unhomogenised, pasteurised, 4% fat milk was passed through a cavitating centrifugal pump (Fang, 1998).

Sensory Consequences

The sensory characteristics of milk and other dairy products are of great commercial importance as they determine consumer acceptability. Many studies on lipolysis have included assessment of sensory quality (e.g. Suhren et al., 1975; Kuzdzal-Savoie, 1975; Schmidt et al., 1989; Escobar & Bradley, 1990; Duncan et al., 1991). It is known that the production of FFAs gives rise to bad flavours and therefore may relate to NMFGM damage. Poor milk flavour is often described as rancid, however this can be produced by the process of lipolysis (lipolytic rancidity), or by oxidation by free radicals present in the milk forming carbonyl compounds (oxidative rancidity). In addition, unacceptable flavours also arise from enzymatic attack on the proteins present in the milk (e.g. Deeth et al., 2002). Therefore a sensory evaluation of the milk product can only show that the NMFGM has been damaged if the correct flavours have been detected. However, because of this multiplicity of production mechanisms of bad flavours, sensory evaluation cannot be used as a reliable measurement of the extent of NMFGM damage. Therefore sensory aspects of NMFGM damage will not be discussed in detail in this review.

2.2.3   Tests based on chemical consequences of NMFGM damage

All of the tests described in this section involve the measurement of FFAs. While these tests give a method for detection of NMFGM damage, they may not measure quantitatively the amount of damage because the lipolysis of damaged fat may not have been completed by the lipolytic enzymes present in the sample. Thus the two are not equivalent. The measurement of NMFGM damage as opposed to the measurement of FFAs present at the moment of sampling will be discussed later in this section.

Tests developed for the determination of FFAs in dairy products involve two major steps:

1. The extraction of FFAs from the milk into a single-phase extract.
2. The determination of FFA levels in the extract.

FFAs in the solvent extract can be easily determined by titration, normally with a caustic solution of either sodium or potassium hydroxide, but colorimetric techniques have also been used (e.g. Joerin & Bowering, 1973; Lindqvist et al., 1975; Heeschen et al., 1975; Koops & Klomp, 1977; Shipe et al., 1980). Most work on FFA tests deals with the issue of extraction of FFAs from the product rather than the determination of FFAs in the extract.

The extraction of FFAs from milk is complex because the triacylglycerols that make up the vast majority of milkfat contain a large range of fatty acid chain lengths from 4 to 18 carbon atoms in length (Walstra et al., 1999). When these fatty acid chains are cleaved by lipolytic enzymes, becoming FFAs, their distribution among the fat and serum phases is very complex (Anderson et al., 1991). The methods used to extract these FFAs can be separated into two groups:

- Extraction of FFAs from the fat phase only (e.g. BDI method: Thomas et al., 1955a; Anderson et al., 1991).
- Extraction of FFAs from both the fat and aqueous phases (e.g. Perrin & Perrin, 1958; Lindqvist et al., 1975; Deeth et al., 1975; Koops & Klomp, 1977).

There is clear evidence that short chain FFAs occur more in the aqueous phase than longer chain FFAs, therefore extraction from the fat phase only gives an incomplete description of all the FFAs present in the milk (e.g. Hemingway et al., 1970a; 1970b). In addition, the International Dairy Federation (Anderson et al., 1991) proposed that tests designed in principle to extract all the FFAs from a milk sample, but which in practice extract varying proportions, should be called measures of "global acidity", not "total acidity".

The other major issues that have been addressed are the choice of solvents used to extract the FFAs and the method of extraction used to ensure as complete a recovery of FFAs present as possible. Even then, the recovery of the FFAs, by the 14 different test methods reviewed by Collomb & Spahni (1995), is still far from complete and varies significantly between different published works as shown in Table 2.2. Not only the recovery, but also the relative mixture of molecular weights of different FFAs recovered varies so widely among these 14 works that a comparison between the methods is not useful.

BDI and Copper Soap Methods

The BDI (Bureau of Dairy Industries) method, also referred to as Acid Degree Value (ADV), is an internationally recognised method for determining the titratable acidity of milk and dairy products. It was originally proposed by Thomas et al. (1955a) and has since gone through a series of modifications culminating in an IDF standard (Anderson et al., 1991).

The basic principle of the BDI method is to separate the fat phase from the milk emulsion by addition of the BDI reagent, a mixture of sodium tetraphosphate and non-ionic surfactant, Triton X-100, then heat the sample in a boiling water bath. A measured quantity of the separated fat is dissolved into an organic solvent, usually light petroleum containing thymol blue as an indicator. The solvent mixture is titrated with tetra-n-butyl ammonium hydroxide or potassium hydroxide. Fat separation can be incomplete and the IDF standard (Anderson et al., 1991) suggests refrigeration of the sample after heat treatment and centrifugation to improve separation when necessary. However, Evers (2003) reported incomplete separation in a few cases from several hundred farm tank

samples. He speculated on possible reasons for this failure, but the results point clearly to a possible failure of the BDI method under unusual and as yet undefined conditions.

Table 2.2: Examples of recovery of individual FFAs by various methods (%).

Method	Product	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1
Johnson & Gould[1]	cream (30%)	7	28	n.d.	76	n.d.	n.d.	n.d.	n.d.	87
Johnson & Gould – Churning[1]	cream (30%)	1	12	85	n.d.	n.d.	n.d.	n.d.	n.d.	82
Johnson & Gould[1]	dilute milk	9	42	n.d.	84	n.d.	n.d.	n.d.	n.d.	90
Johnson & Gould (acid)[2]	dilute milk	31	72	n.d.	102	n.d.	n.d.	n.d.	n.d.	103
Frankel & Tarassuk[3]	milk	29	38	n.d.	n.d.	n.d.	n.d.	96	100	97
Harper et al.[4]	milk	99	100	93	94	n.d.	n.d.	90	n.d.	96
Perrin & Perrin (1:3)[5]		49	63	90	92	93	97	99	100	81
Perrin & Perrin (2:1)[5]		63	85	91	93	94	95	96	97	78
Deeth et al., 1975[6]	milk	34	57	73	85	90	90	99	102	97
BDI[6]	milk	2	4	14	42	71	78	80	81	77
BDI[7]		9	7	8	30	32	n.d.	n.d.	n.d.	n.d.
Salih et al.[8]		100	n.d.	n.d.	105	n.d.	n.d.	n.d.	101	105
BLM-pedia[9]	milk	35	61	72	92	90	92	95	98	96
Lipo R (RS & RL)[10]	milk	38	72	74	92	90	92	95	98	96
modified Dole – Auto Analyser II[11]	milk	60	65	72	77	82	94	98	101	n.d.
Copper Soap[12]	milk[a]	0	1	53	120	108	n.d.	n.d.	n.d.	n.d.
Christen & Shen[12]	milk[a]	50	64	86	46	43	n.d.	n.d.	n.d.	n.d.
Deeth et al., 1983[13b]	milkfat	92	98	95	100	100	99	107	103	104

Sources: 1 – Johnson & Gould (1949a); 2 – Johnson & Gould (1949b); 3 – Frankel & Tarassuk (1955); 4 – Harper et al. (1956); 5 – Perrin & Perrin (1958); 6 – Deeth et al. (1975); 7 – Duncan & Christen (1991); 8 – Salih et al. (1977); 9 – Mouillet et al. (1981); 10 – Mahieu (1985); 11 – Anderson et al. (1991); 12 – Christen et al. (1993); Deeth et al. (1983).

n.d. = no data, [a] = homogenised, [b] = by gas chromatography.

The Copper Soap method, originally proposed by Ayers (1956) for the determination of FFAs in blood was subsequently improved and applied to FFAs in milk by Koops & Klomp (1977). It is based on the formation and selective transfer of copper salts of the FFAs present in the milk, to an organic phase, usually a mixture of chloroform, heptane and methanol. A coloured complex is formed between the copper salts and sodium diethyl dithiocarbamate and a colorimetric determination of the FFAs is obtained. The separation of the two phases is quick and complete with no need for centrifugation. The fat and FFAs are obtained by elution with chloroform containing 5% butanol.

It is reported that the short chain FFAs are not taken up in the organic phase used in the Copper Soap method (shown in Table 2.2) and therefore the results of the BDI and copper soap methods are quite comparable when applied to unhomogenised milk (Koops & Klomp, 1977; Driessen et al., 1977; Shipe et al., 1980). The correlation between the BDI and Copper Soap methods applied to homogenised milk are much poorer. The IDF (Anderson et al., 1991) suggested that the Copper Soap method may not allow the complete extraction of FFAs from homogenised milk since the BDI method is not affected by homogenisation.

The Copper Soap method has been reported by some authors to have a CoV of 4.2% (Koops & Klomp, 1977; Anderson et al., 1991). Shipe et al. (1980) reported that the BDI and Copper Soap methods were repeatable within a coefficient of variation (CoV) of 2.4 to 2.6%. To this end, the test has been validated for milk powders (Evers et al., 2000a) and for other fat containing products including milk, cream and butter (Anderson et al., 1991).

Extraction from Fat and Aqueous Phases

Extraction from both the fat and aqueous phase of the milk emulsion gives a more complete recovery of the spectrum of FFAs than the tests described previously, in particular the C4-C8 FFAs. This is achieved by combining the sample with a mixture of organic solvents. Short chain FFAs are dominated by their hydrophilic carboxyl group and therefore exist in greater proportion in the skim milk phase; the reverse is true for longer chain FFAs where the carbon chain makes them more lipophilic. Therefore an effective solvent must extract both lipophilic and hydrophilic FFAs. In addition, the

FFAs in the aqueous phase (especially short chain FFAs) are in the dissociated form and it is far easier to extract them with a polar solvent (e.g. Perrin & Perrin, 1958). These authors considered diethyl ether, a polar solvent, and petroleum ether, a non-polar solvent, and tested different ratios of each for their ability to extract short and long chain FFAs. They chose a 2:1 mixture of diethyl ether to petroleum ether. No single solvent can recover all the FFAs present in the milk because of the large range of molecular weights between different FFAs (C4-C18).

Different authors have experimented with different mixtures of solvents. For example, Frankel & Tarassuk (1955) added a 2:3 mixture of diethyl ether and petroleum ether to a solution of milk, ethanol and sodium chloride. Harper et al. (1956) fixed cream or milk with silicic acid and transferred the mixture to a chromatography column. Other variations of the silicic acid approach have also been proposed (McCarthy & Duthie, 1962; Kuzdzal-Savoie & Petit, 1966; Woo & Lindsay, 1980).

Dole (1956) used a mixture of heptane, isopropanol, and 1M sulphuric acid (10:40:1) and applied this solvent mixture to FFA determination in blood plasma. The process consisted of adding 10 mL of serum to 2 mL of solvent mixture, mixing them together and decanting. A further 4 mL of isopropanol and 6 mL of heptane were then added. After further mixing, the upper phase was decanted again. The FFAs collected in the upper phase were determined. Heeschen et al. (1975) and Woorstoft et al. (1972) have applied this two stage extraction process to milk.

Kashket (1971) recommended a single extraction; the solvent mixture was modified to (1:1:0.08) isopropanol: heptane: 1 mol/L sulphuric acid. After mixing, phase separation occurred quickly. This simplification was successfully adopted by Lindqvist et al. (1975) produced the Auto Analyser determination, which is widely used in Europe. Deeth et al. (1975) remained faithful to the two-stage extraction method of Dole & Meinertz (1960) but modified the solvent mixture, replacing heptane with petroleum ether, and replacing 1M with 4M sulphuric acid. Stetina & Lück (1976) extracted the FFAs with benzene in the presence of potassium oxalate, and added Rhodamine 6G reagent to the separated benzene layer. The resulting change in colour was determined spectrophotometrically at 535 nm and used to estimate the FFA content.

Koops & Klomp (1977) and Shipe et al. (1980) used a solvent mixture of chloroform, heptane and methanol first proposed by Duncombe (1963) in the ratio 49:49:2, but did not explain the reason for the solvent mixture. Indeed many of the previous authors did not explain in detail the rationale for changing the solvent mixtures used in their extractions techniques. For example, the procedure of Deeth et al. (1975) was merely proposed as a "convenient" method using simple equipment and small volumes of sample with small cost to the experimenter. No reason was given for replacing heptane with petroleum ether.

Salih et al. (1977) attempted to isolate the quantity of free butyric acid (C4) together with other FFAs while avoiding any interference from lactic acid. The medium was strongly acidic, no alcohol was used and the extraction solvent was diethyl ether. This technique has been used by other workers (Needs et al., 1983; Deeth et al., 1983) for the determination of individual FFA content.

In addition, there is evidence that the correlation between the main global FFA tests is good, as shown in Table 2.3 reproduced from Collomb & Spahni (1995). Therefore the global FFA tests seem adequate to measure a representative amount of FFAs in a sample.

A number of procedures exist to determine the amount of individual FFAs in milk and dairy products (Collomb & Spahni, 1995). This differentiation is important because the occurrence of bad flavours is linked to some particular FFA chain lengths (C4-12) more than others (e.g. Scanlan et al., 1965; Kuzdzal-Savoie, 1975). These tests involve basically the use of Gas-Liquid Chromatography (GLC) (e.g. Harper et al., 1956; Salih et al., 1977; Deeth et al., 1983) under many different conditions. Collomb & Spahni (1995) discuss the isolation of individual FFAs and the relation to sensory properties in some detail. In general the use of GLC has focused on sensory properties of FFAs rather than NMFGM damage.

Table 2.3: Correlations between different global FFA tests. From Collomb & Spahni (1995).

Methods	Product	Units	n	Regression equation	Correlation Coefficient	Reference
MSDA/ IDF	butter	mmol / kg fat	35	y(IDF) = 1.03 x(MSDA) +0.69	0.965	Collomb, Spahni (1991)
mod. MSDA/ IDF	butter	mmol / kg fat	35	y(IDF) = 0.97 x(MSDA pot.) + 0.41	0.991	Collomb & Spahni (1991)
BLM-Pedia/ BDI	milk	mmol/L milk	40	n.d.	0.97	Mouillet (1981)
Frankel & Tarassuk/ BDI	milk	mmol/ 100g fat	135	y(FT) = 1.845 x(BDI) + 0.64065	0.96	Pillay (1980)
BDI/ MSC	raw milk	mmol/ 100g fat	81	y(BDI) = 0.9689 x(MSC) – 0.0346	n.d.	Koops & Klomp (1977)
MSC/ BDI	unhomog-enised milk	MSC: mmol/L milk BDI: mmol/ 100g fat	124	y(MSC) = 0.398 x(BDI) + 0.002	0.90	Shipe et al. (1980)
MSC/ BDI	homog-enised milk	MSC: mmol/L milk BDI: mmol/ 100g fat	109	y(MSC) = 0.207 x(BDI) + 0.046	0.88	Shipe et al. (1980)
Deeth/ BDI	activated milk *	Deeth: mmol/L milk BDI: mmol/ 100g fat	71	y(Deeth) = 0.62 x(BDI) + 0.07	0.97	Deeth et al. (1975)
Deeth/ mod. Deeth	milk and cream	mmol/ kg fat	130	y(Deeth) = 0.90 x(mod. Deeth) + 0.62	0.980	Bosset et al. (1990)
Auto Analyser/ BDI	raw milk	mmol/L milk	21	y(Auto) = 1.08 x(BDI) + 0.216	0.975	Lindqvist et al. (1975)
ENZ/ BDI	raw milk	mmol/ 100g fat	67	y (ENZ) = 1.72 x(BDI) – 0.02	n.d.	Koops (1990)

n.d. = not determined

* = milk activated with different levels of lipolysis

"Lipolysable Fat" tests

Anderson (1983) used the term "protected fat" (PF) to describe milk that cannot be converted to FFAs by the endogenous milk lipase, LPL. Thus, the fraction of the milk fat that can be hydrolysed by the endogenous milk lipase should be called unprotected fat (UPF). Deeth & Fitz-Gerald, used this term but Miller & Puhan (1986a) called it "lipolysable fat". Current measurement techniques for FFAs described previously do not indicate that a complete conversion of the UPF occurred and indeed the lack of investigation into the enzyme activity present and incubation conditions used makes these measures quite possibly unrepresentative of the potential FFAs in a sample. Deeth & Fitz-Gerald (1978) realised this point and added an exogenous lipolytic enzyme from *Candida cylindracea* to agitated milk and incubated at 5°C for 20 hours. They concluded that this technique showed the greatest promise for predicting the amount of "fat available for lipase action and the lipolytic potential" of the samples (i.e. UPF, under the present nomenclature).

Miller & Puhan (1986a) followed up on the work from Deeth & Fitz-Gerald in a lipolysable fat test. They commented that incubation at 5°C was not optimal for LPL activity and introduced an incubation regime at 37°C. Under these conditions they did not introduce an exogenous lipase like Deeth & Fitz-Gerald. They showed that the FFA level in samples, carefully milked manually and then mechanically treated using a centrifugal pump, increased with the duration of incubation but eventually levelled off. They proposed that a FFA test could be conducted by incubating a milk sample for 48 hours at 37°C; a 98% conversion efficiency based on the maximum FFA level obtained after 7 days incubation. Samples not mechanically treated did not have any increase in FFAs.

Miller & Puhan were aware that the bacteria in milk samples could multiply rapidly due to the incubation process and lead to the production of bacterial lipolytic enzymes not present in the original sample, and therefore proposed the use of preservatives. Four preservatives were tested (potassium dichromate, sodium azide, formalin and hydrogen peroxide) for the following performance criteria: bactericidal efficiency, no interference on lipase activity, no interference on the fat phase and no interference with the determination of FFAs. Hydrogen peroxide was reported to be the best choice though Miller & Puhan acknowledged it might reduce the lipase activity. However, only a few

researchers in the field have followed up or used a similar approach to Miller and Puhan to measure FFAs (Bosset et al., 1990; Imhof & Bosset, 1995).

The reliance on LPL to perform selective lipolysis of UPF in the work of Miller and Puhan meant that the test could not be applied to pasteurised milk and evaporated milks because these actions deactivate LPL. Both Deeth & Fitz-Gerald (1978) and Fang (1998) proposed that the best way to measure lipolysable fat (or lipolytic potential) in milk was to add an exogenous lipase that was selective in the sense that it would attack only the UPF but would not be active against the NMFGM itself. For this purpose an exogenous lipase, pig pancreatic lipase, PPL, was chosen because of its similar properties to milk LPL (Kuzdzal-Savoie, 1980; Girardet et al., 1993). In particular, the curve showing the course of lipolysis by PPL levelled over time like that of LPL (Hemingway et al., 1970a) indicating that lipolysis of milk incubated with these added enzymes was substrate limited. To further validate the use of PPL, Fang added PPL and calf lipase (CL) to samples of milk damaged by pumping, incubated them at 30°C for 24 hours and determined the FFAs using the New Zealand Dairy Division method (1980), a modification of the procedure of Perrin & Perrin (1958) with recommendations from Joerin & Bowering (1973). Fang showed that the level of FFAs produced using CL was considerably higher than that using PPL, and did not change with the level of pumping damage, suggesting that the former had created damage to the PF during incubation. Fang concluded that PPL was substrate selective in the sense that it did not attack PF but only UPF and called the level of FFAs produced in his test with PPL a measure of "lipolysable free fat". Whilst both Fang and Miller & Puhan (1986a) attempted to measure lipolytic potential there is no guarantee that they measured exactly the same thing. Fang showed that the results of the lipolysable free fat test over six pumping runs correlated well with trends observed in tests based on physical consequences of NMFGM damage like a solvent-extraction FF test, particle size distribution test and microscopic analysis of shape described in the next section. Miller & Puhan (1986b) did not find this correlation between their lipolysable test and a centrifugal FF test.

Despite the relative success of these attempts to measure NMFGM damage using one single method there are other factors not yet monitored or controlled that can cause problems. McKay & Beacham (1995) showed that a particular strain of *Pseudomonas*

bacteria (LS107d2) taxonomically similar to *Pseudomonas fluorescens* can consume FFAs during incubation as well as hydrolyse milkfat. Since Fang (1998) did not add a preservative to his sample the actual numerical value of FFAs obtained may not reflect the true amount of UPF in the milk. In addition, the choice of a selective lipase in the work of Fang did not cover the large field of lipases available and clearly more tests were required. A joint project between Massey University and the New Zealand Dairy Institute was set up to follow up that work but has not been completed yet.

2.2.4   Tests based on physical consequences of NMFGM damage

Centrifugal Free Fat tests

Halter et al. (1978) defined FF as "the portion of glycerides (in particular triglycerides) exuded from the fat globules as a result of the mechanical damage of the fat globule membrane (NMFGM)". Fink & Kessler (1983) referred to FF as the "fat not completely surrounded anymore by a membrane" and sketched diagrammatically the concept of linkage among damaged fat globules, as shown in Figure 2.3. However, fat in milk is without a membrane only for a short time. Mulder & Walstra (1974) reported that casein and serum proteins tended to cover the exposed fat globule surface within 0.01 to 0.1 seconds of removal of the NMFGM, making the definition of Fink & Kessler not practically useful.
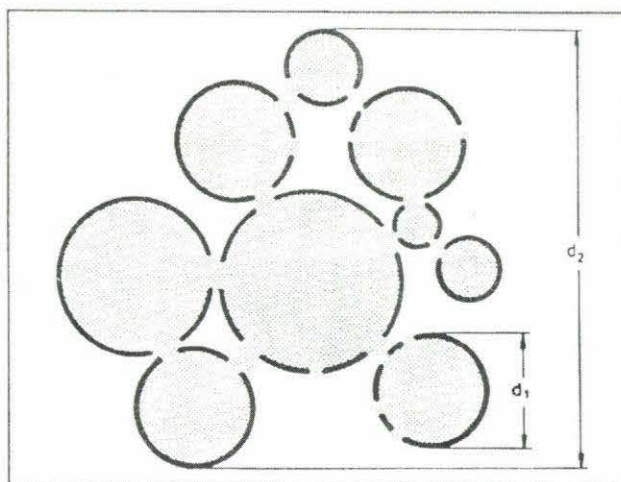
Figure 2.3: Diagram illustrating concept of "free fat" in forming a "clump" of diameter d_2 by linkages of free fat between individual fat globules. From Fink & Kessler (1983).

In centrifugal FF tests (Webb & Hall, 1935; Rothwell, 1962; Te Whaiti & Fryer, 1975; Halter et al., 1978; Fink & Kessler, 1983) the sample is heated to approximately 60°C, centrifuged at that temperature in a long necked container and the size of the resulting oily layer measured. Halter et al. (1978) heated milk or cream to 65°C and used a container known as a FF butyrometer to measure the amount of FF. The supernatant fat layer was made visible with a fat-soluble dye. When this test is used for cream it is necessary to dilute the cream because the high fat content of the sample makes a sharp separation impossible. Fink & Kessler (1983) pointed out that the degree of dilution and the centrifugation time were inter-related and the pH of the diluted sample was also an important variable in determining the FF content.

The centrifugal FF test of Te Whaiti & Fryer (1975) was based on the methods of Webb & Hall (1935) and Rothwell (1962) for measurement in milk and cream. Te Whaiti & Fryer used a Babcock tube instead of a FF butyrometer and heated the milk or cream to 50-60°C. No fat-soluble dye was added and the centrifuged sample was stored overnight at 13°C before it was re-warmed to 60°C. This test has been used with varying success. Te Whaiti & Fryer (1976a; 1976b) reported that the FF test was capable of showing differences between various treatments of cream. Deeth & Fitz-Gerald (1978) reported that the method of Te Whaiti & Fryer (1975) was the least useful test for measuring NMFGM damage in milk of the 6 tests they considered.

In addition, Miller & Puhan (1986b) found that the Halter et al. (1978) FF test was not sensitive enough to detect damage in raw milk from milking in contrast to their lipolysable fat test. Most successful uses of centrifugal FF tests have been on cream products. For example, Fink & Kessler (1983) showed differences in their test results arising from different pumping regimes.

Solvent Extraction Free Fat tests

A number of authors have used so-called solvent-extraction FF tests (e.g. Thomas et al., 1957; Tamsma et al., 1959; Buma, 1971; Aule & Worstorff, 1975; Fink & Kessler, 1983; Fang, 1998). In this FF method the sample is mixed with an organic solvent that dissolves the fat outside the membrane. Fink & Kessler (1983) claimed that the solvent also penetrated fat globules with a partially damaged membrane that have become "permeable" and so dissolve the fat from the interior of the fat globules. The

supposition is that the fat from intact globules (those with a complete NMFGM) is not extracted, however, there is no real data supporting this supposition.

In the method proposed by Fink & Kessler (Fink & Kessler, 1986a) the sample is mixed with petroleum ether at 20°C, shaken for 5 minutes and the fat and aqueous phases are separated with silicone coated filters. The solvent is then evaporated and the FF weighed. Other extractive processes have been proposed mainly on the basis of different solvents and extraction periods. Fink & Kessler (1983; 1986b) reported that diethyl ether gave elevated FF levels whilst carbon tetrachloride and petroleum ether did not. Evers et al. (2001) showed that the results of the solvent-extraction FF test depend on the extraction time, the degree of agitation, the number of extractions, the ratio of sample to solvent, the temperature and the type of solvents used (five tested). They reported that the amount of "free fat obtained over the substantial permutations of conditions ranged from 2-40% of total fat from milk and 2-70% for cream. The authors concluded that the "free fat results are partly or wholly an artefact of the method and the supposition underlining the method does not seem plausible".

A disturbing factor to consider is evident in the results of Fink & Kessler (1983) who compared the results of centrifugal and solvent-extraction FF tests. They found that the amount of FF obtained by the centrifugal method was much lower than by the extraction method. For example, pasteurised cream pumped through a vane pump showed an increase in centrifugal FF from 0.04 to 0.075% of fat content whereas the extraction FF content remained unchanged at 2.1%. Fink & Kessler concluded that the two methods measured different amounts and types of FF.

Some researchers have attempted to quantitatively measure both the physical and chemical consequences of NMFGM damage. These methods have the aim of accurately measuring all consequences of NMFGM damage rather than simply measuring a particular consequence precisely and reproducibly. Halter et al. (1978) have investigated the addition of two separate NMFGM damage tests to give a composite result. This included a centrifugal FF test and a solvent-extraction FFA test (Deeth et al., 1975). The principle of the test was to measure the FF and FFAs independently for the same sample. Next, the FFA value was converted into equivalent FF units. The two FF numbers were then added together. Halter et al. did not give any proof that the

combination of results from the centrifugal FF test and the FFA test equated to the total amount of equivalent FF in the milk. In the author's view there is no guarantee that FFAs are absent from the FF layer separated in the centrifugal FF test. Thus some FFAs might be counted twice. In addition, there is no guarantee that the centrifugal FF test has extracted the entire amount of FF available in the milk since it gives different results from the solvent-extraction FF test. Other researchers in the field have not used the combined FF / FFA method of Halter et al.

Particle Size Distribution Tests

Many researchers have analysed milk particle size distributions (PSDs) but few have used them to look at damage to fat globules. Walstra produced a number of papers aimed at describing more accurately the PSD of milk. Various tools were used including coulter counting, fluorescence spectroscopy and spectral turbidity (Walstra, 1969a). In particular, Walstra showed that the spectral turbidity technique correlated well with particle size measurements. He also divided the particle size distribution into three sub-distributions: a small fat globule distribution of sizes less than 1 µm, a main sub-distribution, comprising most of the fat (e.g. 94%) and a sub-distribution of very large particles greater than 10 µm (Walstra, 1969b) and proposed a mathematical model for these distributions (Walstra, 1969c). However, Walstra did not relate PSD and damage to fat globules or the NMFGM.

Michalski et al. (2002a) used laser light scattering and dynamic light scattering to analyse fat globule PSDs of milk, mechanically damaged, and argued that the occurrence of sub-micron particles could be used as an indicator of bad handling, pumping or homogenisation of milk. Michalski et al. (2002b) used the zeta potential measured by laser Doppler electrophoresis to assess homogenisation of raw milk fat globules. Essentially, the change in apparent zeta-potential is linked with the attachment of plasma proteins to fat globules from which the NMFGM has been stripped. The technique is interesting but still needs much refining before it can be applied.

Fang (1998) showed that a peak of large particles can be created in addition to the original PSD by pumping milk under cavitation conditions. He corroborated that finding with confocal laser photographs (Figure 2.2) that showed that some larger globules, sometimes of irregular shape, were created after pumping.

Since larger and smaller globules, compared to the original "normal" fat globule sizes, can be created by mechanical damage a measurement of average size of particles would not give a reliable measurement of NMFGM damage, however a PSD would be able to capture both the smaller and larger size changes. Cases of changes in PSD, illustrated by Michalski (2002b) and Fang (1998), can give interesting evidence that some damage exists but do not form a quantitative measure of damage. In fact the comparison of PSDs before and after mechanical processing has only been qualitative.

2.3 Factors affecting NMFGM damage

The factors that affect NMFGM damage can be placed into the following categories:

- Enzymatic.
- Compositional and structural.
- Process-related (including transportation and storage).
- Chemical and physical.

2.3.1 Enzymatic Factors

It is well established that damage to the NMFGM in raw milk can lead to lipolysis of milkfat and the production of FFAs. Poor sensory properties in milk products have also been linked to the production of FFAs. The production of these FFAs is a result of fat hydrolysis by lipolytic enzymes. Chen et al. (2003) have published an excellent review on enzymes in milk and milk powders. The measurement of lipase activity in milk has been the subject of numerous research papers (e.g. Driessen & Stadhouders, 1975; Downey, 1975; Anderson, 1983; Sundheim & Bengtsson-Olivecrona, 1985; Ahrné & Björck, 1985; Cartier & Chilliard, 1989a; Cartier et al., 1989b) including mechanisms of inhibition (e.g. Girardet et al., 1993) and measurement (e.g. Antonelli et al., 2002).

Lipolytic enzymes are defined as carboxylesterases that hydrolyse acylglycerols (e.g. Jaeger et al., 1994) and most of the fat in milk is found in the form of triacylglycerols (Figure 2.4). Therefore the role of lipolytic enzymes in milk is very important. Lipolytic enzymes have been grouped into three categories. The first category, non-specific lipolytic enzymes, release FFAs from all the three positions of the acylglycerol moiety

and therefore completely hydrolyse triacylglycerols to FFAs and glycerol. The second group of lipolytic enzymes is 1,3-specific, releasing FFAs from the outer sn-1 and sn-3 positions of the acylglycerol moiety. This can leave final products of 1,2 diacylglycerols, 2,3-diacylglycerols or 2-monoacylglycerols. However, because these acylglycerols are chemically unstable they can undergo acyl migration, where the fatty acid chain at the sn-2 position moves to the sn-1 or sn-3 position (Jaeger et al., 1994). Given a long enough incubation period the triacylglycerol can be completely converted to FFAs. The lipolytic enzymes of the third group have a preference for particular fatty acid chains, but no bacterial lipase is known to show this type of specificity (Chen et al., 2003).
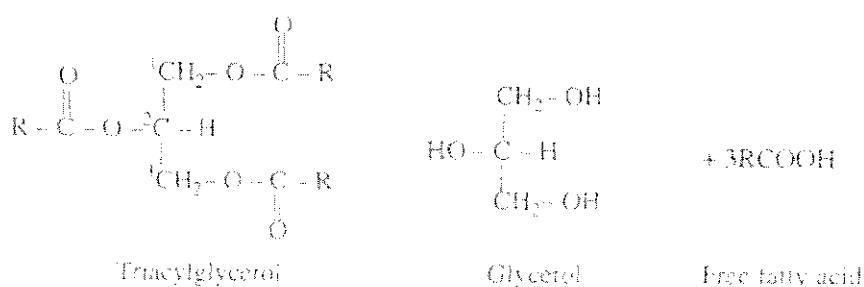
Figure 2.4: Diagram of molecular conversion of a triacylglycerol molecule into FFAs. The subscripts 1,2 and 3 represent the sn-1, sn-2 and sn-3 fatty acid chains. Adapted from Chen et al. (2003).

True lipases act on a lipid-water interface (Mulder & Walstra, 1974; Brockman, 1984). As a consequence, the concentration of the substrate (triacylglycerols) and the lipase on the interface is more important than their total concentrations in the milk. Both the substrate and enzyme must adsorb to the interface for any reaction to occur, and this reaction is further complicated by the possible need for enzyme cofactors or the modification of the enzyme once it has adsorbed to the interface (Figure 2.5). The interface allows high concentrations of substrate and enzyme to meet and in the correct orientation, which is more difficult in solution (Brockman, 1984).

The NMFGM is important as it acts as a barrier to lipolysis (Mulder & Walstra, 1974; Deeth, 1995). There is little information on why the NMFGM protects against some enzymes (e.g. LPL, Deeth, 1995) and not other enzymes (e.g. lingual lipase, Hamosh, 1984).
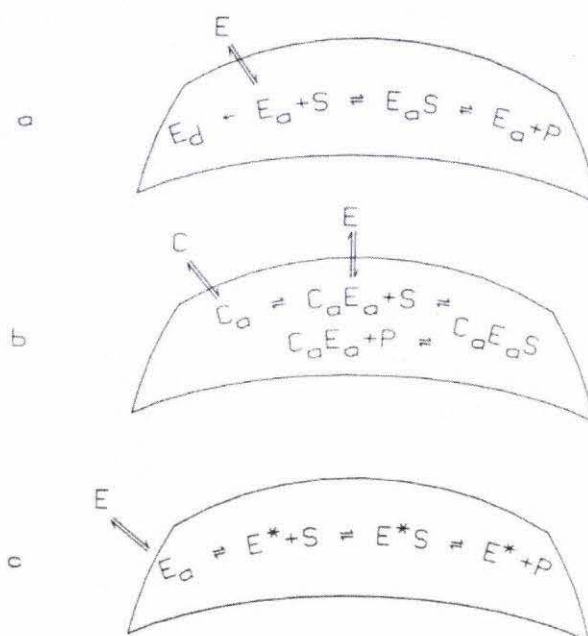
Figure 2.5: Variations of the basic scheme for lipolysis. Symbols: E, enzyme in solution; E_d, denatured enzyme; E^*, activated enzyme; E_a, adsorbed enzyme; C, protein cofactor; C_a, adsorbed protein cofactor; S, substrate; P, products. Adapted from Brockman (1984).

Sources of Lipolytic Enzymes in Milk

Lipases in milk have two main sources:

1. Endogenous, from the secretions from the mammary glands of the bovine cow.

2. Bacterial, from contamination at any point in the process of handling the milk after it has been taken from the cow.

Endogenous Lipolytic Enzymes

Endogenous LPL is the main source of lipolytic enzymes in raw milk (Downey, 1975; Chen et al., 2003). Most LPL in milk is not associated with the NMFGM upon secretion of the fat globules but is mainly associated with casein micelles (Downey & Murphy, 1970; Brockerhoff & Jensen, 1974; Downey & Murphy, 1975; Anderson, 1982; Hohe et al., 1985). LPL is specific for the sn-1 and sn-3 fatty acid sites of the triacylglycerol (Figure 2.4).

In normal milk, fat globules are relatively resistant to LPL (Sundheim & Bengtsson-Olivecrona, 1987b) because native milk fat globules are effectively packaged in, and protected by, the NMFGM (Chen et al., 2003). However, blood serum allows lipolysis

to proceed (Castberg & Solberg, 1974; Jellema & Schipper, 1975). The mechanism of this reaction is unknown. Some authors described mechanical handling, such as agitation, as an activator for raw milk lipolysis (e.g. Deeth & Fitz-Gerald, 1978) and termed this phenomenon as induced lipolysis. By this, Deeth & Fitz-Gerald meant that LPL must be in contact with the fat globules for lipolysis to occur, and the mechanical handling enhances this contact. The activity of LPL is really a function of the temperature and pH that the enzyme operates at and the balance between inhibitors and activators in the milk (Deeth & Fitz-Gerald, 1975; Anderson, 1981; 1983; Miller & Puhan, 1986a; Sundheim & Bengtsson-Olivecrona, 1987a; 1987b; Deeth, 2002).

Apolipoprotein CII is the activating protein found in blood serum and is often associated with mastitis in cows (Balasubramanya et al., 1988). The activity of LPL can be deactivated by inhibitors (Anderson, 1981; 1983; Sundheim & Bengtsson-Olivecrona, 1987a; 1987b; Deeth, 2002). These include:

- Proteose-peptone fractions
- Inorganic salts
- Product inhibition by FFAs produced from lipolysis

The term 'spontaneous lipolysis' describes an increase in FFAs of freshly drawn raw milk as a result of simply cooling the raw milk without agitation (e.g. Tarassuk & Frankel, 1957; Sundheim & Bengtsson-Olivecrona, 1985; Ahrné & Björck, 1985). This is one of the earliest topics of interest in milk lipolysis and is covered in a number of good reviews (Fleming, 1979; Downey, 1980; Olivecrona, 1984; Deeth & Fitz-Gerald, 1995). The mechanism by which cooling leads to spontaneous lipolysis is still not well understood but a number of factors have been identified. The rate and extent of cooling have been reported to influence significantly the rate of development of spontaneous lipolysis (Johnson & Von Gunten, 1962). The main enzyme involved in spontaneous lipolysis is LPL (Deeth & Fitz-Gerald, 1976; Olivecrona, 1980; Downey, 1980; Chilliard, 1982). However, the amount of spontaneous lipolysis does not correlate well with the amount of LPL present (Driessen & Stadhouders, 1974; Deeth & Fitz-Gerald, 1976; Murphy et al., 1979; Downey, 1980; Ahrné & Björck, 1985) but correlates well with the amount of LPL bound to the NMFGM (Tarassuk & Frankel, 1957; Wang & Randolph, 1978; Sundheim & Bengtsson-Olivecrona, 1985; Ahrné & Björck, 1985).

Downey & Murphy (1970) and Cartier & Chilliard (1989a) reported that the process of cooling dissociates the casein micelles to free LPL into the milk plasma. However, Olivecrona (1984) and Sundheim & Bengtsson-Olivecrona (1985) also reported that mechanical handling or the addition of salts of heparin, dissociates the casein micelles. Thus, Deeth and Fitz-Gerald (1976) argued that the balance between activators and inhibitors is the main factor affecting spontaneous lipolysis. For example, addition of apolipoprotein-CH greatly increased lipolysis (e.g. Bengtsson & Olivecrona, 1982). Removal of some of the proteins from the NMFGM with guanidine hydrochloride made the fat globules more accessible to LPL (Sundheim & Bengtsson-Olivecrona, 1987b) but an addition of these extracted proteins to milk hindered lipolysis (Sundheim & Bengtsson-Olivecrona, 1987c). Skim milk was also reported to inhibit spontaneous lipolysis (Sundheim & Bengtsson-Olivecrona, 1987a). Thus even though cow's milk contains a maximum lipase activity of about 2 μmol FFA released/min at 37°C (Murphy et al., 1979), the available activity may be as low 0.002 μmol FFA released/min at 37°C (Anderson, 1983).

The complexity of spontaneous lipolysis and the difficulties in controlling the factors that affect it means that it cannot be used as a reliable measurement of the amount of UPF. To this end, induced lipolysis is much more useful, but in raw milk it is unclear from the literature how induced and spontaneous lipolysis can be differentiated. The majority of authors agree that pasteurisation deactivates LPL (Deeth & Fitz-Gerald, 1976; Anderson, 1983; Shamsuzzaman et al., 1987; 1995; Deeth et al., 2002). This effectively means that studies of lipolysis in pasteurised or heat-treated milks are not based on the activity of LPL (and therefore there is no spontaneous lipolysis) but of bacterial lipolytic enzymes.

Bacterial Lipolytic Enzymes

Bacterial lipolytic enzymes are relatively diverse because they are produced from a vast number of bacteria that can grow in milk. Matta & Punj (1999) reported that raw milk contained lipolytic psychrotrophic spore forming bacteria in 48 out of 100 milk samples such as *Bacillus* (*B.cerus*, *B. polymyxa*, *B. licheniformis*, *B. circulans*, *B. subtilis*, *B. laterosporus*, and *B.coagulans*). Shelley et al. (1987) selected 205 strains of lipolytic psychrotrophic strains characterised with 86 taxonomic tests and found that only two

strains, *Pseudomonas fluorescens* and *Ps. fragi* accounted for 63.9% and 31.2% of the isolates, respectively. Little information has been published on the lipolytic enzymes produced by *Bacillus* species found in milk and milk powders (Chen et al., 2003). Unlike milk LPL, bacterial lipases are capable of hydrolysing intact milk fat globules. However, the mode of access and mechanism of this activity are not yet known (Deeth & Fitz-Gerald, 1995).

The lipases of the *Pseudomonas* and *Bacillus* species are relatively heat stable (Chen et al., 2003). For example, lipases of *Pseudomonas* species isolated from raw milk retained 55-100% activity after heat treatment at 63°C for 30 minutes (Law et al., 1976). Activity from 75%-100% was retained after heat treatment of skim milk at 100°C for 30 seconds (Fitz-Gerald et al., 1982). Jaeger et al. (1994) found activity in *Bacillus* species in a cell-bound form at 100°C. Shamsuzzaman et al. (1987) found no loss in lipase activity in a spray dried non-fat milk powder containing lipase from *P. fluorescens* B52 after storage at 20°C for 2 months. Kishonti (1975) found that some lipolytic enzymes required temperatures as high as 130°C for 5-10 minutes to reduce their activity to 1% of their original. Celestino et al. (1997a; 1997b) found that lipases in pasteurised milk could survive all the heat treatments during the manufacture of milk powder and even subsequent ultra high temperature treatment. The enzymes remained active in the milk even after 6 months storage at 25°C. For example, Chen (2000) reported that the level of short chain FFAs increased in whole milk powders stored at 37°C. The poor flavour threshold was exceeded after only 2 weeks storage. Therefore the presence of lipolytic enzymes in pasteurised milk is a serious and real issue for the quality of milk products.

The number of bacteria present in milk is a well-studied subject. In the literature, a bacterial count above 10^6-10^7 cfu/mL (colony forming units per mL) is needed to produce spoilage in milk products (e.g. Deeth & Fitz-Gerald, 1976; Downey, 1980). Since the enzymes produced from bacteria are unlikely to be inactivated by any heat treatment, the control of bacterial growth in raw milk is essential.

Other Enzymes

Proteins in the NMFGM are potentially vulnerable to attack by proteolytic enzymes. Good reviews on proteolytic enzymes have been published by Jaeger et al. (1994; 1999) and Beisson et al. (2000). Some researchers (e.g. Kobylka & Carraway, 1973; Shimizu

et al., 1979) attacked the NMFGM with proteolytic enzymes, such as trypsin, Pronase and papain to study its structure but did not discuss its disruption. Shimizu et al. (1980) reported that phospholipase C from bacterial sources except *Pseudomonas* caused severe coalescence in cream.

Xanthine oxidase is an endogenous enzyme found in the NMFGM itself. Xanthine oxidase can cause the catalysis of a reaction to form superoxide (McPherson & Kitchen, 1983). This reaction can damage phospholipids found within the NMFGM and lead to instability in the membrane and become a precursor to fat lipolysis. Houlihan (1992) reported 21 enzymes other than lipase found in cream that exist predominantly as components of the NMFGM but did not discuss any possible interaction with lipolysis of fat in the globules.

2.3.2 Compositional and structural factors

The potential damage to the NMFGM is a function of its strength and stability as well as the external forces applied to it. However, there are no reports in the literature of work that has ever attempted to survey the strength of the NMFGM from various sources. This may be partly due to the lack of a successful method of measurement of NMFGM strength. Thus most inferences made on NMFGM strength come from spontaneous lipolysis studies.

Spontaneous lipolysis, as discussed previously, is a function of many factors some of which, such as balance between activators and inhibitors in enzymes, are not directly related to the strength of the NMFGM. The factors that have been linked with the susceptibility of milk to spontaneous lipolysis include: stage of lactation, cow dietary intake, cow health, milk yield, occurrence of mastitis and breed of cow (Deeth & Fitz-Gerald, 1976). For example, spontaneous lipolysis has been reported to be more prevalent at the end of the lactation period (e.g. Anderson & Cheeseman, 1975; Jellema & Schipper, 1975). Anderson & Cheeseman reported that variations in stage of lactation affected the yield of NMFGM.

Mastitis, a well-known bacterial infection of the udder, has been extensively studied with regard to the composition of the NMFGM. McPherson & Kitchen (1983) reported

that mastitic milk contained less phospholipids and less total protein. In addition, incomplete milk fat synthesis was reported. This has significant implications to NMFGM damage since phospholipids help to stabilise the NMFGM (e.g. Deeth, 1997). Blood serum is often found in mastitic milk. Balasubramanya et al. (1988) reported a decrease in membrane material and an increase in the amount of lipase present when blood serum was added.

2.3.3   Process-related factors

There are many unit operations in milk manufacturing operations that have the potential to damage the NMFGM and lead to lipolysis (e.g. Fitz-Gerald, 1974). This section discusses the fundamental factors behind these manufacturing operations including external forces, air inclusion and temperature. The work in this area is mostly preliminary in nature and there are few in-depth discussions of the mechanisms behind these factors.

External Force

The NMFGM can be damaged by the application of external forces, both pressure and shear. Mulder & Walstra (1974) point out that the fat globule must first be deformed before it can be broken, with clear implications of damage to the NMFGM. For deformation to occur the force applied to the globule must overcome what is known as its internal pressure. Mulder & Walstra illustrated that a globule of 2 μm in diameter would produce a pressure difference of approximately 10 kPa across the globule. Since this pressure difference would occur over a distance of 1 μm (i.e. the radius of the globule) then a pressure gradient of 10,000 MPa/m would exist. This gradient would require a shear rate of $5 \times 10^6 \text{ s}^{-1}$ to overcome the internal pressure. Mulder & Walstra pointed out that even this figure is just an estimate, as the calculation does not take into account an increasing interfacial tension as the globule is deformed (since the surface area increases) and also does not take account of the presence of the NMFGM. In addition, any solid fat in a globule will be more resistant to deformation.

Turbulent flow creates eddies but has to reach a certain level before the eddies become the same size as the fat globules. If the eddies are large (low level of turbulence) the globules are entrained in the eddies and cannot be deformed. When the eddies are about

the same size as the fat globules they create a pressure gradient across the globules that is large enough to disrupt them. Homogenisation is a favourite method for disrupting milk fat globules (e.g. Goulden & Phipps, 1964). Mulder & Walstra (1974) report that homogenisation can produce eddies as small as 0.3 μm whereas other processes cannot.

Cavitation has also been reported to damage fat globules (Rudzik, 1987; Fang, 1998). Cavitation is characterised by the formation and collapse of vapour bubbles within a fluid due to changes in pressure within the fluid system. For example, if the inlet pressure to a centrifugal pump drops below the vapour pressure of the fluid then vapour bubbles will form spontaneously through flashing. But as the bubbles travel to the exit of the pump impeller the pressure is increased again and the vapour bubbles collapse suddenly creating a large number of small vacuums that suck in tiny jets of liquid. These jets of liquid can create pressures estimated to be 10,000 atmospheres (Rudzik, 1987).

A similar situation occurs during steam injection operations because the steam bubbles collapse almost instantaneously due to the cooling action of the milk to be heated.

Three common manufacturing operations that involve external forces are pumping, agitation and homogenisation. These operations vary in the degree of shear or pressure they impart to the fat globules in the milk.

Air Inclusion

Most researchers agree that air inclusion is a factor which increases NMFGM damage, or one of its consequences, lipolysis (e.g. Mulder & Walstra, 1974; Deeth & Fitz-Gerald, 1976; Walstra et al., 1984; Needs et al., 1986; Grindal, 1988). Air inclusion is often manifested as foaming in milk, however the amount of foam present in experiments on NMFGM damage is rarely reported in the literature. Mulder & Walstra (1974) suggested a mechanism for the process by which fat globule can attach to an air bubble (Figure 2.6). The subsequent spreading and collapse of the bubbles can disrupt the fat globule and create NMFGM damage.
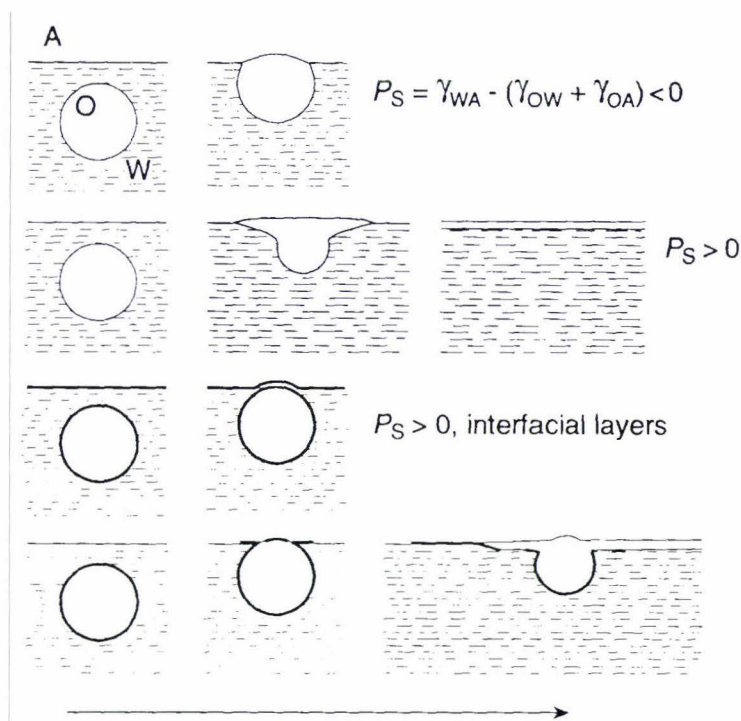
Figure 2.6: Interactions between an oil droplet (O) and the air-water (AW) interface, as a function of the spreading pressure, Ps, (γ = interfacial tension). Interfacial adsorption layers (e.g. NMFGM) are shown by the heavy lines. The bottom row shows a fat globule approaching a newly formed plasma-air interface. From Mulder & Walstra (1974).

Temperature applied to the milk

Cooling of milk is reported to change the composition of the NMFGM (e.g. Anderson & Cheeseman, 1975) and lead to spontaneous lipolysis. It is claimed that temperature cycling, the successive heating and cooling of milk, does create NMFGM damage (Fitz-Gerald, 1974; Puhan, 1989).

Most workers have discussed the effect of temperature on NMFGM damage in terms of the proportion of liquid and solid fat in the milk. Milkfat is a mixture of solid and liquid at temperatures between $-40°C$ and $40°C$ (Deeth & Fitz-Gerald, 1978; Miller & Puhan, 1986b; Kon & Saito, 1997). Walstra et al. (1984) claimed that milk fat was usually totally solid below $5°C$. Some researchers believe a mixture of solid and liquid fat allows more mechanical damage to the NMFGM than when the fat is fully liquid or solid (Deeth & Fitz-Gerald, 1978; Hinrichs, 1998). Some claim there is a critical solid to liquid ratio for maximum NMFGM damage (Miller & Puhan, 1986b). Mulder &

Walstra (1974) suggested that fat crystals could form, pierce the NMFGM, and then upon thawing melt to allow exposure of the fat globules to potential lipolytic attack.

There have been reports that heating causes changes to the composition of the NMFGM. For example, Greenbank & Pallansch (1961) reported that agitation caused a migration of phosphatides from the NMFGM to the skim milk. This migration increased substantially when the agitation temperature was raised from 10°C-20°C then decreased slightly as the temperature rose to 40°C and 50°C. Fink & Kessler (1985) found that solvent-extraction FF levels reached a maximum at about 125°C when milk was heated in the range from 85°C to 155°C. They concluded that between 105°C-125°C the NMFGM became progressively more permeable, giving a higher amount of solvent-extraction FF. Dalgleish & Banks (1991) and Houlihan et al. (1992) reported gains in whey proteins, predominately β-lactoglobulin, at temperatures above 70°C. No changes in caseins were reported. α-Lactalbumin was found at temperatures above 85°C. Dalgleish & Banks proposed that the reason for these changes was because the whey proteins had become denatured and were able to attach to the NMFGM. Van Boekel & Walstra (1989) agreed that membrane proteins will denature above 70°C and expose reactive residues that allow attachment of whey proteins to the NMFGM. But van Boekel and Walstra disagreed fundamentally with Fink and Kessler and argued that the higher FF obtained by Fink and Kessler may simply be a result of changes in fat globule sizes, not changes in the NMFGM. Van Boekel & Folkerts (1991) found that with indirect heating from 90°C to 150°C, there was no change in the fat globule size distribution, and the stability of natural fat globules against coalescence was good. Their measurements could not confirm the FF results of Fink and Kessler.

2.3.4   Chemical and physical factors

Chemical and physical factors that can facilitate enzymatic attack and mechanical action include copper, haem proteins in the NMFGM, oxygen, superoxide, light and milk pH. These chemical and physical factors mainly facilitate the oxidation of unsaturated lipids. Copper plays a crucial role in catalysing autoxidation in phospholipids, triglycerides and FFAs (e.g. McPherson & Kitchen, 1983; Walstra et al., 1984; Allen & Joseph, 1985). Autoxidation leads to conversion of unsaturated lipids to hydroperoxides and more importantly bad flavours from carbonyl compounds (Walstra et al., 1984) but

also the destruction of the phospholipids, one of the main stabilising systems of the NMFGM (McPherson & Kitchen, 1983). Work by Bernstein (1977) indicated that haem proteins may also be involved in the initial reactions producing oxidative rancidity in milk. This reaction is also enhanced in the presence of oxygen (Allen & Joseph, 1985). Superoxide, a product of a reaction catalysed by xanthine oxidase (Bors et al., 1974) causes extensive lipid peroxidation. Light can catalyse lipid oxidation, in particular when oxygen is present (Allen & Joseph, 1985).

Little research has been conducted on the influence of milk pH on NMFGM damage. Kammerlehner & Kessler (1980) studied the affect of pH on cream ripened at 22°C for 18 hours. They found a 20% increase in FF at pH 6.52 and a 120% increase at pH 4.11.

2.4   Process operations which affect NMFGM damage

There is not yet a full understanding of NMFGM damage in all dairy operations, however, a limited number of unit operations have been identified and are discussed in this section. These include pumping, agitation, homogenisation and heating processes. Unfortunately, there is a limited understanding of the interaction between different parameter settings and the factors that cause NMFGM damage in each individual operation, probably due to the small number of papers devoted to the topic.

2.4.1   Pumping

Pumping is the basic method used in the dairy industry to transport a fluid from one place to another. Centrifugal pumps are the most common pumps used in the dairy industry and almost all of the work on NMFGM damage by pumping has been studied using these types of pumps (Deeth & Fitz-Gerald, 1976; Kammerlehner & Kessler, 1980; Miller & Puhan, 1986b; Rudzik, 1987; Escobar & Bradley, 1990; Fang, 1998). Centrifugal pumps in the dairy industry generally run at impeller speeds between 1000 rpm to 3000 rpm and this is reflected in the three research papers on impeller speed (Kammerlehner & Kessler, 1980; Rudzik, 1987; Escobar & Bradley, 1990). Escobar & Bradley found a higher FFA level after pumping raw milk with a 3500 rpm pump compared to a 1750 rpm pump and Rudzik found FFA levels and FF levels increased as pumping was conducted for 3 minutes or more at 1000, 2000 rpm and 3000 rpm. Under

shorter pumping times no significant change was observed. Neither author offered a discussion on what might be happening to the NMFGM or fat globules during pumping. Kammerlehner & Kessler (1980) reported that 36% fat cream pumped through a throttled centrifugal pump at a performance level of 10% gave an increase of 50%-90% in centrifugal FF as a proportion of total fat. However, they did not separate the possibility of damage due to the valve from damage due to the pump. They postulated that pumps where fat globules were "soaked" between two walls at differential speeds would create larger levels of fat damage due to shear and frictional forces. The centrifugal pumps used in their experiments created lower levels of NMFGM damage than other pumps except at 10% performance. Most other pumps did not use a valve but a speed controller; therefore a comparison is difficult to make.

If shear is the major factor then NMFGM damage must be a function of the flow rate, impeller design and speed (McCabe et al., 1993). However, the experiments mentioned above did not separate the effect of impeller speed and flow rate, or even measure the flow rate at times.

Cavitation in Pumps

Cavitation is characterised by erosion of impeller surfaces (e.g. Rudzik, 1987) and creates a characteristic noise inside pumps but is very difficult to quantify (Instrument Society of America, 1995). Some researchers have claimed to measure the effect of cavitation in a centrifugal pump on NMFGM damage (Rudzik, 1987; Fang, 1998). Fang passed milk through a throttled centrifugal pump from one tank to another (described as one pass), whereas Rudzik recycled milk through a throttled centrifugal pump for a set period. Rudzik reported that cavitation regulated pumping (where cavitation was measured by assessing the pressure jump using an ultrasound signal) gave lower levels of FF and FFAs than unregulated pumping. This did not separate the effect of impeller speed, which reduced when the regulated pump sensed cavitation conditions. However, Rudzik quoted the average speed of the regulated pump as 2300 rpm, and 2000 rpm for unregulated pumping.

Pump Design

Different pumps often have different impeller designs and therefore the design will influence the level of NMFGM damage. Only Kammerlehner & Kessler (1980) have dealt with this issue. They reported on the behaviour of two different centrifugal pumps compared to ten other types of pump operated at performance levels of 10, 35, 65 and 100% with cream. Centrifugal pump 1 gave increases in centrifugal FF levels of 33%, lower than all of the other pumps except a diaphragm pump (which is not used widely in the dairy industry) between performance levels of 35% and 100%. In contrast, Centrifugal pump 2 was reported to give an increase of 67% at the same performance levels. However, Kammerlehner & Kessler did not elaborate on the differences in design between the two centrifugal pumps, thus it is unclear what design factors were important.

Pumping Temperature

Kelley & Dunkley (1954), Herbst et al. (1984), Miller & Puhan (1986b) and Kessler & Fink (1992) have looked at the impact of pumping temperature on NMFGM damage. Kelley & Dunkley found more lipolysis in milk pumped at 10°C than at 20°C. Herbst et al. found marked "oiling off" at temperatures between 20-30°C when FFA levels were lowest. Kessler and Fink found that the greatest amount of damage occurred at 25°C. Miller & Puhan re-circulated two litres of milk for 10 minutes with a small centrifugal pump through a tube immersed in a water bath. Three temperatures were tested: 5°C, 20°C and 40°C. The milk was tested for both centrifugal FF and FFAs using their lipolysable fat test. There was no detectable FF in the sample pumped at 5°C but a maximum in the 20°C sample, which was twice the value of the 40°C sample. The trend in FFAs was different from the trend in FF. It showed that the FFA level after pumping at 40°C was three times the level at 20°C. Miller & Puhan explained the differences as the result of different proportions of liquid to solid fat at the different temperatures.

Pumping with Air Inclusion

Only two research teams (Aule & Worstorff, 1975; Miller & Puhan, 1986b) have published work on air inclusion during pumping. Miller & Puhan report no measurable lipolysable fat or centrifugal FF from raw milk pumped without air at 40°C for 30 minutes but a high value with air inclusion even after only 10 minutes of pumping at

40°C. The air level was not specified, so no comparison of the level of air inclusion is possible. Aule & Worstorff carried out a more comprehensive study by testing milk samples at two temperatures, 5°C and 32°C with three levels of air inclusion: 0%, 2% and 10%. Raw milk was circulated through a plant at 600 L/hr and analysed for FFAs, both immediately after pumping and after storage at 5°C for 24 hours, and for solvent-extraction FF. The efficiency to separate the milk into skim and cream fractions was also measured. Aule & Worstorff reported that the fat content in the skim milk fraction increased with the amount of air injection and was considerably higher when pumping was conducted at 32°C than at 5°C. These results were mirrored in the trends of FFAs measured both before and after incubation. The amount of FF increased with the amount of air inclusion when pumping was conducted at 5°C, but showed no effect when pumping was conducted at 32°C.

2.4.2 Agitation

Many applications involve some form of agitation, for example: farm vat stirring, circulation in factory silos and manipulation of recombined products. Agitation has been a preferred method of creating NMFGM damage in the laboratory by many research teams as a way to study so called induced lipolysis (e.g. Krukovsky & Sharp, 1938; Tarassuk & Frankel, 1955; Kitchen & Aston, 1970; Fitz-Gerald, 1974; Deeth & Fitz-Gerald, 1977; Deeth & Fitz-Gerald, 1978; Kammerlehner & Kessler, 1980; Miller, 1981; Miller & Puhan, 1986b). There is wide agreement among authors in the literature that agitation does create "physical disruption of the protective fat globule membrane [NMFGM] thereby exposing the fat to lipase action" (Fleming, 1979). However, there are differences between the results of different groups, mainly because the consequence of agitation is measured through so-called induced lipolysis, which is a function of the amount and type of lipase present in the milk as well as the conditions of incubation, which vary between different authors.

Impeller Design

Impeller shapes are designed for their ability to create different shear levels, an important parameter clearly connected with other factors that create NMFGM damage. Miller (1981) looked at the design and operation of top entering agitators in whole milk storage tanks. Miller measured the amount of centrifugal FF in the milk and the mixing

efficiency by determining the fat content at different positions in the tank agitated with two types of impeller - pitched blade and paddle - using 12 impeller speeds from 15 to 150 rpm. He indicated that the paddle design might create more FF, however the results were very scattered and preliminary in nature. He proposed a correlation between impeller speed and mixing time to obtain uniformity of the milk in the vat. Kammerlehner & Kessler (1980) used four types of impeller to stir cream and claimed to show a difference between the types of impeller - anchor, propeller, disk and surface-moving stirrer - each at a different selected speed respectively - 65, 360, 1420 and 10 rpm. FF results indicated that "surface moving stirrers at a low speed cause only very little fat damage, even after a stirring period of 24 hours. After 20 minutes stirring with a surface-moving anchor mixer the FF content also hardly increased. The increase within the same period was higher in a propeller mixer and even considerably higher in a disk mixer after a stirring period of only three minutes." However, one cannot differentiate between the results whether the major effect was impeller speed or design. Thus the work so far indicates tentatively that impeller design may be a factor in NMFGM damage, but the evidence is very preliminary in nature.

Speed of Agitation

Deeth & Fitz-Gerald (1977) agitated milk with four commercial mixers: a Sorvall Omnimixer, a 2-speed Waring commercial blender, and Kenwood Chefette Liquidizer and a 2-stage APV homogeniser. The Sorvall Omnimixer was run at four speeds, 4000, 8000, 11000 and 13000 rpm and FFA measurements of the milk showed a clear increase in FFAs with agitator speed. Deeth & Fitz-Gerald (1978) agitated two litres of milk with a Sorvall Omnimixer at speeds of 4000 and 15000 rpm. They used six indicators of NMFGM damage: degree of activation of lipolysis, susceptibility to lipolysis by a lipase from *Candida cylindracea*, fat content in skim milk, alkaline phosphatase activity in skim milk, solvent-extraction FF and centrifugal FF. In all cases the indicator showed an increase in NMFGM damage at the higher speed. Miller (1981) agitated the milk at six speeds between 15 and 40 rpm for six hours and six speeds between 50 and 150 rpm for 20 minutes. The results indicated clearly that the FF generated by mixing increased with agitation speed in general, but the data was scattered.

It is difficult to compare the results of these authors because of the wide differences in impeller design and agitation speeds used as well as differences in analytical technique.

All that can be concluded is that there seems to be general agreement that NMFGM damage increases with agitation speed, but the detailed quantitative correlation even for one specific configuration of mixer is not possible because of too few data points in any particular set of conditions, and because the measurement used may not correlate directly with NMFGM damage.

Duration of Agitation

The effect of agitation time on induced lipolysis or the creation of FF has often been coupled with agitation speed (Kammerlehner & Kessler, 1980; Miller, 1981). Only Deeth & Fitz-Gerald (1978) have isolated the effect of agitation time by keeping other conditions such as speed and temperature constant. Their data indicated that amount of FF, skim alkaline phosphatase activity and FFAs in the milk all increased with agitation time in a Sorvall Omnimixer run at two different speeds (4000 and 15,000 rpm) and three temperatures (5, 20 and 40°C).

Agitation Temperature

Kitchen & Aston (1970) investigated the effect of agitation temperature in a high speed Waring blender, and cooling rate, on the activation of lipase after storage at 5°C for 20 hours. The amount of FFAs reached a maximum at an agitation temperature of 37°C and fell both at low temperatures (15°C) and high temperatures (50°C). Fitz-Gerald (1974) showed that the ADV value (FFAs measured by the BDI method) after incubation at 5°C for 20 hours went through a maximum around agitation temperatures of 40°C and 50°C. She also noted that the previous history of the milk had a pronounced effect on the results. Deeth & Fitz-Gerald (1977) followed up with a more comprehensive study. The measurement of NMFGM damage was obtained by determination of FFAs after incubation for 20 hours at 5°C by the solvent-extraction method of Deeth et al. (1975). The FFAs measured showed a dependence on the temperature curve reminiscent of those obtained by Fitz-Gerald (1974) but "universally applicable." Deeth & Fitz-Gerald noted that the shape of the Fitz-Gerald (1974) curve was linked with relatively low energy treatments especially those with incorporation of air or other gases. The curve was much smoother with high energy inputs, such as homogenisation. This difference appears to be attributable to the effect of energy input on disruption and flocculation/coalescence of fat globules (Mulder & Walstra, 1974).

Deeth & Fitz-Gerald noted that the extent of induced lipolysis also depended on the breed of cows used.

One particularly interesting result in this work of Deeth & Fitz-Gerald is the proof that there is a redistribution of lipase activity between the aqueous and fat phases of raw milk after agitation. The amount of lipase transferred to the fat phase represents "several times that which is normally associated with the fat and represents over half of the initial lipase present in the milk." In a later study, Deeth & Fitz-Gerald (1978) agitated milk again in a Sorvall Omnimixer at 4000 and 15000 rpm under different conditions of temperature and for different durations of time, as reported previously. One notable progression of thinking of these authors was that the realisation that the use of the BDI method (Fitz-Gerald, 1974) or the FFA measure (Deeth et al., 1975) may not have captured the quantitative essence of NMFGM damage by agitation because of incomplete conversion of UPF into FFAs. They introduced a new test involving a microbial lipase from *Candida cylindracea* that was claimed to only attack only UPF. The addition of this lipase increased the amount of FFAs produced after storage of the sample at 5°C for 20 hours compared to storage without the added lipase but did not change the general shape of the curve with respect to time or temperature.

Interestingly, Deeth & Fitz-Gerald reported that the amount of solvent-extraction FF produced at 4000 rpm increased steadily with agitation temperature between 5°C and 40°C but the FF temperature curve for 15000 rpm showed a maximum at 25°C, reminiscent of the ADV temperature curve of Fitz-Gerald (1974). The shape of the FFA temperature curve under the same conditions was completely different from the FF curve and showed a continuous increase in FFAs from 5°C to 40°C for the higher agitation speed, for example. The ratio of the activity of alkaline phosphatase in the skim milk compared to the whole milk showed a minimum at 25°C for both speeds. In summary, the results of Deeth & Fitz-Gerald highlight:

1.   "Classical methods of assessing milk fat globule damage (up to that time -1977) are unsuitable for predicting the amount of fat available for lipase action" and there was an urgent need to develop tests that can be universally adopted.

2.   The understanding of NMFGM damage by agitation at different temperatures, rotational speeds and time is far from complete given this lack of suitable measurement tools.

The temperature dependence of FF obtained in the agitation experiments of Deeth & Fitz-Gerald (1978) and the pumping experiments of Miller & Puhan (1986b) are very similar which is not unexpected as both unit operations create rotational shear by an impeller.

Air Inclusion

All authors agree that air inclusion during agitation increases damage to the NMFGM (e.g. Tarassuk & Frankel, 1955; Fitz-Gerald, 1974; Mulder & Walstra, 1974; Te Whaiti & Fryer, 1975; Deeth & Fitz-Gerald, 1977; Miller & Puhan, 1986b). Tarassuk & Frankel (1955) argued that foaming, a consequence of air inclusion, is necessary to produce induced lipolysis. Fitz-Gerald (1974) bubbled oxygen-free nitrogen during agitation to produce induced lipolysis. However, there is little quantitative data that links the amount of air included with the amount of FF or FFAs produced. In addition, while the study of foam structure, for example in whipped cream (e.g. Brooker et al., 1986) showed clearly the clumping of damaged fat globules at the surface of air bubbles there are clear indications that the amount of lipolysable fat in the foam is three to four times higher than in the liquid milk (Miller & Puhan, 1986b), one cannot say conclusively from the evidence whether a lot of the damage to the fat globules has simply moved from the milk to the foam layer or whether it is the phenomenon of foaming that causes the damage in the first place.

2.4.3   Homogenisation

Homogenisation can reduce the size of fat globules (Walstra et al., 1995). Homogenisation is not found in farm operations or in the manufacture of some dairy products, but is used in the manufacture of milk powders. It has mainly been studied in terms of particle size (for example, to investigate the rate of creaming) and any research studying NMFGM damage has looked at homogenisation as an adjunct to the main topic. Those authors who discuss NMFGM damage (e.g. Fink & Kessler, 1983; Cartier & Chilliard, 1989a; Deeth, 2002) also agree that homogenisation disrupts the NMFGM.

Fink & Kessler (1983) reported a higher level of FF in cream after homogenisation. Their work also indicated the order of high temperature heating and homogenisation does have an effect on the FF in cream. Lower FF levels were measured with cream heated then homogenised than in cream homogenised then heated. Deeth (2002) found that raw milk went rancid within a matter of minutes after homogenisation. Cartier & Chilliard (1989a) found a six-fold increase in FFA in raw milk after homogenisation but the data also showed large variations of up to ±67%.

2.4.4   Impact of temperature history

Because milk is stored at low temperatures at the farm and is later heated again for further processing there has been a lot of interest in the role of temperature history of the milk in terms of creation of bad flavours through so-called spontaneous lipolysis. It has been reported that the mere action of chilling changes the composition of the NMFGM (Anderson & Cheeseman, 1975). Krukovsky & Herrington (1939) concluded from their experiments that maximum spontaneous lipolysis was obtained by cooling the milk to 5°C, pre-warming to 30°C, then cooling to 10°C. Herrington & Krukovsky (1942) noted that the rate of cooling, not just its temperature, had an effect on the subsequent rate of lipolysis in milk. Mulder & Walstra (1974) warmed milk from 5°C to 30°C then cooled it to 5°C and found an increase in viscosity. Miller & Puhan (1986b) found increases in lipolysable fat of 50% when fresh warm milk was added to cooled raw milk in a farm vat. Fitz-Gerald (1974) found that the temperature history of the milk affected the ADV of milk agitated with air inclusion. In their review, Deeth & Fitz-Gerald (1976) stated that re-warming of milk or cream to 25-30°C, then re-cooling, was found to increase the level of FFAs in the sample.

2.5   Industrial dairy applications involving milk fat globule membrane damage

Model laboratory experiments can identify the factors that can cause NMFGM damage in a particular unit operation. Of additional interest is where the damage occurs in-line during the manufacture of a particular dairy product.

Surveys of NMFGM damage on farms or in factories are not widely reported. Anderson (1983) is one of the few researchers to cover farm milk handling comprehensively. Other researchers to a lesser extent cover aspects of farm or factory processing (Te Whaiti & Fryer, 1975; Escobar & Bradley, 1990; Evers & Palfreyman, 2001). The reason may be that it is more difficult to assess NMFGM damage in industrial plants and dairy farms as opposed to model experiments since in these cases it is difficult to control all the variables that affect NMFGM damage. For example, the flow rate is often less stable, making repeat sampling difficult. In addition, the stability of the process in previous unit operations influences replicate sampling and therefore the factors affecting a particular unit operation can be masked by variations in previous unit operations.

2.5.1   Milk handling

Milk handling is a general term that can refer to all operations that transport milk from one point to another. The survey of Anderson (1983), summarised in Table 2.4, clearly shows that milking was the largest source of NMFGM damage on the farm, as shown by a 45 point increase in relative FFA, but transportation and storage at the factory also contributed a significant increase in lipolysis as did separation of the cream from the skim milk. However, Anderson claimed that pasteurisation would eliminate the damage from separation, implying that LPL activity was the main reason for the increase in FFAs at this point. The remainder of Section 2.5.1 will cover the individual unit operations where there are details on the factors that influence NMFGM damage in them.

Table 2.4: Relative changes in raw milk FFA levels during milking, milk handling and cream processing from U.K. data (adapted from Anderson, 1983).

Location in process	Relative FFA level
Cow	5
After milk machine	50
After receiving jar	78
After pump to bulk tank	80
After bulk tank	100
At factory silo	150
Before pasteurisation	155
After pasteurisation (bottled milk)	145
Preheating before separation*	165
Cream after separation*	330

*These values follow on from the 'before pasteurisation' value (155) and do not include pasteurisation.

2.5.2   Milking

Milking is quite simply the process of taking the milk from the cow. Historically this has been carried out manually, however, in countries where milk production is high and microbial quality important this task is currently carried out with machines. The increased mechanisation of milking has been accompanied by a rise in NMFGM damage compared to manual milking. There are two broad categories of milking machines. Pipeline milking machines use a pulsating vacuum to squeeze the milk out of the mammary glands and air admission to transport the milk toward the milk-receiving container. Bucket milking machines are the other type but are not used in New Zealand and will only be discussed as a comparison to pipeline milking in this review. A schematic diagram of a typical pipeline milking plant is shown in Figure 2.7.
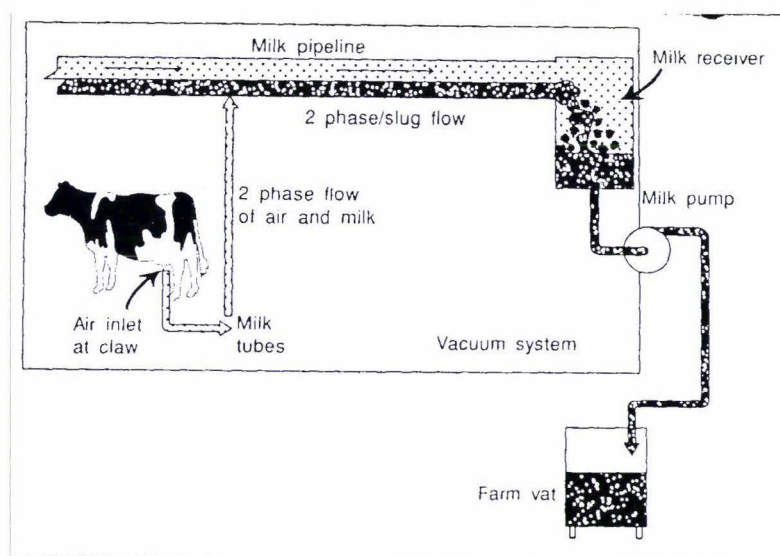
Figure 2.7: Schematic of pipeline milking system. Reproduced from Woolford (1993).

There are numerous field studies of milking systems using FFA levels as a measure of damage (Gander, 1955; Thomas et al., 1955b; Jokay & Jensen, 1956; Chen & Bates, 1962; Johnson & Von Gunten, 1962; Hunter, 1966; Worstorff et al., 1972; O'Halloran et al., 1975; Pillay et al., 1978). These studies showed a large measure of agreement on the critical features of milking systems affecting NMFGM damage, including:

1. Bucket milking machines create less damage than pipeline milking machines.

2. Air incorporation greatly increases the level of damage.

3. The height of the risers (vertical milk line sections) has a strong effect on damage in pipeline systems, the lower the less damage created.

4. Worstorff et al. (1972) observed that high milk pump capacity and narrow pipeline diameter in dual pipeline systems created unusually high levels of damage. These features are compatible with pump cavitation.

5. Interestingly enough there was a significant difference in damage between morning and evening milking and a strong seasonal trend.

Fleming (1979) has included details of these surveys in an excellent review on lipolysis of bovine milk. The use of induced lipolysis for assessing NMFGM damage in these early surveys is more indicative than quantitative since these two variables are different (Section 2.2.3). So far only Miller and Puhan (1986b) have given a more accurate estimate of NMFGM damage in milking systems by measuring both centrifugal FF and lipolysable fat. The FF test was too insensitive to apply in most samples but the LF test confirmed the conclusions of the previous surveys.

A number of researchers performed controlled experiments to validate the conclusions of these surveys. For example Jensen et al. (1958) found that pipeline milkers without risers or air leaks produced mean ADV of 0.87 meq/L in summer compared to 1.31 in winter. Gander & Jensen (1960) confirmed that air leaks and milk lift contributed to lipolysis. Gholston et al. (1966) found mean ADVs of 0.68 and 1.16 when milking pipelines were located respectively 2 feet below and 4 feet above normal udder level. Riser level manipulation was also performed by Downes et al. (1974), Judge et al. (1977) and Connolly et al. (1978) with similar results. Escobar & Bradley (1990) operated a milking system with two different pumps run at 1750 rpm and 3500 rpm and showed a greater amount of FFA induced at the higher speed. Salvatierra et al. (1978) investigated the performance of two milking claws and concluded that the difference in FFA levels produced can be directly explained by different amounts of air inclusion resulting from the two different designs. O'Brien et al. (1998) investigated the effect of pump operation, air admission and claw design on FFA content. They reported that the greater level of FFA observed in evening milking was linked to a low throughput of milk (compared to morning milking) forcing the pump to run in a "starved condition", presumably cavitation. They found that a claw air admission of 13 L/min increased FFA levels significantly compared with 6 L/min.

With the introduction of automatic milking systems that are used by hundreds of farms in western Europe (Klungel et al., 2000), a new interest in milk quality developed. In their surveys, Wirtz et al. (2002) and Klungel et al. (2000) both found that the level of FFAs in milk from automatic milking machines was higher than that in milk from a conventional milking parlour. The schedule of milking (2 or 3 times a day) also had a small effect on FFAs (Klei et al., 1997; Klungel et al., 2000).

The results from the surveys and controlled studies of milking systems have not remained unnoticed by manufacturers and continuous improvements have been made. One of the latest developments is the introduction of milking systems where milk is transported by vacuum rather than air lift (Carter, 1990).

Bulk Farm storage

Because of the consolidation of dairy companies in the world, milk is now stored for days before it is picked up and processed. There are conflicting reports in this area on the severity of NMFGM damage. In the review by Deeth & Fitz-Gerald (1976), they discouraged the use of top filling milk vats. However Evers & Palfreyman (2001) found no statistical difference ($p > 0.05$) between bottom and top filling vats. All authors studying farm vat and silo storage agreed that agitation of milk in tanks promoted some lipolysis, especially if air and foaming were included. Deeth & Fitz-Gerald recommended ceasing agitation when the milk was about level with the impeller to avoid foaming. Kirst (1980) reported that over-vigorous agitation led to unnecessary power consumption and induced lipolysis. However Miller (1979), found that liquid depth, agitator blade width and clearance between the impeller and the vessel base were of lesser importance than impeller design. He recommended the use of a two bladed paddle over a pitched blade impeller (Miller, 1981).

Transportation to the Factory

The survey of Anderson (1983) found that the increase in FFAs during transportation between the farm and the factory in the United Kingdom was approximately half of that found at the farm (see Table 2.4). On the other hand, Miller & Puhan (1986b) reported no significant increase in lipolysable fat during transport and, Deeth & Fitz-Gerald (1976) claimed that only faulty design produced significant damage.

Separation

The separation process divides the milk into a low fat skim milk fraction and a high fat cream fraction. Anderson (1983) identified this operation as a highly significant for induced lipolysis. Deeth & Fitz-Gerald (1976) claimed in their review that substantial increases in lipolysis occurred if separation temperatures were below 30°C, for raw milk. They explained that the presence of both solid and liquid fat, which is known to give higher levels of FF upon agitation or pumping, was the cause. In the New Zealand dairy industry milk is separated mostly at 55°C, and the fat is fully liquid, giving a lower incidence of FF.

Pasteurisation

The survey of Anderson (1983) showed a small drop in FFA value in the milk after pasteurisation, but a substantial increase in FFAs in the cream portion. Evers et al. (2000b) also reported a drop in ADV level in fat standardised milk after pasteurisation They attributed this drop to the reduction of fat content in the pasteurised milk compared to the raw milk following standardisation.

2.5.3   Milk heating

Three processes in milk powder manufacturing use heat in either a direct (e.g. steam) or indirect form (e.g. through a heated metal surface) to condition the milk proteins and/or reduce water content. These processes are known as preheating, evaporation and spray drying. In terms of NMFGM damage, there is very little information on these processes in the literature. The disruption of fat globules during direct UHT heating has been reported (Zadow, 1969; Ramsey & Swartzel, 1984; Van Boekel & Folkerts, 1991). Evers et al. (2000b) have reported measurements of ADV in the whole milk powder manufacturing process using the BDI method. No major change was found at any stage after pasteurisation (including preheating, evaporation, concentrate handling and spray drying) except after the second fluidised bed. The increase in ADV at the fluid bed was attributed to the addition of lecithin, a wetting agent used in instant milk powder formulations.

2.5.4   Homogenisation

The processes of homogenisation and heating often go together both during manufacture of milk and milk powder products. Van Boekel and Walstra (1989) have discussed the matter at some length. Upon homogenisation, the globules are reduced in size and a great deal more interfacial area between the fat and aqueous phase is produced (Walstra, 1983). According to Darling & Butcher (1978) and Walstra & Oortwijn (1982) this new surface, or membrane as it is referred to, is dominated by casein micelles and to a lesser extent serum proteins. Globules with this new membrane can now participate in any casein reaction, such as renneting, and cause the effective casein concentration of the milk to increase. However, the rate of reaction of caseins adsorbed on the surface of the fat globules may be different from that on non-adsorbed casein micelles (Robson & Dalgleish, 1984). The new membranes may impart less

stability than the NMFGM but the reduced size of the fat globules makes them much more stable, particularly with respect to creaming (Walstra, 1983).

During heating of homogenised milk, heat coagulation is an important phenomenon. The heat stability of homogenised, unconcentrated milk is lower than that of unhomogenised, unconcentrated milk, the more so when the homogenisation pressure is high (Sweetsur & Muir, 1983). Homogenisation of concentrated milk had a detrimental effect on heat stability. Homogenising the milk before concentrating decreased the heat stability much more than homogenisation after concentration (Sweetsur & Muir, 1982). Preheating before concentration tended to give greater heat stability to milk because it encouraged a reaction between β-lactoglobulin and κ-casein, thus preventing the casein micelles from growing further. Preheating before homogenisation gave a better heat stability than preheating after homogenisation (Sweetsur & Muir, 1983).

2.6   Consequences to the final product

Ultimately of most concern are the consequences of NMFGM damage to the final product, be that processed milk, cream, cheese, butter, milk powder or any other dairy product. However the focus of the literature is generally not directed at NMFGM damage, but related issues of microbial spoilage and off-flavours.

Sensory evaluation is a well-known measure of final product quality, and, as discussed in Section 2.2.2 can be the result of lipolysis but also of other factors such as oxidation and proteolysis. This section will not cover sensory issues resulting from these two factors but only those related to lipolysis. In addition, physical consequences of NMFGM on final products will be discussed.

2.6.1   Milk and Cream

Chemical Consequences

The key flavour linked with NMFGM damage is a sour or rancid taste. Traditionally this indicates spoilage. Trained panels are used to distinguish the differences between such flavours as bitter, soapy, cheesy, rancid and sour tastes. Only rancid and sour flavours are the result of lipolysis (or oxidation).

Short chain FFAs (with 4 to 12 carbon atoms) have been linked to flavour defects (e.g. Patton, 1964; Al-Shabibi et al., 1964; Scanlan et al., 1965; Kintner & Day, 1965). Trained sensory panels are expensive to use and this has created interest in correlating flavour scores with FFA levels frequently determined by ADV. Senyk et al. (1985) proposed that with an ADV of 1 meq/100g fat is in danger of becoming rancid. Richardson (1985) reported that an ADV of 1.5 meq/100g fat or greater was "unsatisfactory (extremely lipolysed)", but Earley & Hansen (1982) and Rerkrai et al. (1987) found that farm milk samples with an ADV greater than 1 meq FFA/100g fat did not taste rancid to trained panellists. A number of early works relating rancid flavour and ADV (e.g. Thomas et al., 1955a) identify the FFAs responsible for rancid flavour by using laboratory prepared samples. Duncan et al. (1990; 1991) reported a very poor correlation of 0.13 (p=0.16) between ADV and trained panel score. They also found that rancidity scores assigned to laboratory prepared samples were higher than farm collected milk samples at the same ADV. Anderson (1983) found a good correlation between rancidity and FFAs measured by the copper soap method. He suggested that the reason for poor correlation in some work may be due to:

- Poor extraction of short chain FFA by the FFA test used.
- Flavour perception in fat and aqueous phases is different and creates variability in results.
- Variable detection of flavour.

Since the BDI method determines the FFAs in the fat phase it tends to pick up more of the long chain FFAs that do not contribute bad flavour. Many authors have reported that the concentration of butyric acid has a significant influence on flavour (Kosikowski, 1959; Harper et al., 1961; Kintner & Day, 1965). Hemingway et al. (1970a) showed that lipolysis by PPL, an enzyme with very similar behaviour to endogenous milk LPL, preferentially released short-chain FFAs in the early stages of lipolysis and a high proportion of these passed into the aqueous phase. These would not have been picked up by the BDI method. Clearly there was a need to develop alternative methods to ADV determination for the control of off-flavours. Ukeda et al. (1992), for example developed a microbial sensor to determine free short-chain fatty acids.

Not all chemical consequences affect flavour. Formation of mono- and di-glycerides are the result of partial lipolysis of triacylglycerols. Work by Buchanan (1965) and Deeth (2002) have shown that increased FFA levels produce poor steam frothing of milk. Deeth attributed this to the formation of mono- and di-glycerides. This is an issue in the manufacture of cappuccinos (Deeth & Fitz-Gerald, 1976).

Physical Consequences

The research on physical consequences of NMFGM damage on product quality is mostly qualitative. However, a few researchers have given quantitative results, such as Deeth & Fitz-Gerald (1978) who reported increasing proportions of fat in skim milk separated after agitation for various periods.

The physical consequences are often the result of an increase or decrease in particle size. Some researchers (e.g. Kammerlehner & Kessler, 1980) have made these observations:

- A rapid creaming rate in milk products.
- The occurrence of butter granules in a milk or cream product.
- Reduced whipping in cream as well as reduced foam stability.
- Unwanted butter flocculation in ice cream, found in the form of specks of fat.
- Increased rising of fat in cream tubs which results in "fat-collars" (or cream plug).

2.6.2   Other Dairy Products

There is very little information on other dairy products, including milk powders. In milk powders, a key problem is the development of bad flavours, through both oxidative and lipolytic rancidity. These are linked with the presence of FF on the surface of powder particles (Buma, 1971). Free fat in milk powders can be reduced substantially by homogenisation (e.g. Snoeren et al., 1983). These authors also note that homogenisation substantially changed the viscosity of milk concentrates and that this phenomenon affects the properties of the resulting powder like particle size, bulk density and vacuole formation.

McDonald et al. (1986) conditioned milk by three treatments (1) immediate pasteurisation, (2) and (3) agitation to induce different levels of hydrolytic rancidity

then used it to make cheddar cheese. The ADV for milk in treatments 1, 2 and 3 were respectively 1.06, 1.39 and 1.98 and the yields were affected (0.80, 0.83 and 0.74 kg of 63% solids cheese per kg of milk solids). The fat content of the cheeses decreased with increasing ADV and the flavour score also decreased. Hicks et al. (1990) damaged the milk by pumping through a recycle loop up to 209 times with and without air. They noted that yield losses occurred when air was incorporated. ADV values also increased when milk was concentrated by ultrafiltration.

The effect of NMFGM damage on butter making is also concerned with rancidity and yield. Kammerlehner & Kessler (1980) added that fat damage could cause fat losses in the butter making process due to a higher fat content in the buttermilk.

2.7 Conclusion

Extensive research on NMFGM damage-related issues spanning over half a century has produced a vast number of tests used to measure the chemical and physical consequences of NMFGM damage. Lipolysis has been studied extensively, with much research on lipase activity in raw milk and cream, and research into the difficult topic of spontaneous lipolysis. The effect of the quantity or quality of FFAs on flavour appears to be well understood. However, a number of fundamental concepts remain poorly defined. Research into the effect of heat-resistant lipolytic enzymes on the consequences of NMFGM damage is short in detail, though many lipolytic enzymes have been identified in milk. The inactivation of milk LPL after pasteurisation appears to have limited the research into the effect of NMFGM damage on pasteurised milk in factory processes used to manufacture dairy products.

A direct measurement of NMFGM damage, defined here as a tear or disruption, is some distance away and may not even be feasible. The methods based on the detection in the serum phase of components originally found only in the NMFGM do not work because the NMFGM loses some of its components readily through chilling and washing without necessarily being damaged. Visual techniques of detection of actual damage are logistically difficult to achieve. Thus, the best option is still the monitoring of physical and/or chemical changes as a result of NMFGM damage. However, studies of lipolysis

to date have not been sufficiently controlled to provide a quantitative evaluation of NMFGM damage.

Most tests currently devised appear inadequate in measuring NMFGM damage reliably. What is actually measured in the long-used solvent-extractable free fat (FF) test is highly questionable (Evers et al., 2001), and the use of PSDs has at this stage only been applied in a qualitative way. In pasteurised milk, the milk LPL is likely to be inactivated and traditional FFA measurement tests or the LF test of Miller and Puhan (1986a) are inadequate. The most promising approach appeared to be that of Fang (1998) who added a lipolytic enzyme, PPL, that was reported to attack only the fat made available by disruption of the NMFGM but not attack the membrane itself.

However, this line of approach still needs further validation and refinement. A joint project was set up between Massey University and the New Zealand Dairy Research Institute to perfect that technique used by Fang, so that it could be used in the present work. Unfortunately that project was repeatedly delayed and did not produce any notable results. Industrial measurements attempted in the first year using the procedure of Fang showed that considerable work remained to be performed before a reliable measurement of NMFGM damage could be achieved.

Therefore, the first objective of this project was to develop a technique for measurement of NMFGM damage (Chapter 4).

The research into the factors and process operations that affect NMFGM damage has been carried out by a handful of authors in a limited number of studies and conditions. These studies indicate where NMFGM damage is most significant, but the scope of the work needs to be extended.

Therefore, the second objective was to analyse the factors that affect damage to the NMFGM using the measurement techniques devised (Chapter 5).

To an even greater extent, research into NMFGM damage from processes in industrial sites is very limited, except on farms where coverage of milking systems has been

relatively comprehensive. The impact of NMFGM damage on milk powders has not been researched in any great detail.

Therefore, the third objective was to make case studies of the damage occurring in a number of key operations or equipment found in milk powder plants where the factors studied in the second objective could be found (Chapter 6).

Clearly, the effect of NMFGM damage to the final product, particularly of milk powders, is very important, but in this work could only be objectively analysed once the first three objectives, described above, were achieved. This was not possible within the scope of this work.

Chapter 3
Materials and Methods

The overall aim of this work was to explore native milk fat globule membrane (NMFGM) damage in milk powder processes. This task was broken down into three key objectives as described in Chapter 2. In essence this was to develop techniques for measuring NMFGM damage and apply these techniques to understand better the mechanical factors affecting NMFGM damage and how these factors influence industrial milk powder manufacturing processes.

Obviously, reliable methods of measuring NMFGM damage were required; but these were not readily available, as discussed in Chapter 2. The development of two techniques for measuring, respectively, the physical and chemical consequences of damage to the NMFGM is reported first in this chapter. This task required the largest portion of resources and this is reflected in its emphasis in this chapter. These two techniques combine to give a reasonably comprehensive assessment of the level of damage to the NMFGM. A discussion of sample handling issues is also included because it was found that phase separation of samples caused serious problems with obtaining a representative sample, as did further NMFGM damage during sampling, and the temperature and duration of storage.

The second part of this chapter (Sections 3.4-3.5) focuses on the remaining two objectives of this project and describes the equipment and methods used to study the effect of mechanical factors and unit operations in milk powder processes with respect to NMFGM damage both in controlled experiments in the Massey University milk processing pilot plant and in surveys of industrial milk powder manufacturing plants.

3.1 Measurement of the chemical consequences of NMFGM damage

Measurement techniques were developed and refined gradually over the course of this work (over 30 experimental runs). This was necessary because of inconsistent and

irreproducible results found when applying the techniques of Fang (1998) during a survey of NMFGM damage in industrial dairy plants in the first year of this project.

The failed attempt during these earlier surveys to measure the degree of NMFGM damage at various stages of the milk powder manufacturing process did, however, highlight weaknesses in:

1.   The principles of the test itself, in particular the conditions of incubation and the understanding and defining of different FFAs present or elaborated during incubation.

2.   The existing method of sampling.

3.   The method of handling samples; this tended to create more artefacts than previously realised.

In this first section the principles of a new test to measure FFAs produced during incubation of milk samples (with NMFGM damage) will be described. Details of the test are then described, and finally the development of new protocols for sampling and sample handling are identified and discussed.

3.1.1   Terminology

The measurement of FFAs in milk has been used typically to measure flavour and storage quality as a result of lipolysis in a milk sample (e.g. Kuzdzal-Savoie, 1975). Most previous workers have been interested in using the FFA level to monitor lipolysis at any one point in time, often by storing a milk sample for 20-24 hours at 4-6°C to generate lipolysis in the sample (e.g. Fitz-Gerald, 1974; Deeth & Fitz-Gerald, 1978; Hisserich & Reuter, 1984; Sundheim, 1988; Wiking et al., 2003). Note that both the term 'storage' (e.g. Deeth & Fitz-Gerald, 1978) and 'incubation' (e.g. Fitz-Gerald, 1974) have been used to describe the process of generating lipolysis in a milk sample. In order to differentiate the type of process used to generate lipolysis the term 'storage' will refer to incubation/storage of a milk sample at a temperature of less than 20°C, well below the optimum for most enzymes found in milk (Fox et al., 1989), and 'incubation' will refer to storage/incubation at temperatures above this. Only a few authors have stored milk samples at other than 4-6°C for 20-24 hours. Miller & Puhan (1986a)

attempted to monitor lipolysis by incubating samples at 37°C for 48 hours, whilst Deeth & Fitz-Gerald (1978) stored milk samples at a standard time and temperature (20 hours at 5°C) but added an exogenous enzyme. Fang (1998) applied both modifications suggested by Miller & Puhan and Deeth & Fitz-Gerald by incubating samples at 30°C for 24 hours with an exogenous enzyme. Unfortunately in lipolysis studies the lipolytic enzyme activity in milk samples, in general, is rarely reported and therefore it is almost impossible to compare results from different authors, especially those using different storage/incubation conditions.

Even the terminology used with FFA measurement can be confused and confusing, the same term being used by different writers to explain different concepts. One example is the use of the term *lipolysable fat*. Miller & Puhan (1986a) defined it in terms of the level of FFAs measured in raw milk incubated at 37°C for 48 hours without the addition of exogenous enzymes. However, Fang (1998) added an excess of pig pancreatic lipase (PPL) to a pasteurised milk sample and incubated it at 30°C for 24 hours, in what he called the lipolysable free fat (LFF) test.

The incubation time, and the type and likely activity of lipolytic enzymes, used in the lipolysable fat test of Miller & Puhan and the LFF test of Fang are quite different. Yet the authors of both tests aimed to convert all of the fat with a damaged NMFGM to FFAs. Miller & Puhan describe their results as simply 'lipolysable fat', whereas Fang called his 'lipolysable free fat' suggesting that the two tests actually do not measure the same thing, but Fang did not elaborate. As discussed in Chapter 2, the different methods of storage/incubation encountered in the literature give rise to different levels and types of FFAs. It is therefore essential to have a systematic terminology if the chemical consequences (i.e. FFAs) from NMFGM damage are to be analysed fully.

The author proposes the following terminology for defining the different types of fats and FFAs encountered in the measurements of NMFGM damage. It is applied throughout the remainder of this thesis.

Materials and methods

Fat Content

The total mass of all types of fat found in a sample. Most fat in milk is found in the form of neutral triacylglycerols, a triacylglycerol comprising three fatty acid chains esterified to a glycerol molecule.

Free Fatty Acids (FFAs)

Fatty acids not esterified with glycerol.

Protected Fat (PF)

Fat completely protected by the NMFGM from lipolysis by selective lipolytic enzymes.

Unprotected Fat (UPF)

Fat that is not protected by the NMFGM from lipolysis by selective lipolytic enzymes.

Selective enzyme

A lipolytic enzyme that is active against unprotected fat (UPF) but not protected fat (PF). Note, selectivity in this context means the enzyme has restricted access to the fat substrate (Section 3 of Chapter 2).

Non-selective enzyme

An enzyme that can lipolyse PF.

NMFGM damage

This is defined as the amount of UPF present in the sample when collected.

Original Fatty Acids (OFA)

FFAs present in the sample when collected.

Fatty Acids from Protected Fat (FAPF)

FFAs resulting from the action of non-selective lipolytic enzymes.

Fatty Acids from Unprotected Fat (FAUPF)

FFAs resulting from lipolysis of UPF.

Incubation Fatty Acids (IFA)

FFAs present after incubation of the collected milk sample.

Selective Lipolysis Fatty Acids (SLFA)

FFAs present after incubation of the collected milk sample containing excess added selective enzyme. SLFA was defined by Fang (1998) as *lipolysable free fat*, but this latter term is somewhat misleading as it is equivalent neither to the free fat (FF) content as defined by Halter et al. (1978) nor to the lipolysable fat content as defined by Miller & Puhan (1986a). Hence the introduction of a new term to avoid confusion.

3.1.2   A new test for measuring free fatty acids from UPF

Purpose of the test

The purpose of this test is, ideally, to assess the amount of UPF that is present in a milk sample when collected, in terms of the FFAs produced by the complete lipolysis of the UPF. UPF is, from the definition given above, a measure of the extent of NMFGM damage in the milk from which the sample was taken. It should be clearly understood, however, that the test measures the FFAs produced by lipolysis of the UPF (i.e. FAUPF) and not directly the UPF itself. Because the lipolysis of UPF to FFAs requires a selective enzyme, the name of this test is the 'selective lipolysis test'.

Objective of the Test

The objective of the selective lipolysis test is, ideally, to convert all of the UPF present in the sample on collection, and only this UPF, to measurable FFAs, and to measure these FFAs. This objective cannot be reached in practice, as discussed in the following. Thus, the selective lipolysis test as developed in this work is one that approaches the ideal.

Consider the measurement of FFAs in incubated (including stored) and non-incubated milk samples. The FFAs measured in a non-incubated milk sample, which will be called here original fatty acids (OFA), clearly result from either being present when excreted from the mammary glands of the bovine cow or from enzymatic action prior to the sampling and measurement process. UPF generated by damage to the NMFGM prior to sampling is unlikely to have been fully converted to FFAs by the time the sample is

analysed since there may not have been enough lipolytic enzymes, and optimum time or temperature conditions, for conversion of all of this UPF into FFAs. Even if full conversion did occur, a measure of OFA would still include FAPF as well as FAUPF from lipolysis during the time between sample collection and FFA measurement by lipolytic enzymes present in the milk sample when collected. Thus, OFA cannot be a measure of the UPF present in the milk when sampled.

Upon storage or incubation of a milk sample after collection, the amount of FFAs measured, known here as incubation fatty acids (IFA), will be higher than the OFA. This will be due to the better conditions (i.e. time and temperature conditions) for lipolytic enzymes present in the milk to convert UPF to FFAs. Most workers use a measure of IFA in some form or another. In raw milks, the lipase activity is high (Anderson, 1983), but in pasteurised milks there is little lipase activity (Deeth, 1995). Therefore storage or incubation may not result in all of the original UPF being converted to FFAs. Furthermore, there is a clear possibility that at least some of the enzymes originally present in the sample would be non-selective ones able to lipolyse PF. Thus there would be, after incubation, some FAPF, as well as FAUPF produced from the UPF originally present. Therefore the IFA measured will consist of three components (Equation 3.1):

$$IFA = OFA + FAPF + FAUPF_p \qquad (3.1)$$

where $FAUPF_p$ is FFAs produced by lipolysis of *part* of the original UPF.

The longer the incubation the more FAPF are produced if non-selective lipases are present. This, together with the fact that OFA are present, and the fact that there may be incomplete conversion of UPF to FFAs, means that there is no certainty that IFA will be a good measure of the UPF present in the milk when sampled, and therefore of NMFGM damage. For example, Miller & Puhan (1986a) who made the most thorough investigation of incubation conditions to the author's knowledge implicitly included FAPF in their definition of lipolysable fat, but they did not mention this explicitly, only acknowledging that high microbial contamination would require the analysis to be

repeated, rather than the potential of additional FFAs being produced by lipolysis by non-selective enzymes.

One way to ensure the total conversion of UPF to FFAs is to incubate a milk sample with an excess of selective enzyme as proposed by Fang (1998). To minimise the creation of FAPF the level of added enzyme should be overwhelming, so that full lipolysis of UPF occurs in a short incubation time. This level was substantially increased in the present work from the level proposed by Fang. A detailed description of the final test is given in Section 3.1.3.

When an excess of added selective enzyme is present and suitable incubation conditions are used, there is complete conversion of UPF to FFAs. Incubation therefore yields SLFA:

$$SLFA = IFA + FAUPF_r$$
$$= OFA + FAPF + FAUPF_p + FAUPF_r \tag{3.2}$$

where $FAUPF_r$ is FFAs produced from the UPF that would remain unlipolysed if a selective enzyme was not added under the same incubation conditions.

Thus,

$$SLFA = OFA + FAPF + FAUPF_t \tag{3.3}$$

where

$$FAUPF_t = FAUPF_p + FAUPF_r \tag{3.4}$$

While OFA can be measured (i.e. immediate determination of FFAs prior to any incubation), there is no known way of separately measuring FAPF and $FAUPF_t$ in a single sample. There is thus no way of obtaining an absolute measurement of $FAUPF_t$, and thus of the extent of NMFGM damage, in such a sample. However, when a particular unit operation is studied, for example a pumping step, if it is assumed that

pumping has no effect on the values of OFA and FAPF, an exact measure of the NMFGM damage (in terms of FAUPF) created by the pump can be obtained as the simple difference between the SLFA values for a sample taken upstream (1) and a sample taken downstream (2) of the pump:

$$\text{NMFGM damage} = (SLFA)_2 - (SLFA)_1 = \Delta(FAUPF_i) \tag{3.5}$$

It is assumed that added selective enzyme does not interact with the enzymes originally present in the milk sample, including lipases and proteases. The possible interactions between different enzymes would be highly specific and very dependent on the exact enzyme mixtures found in each individual sample. Since the identification and determination of enzymes in milk samples is expensive and time consuming and no published method is available, it is unrealistic to try and verify this assumption for industrial test purposes. The issue was therefore not pursued further.

Results in this work are expressed in terms of measured FFAs, not quantities of UPF. The only published method that claims to perform a conversion of FFAs into fat units (Halter et al., 1978) uses the average molecular weight of milk fat, and assumes that the fat substrates are all triacylglycerols that are converted to the three FFA chains. Strictly speaking, in order to perform a rigorous conversion one needs to know the exact proportion and molecular weight of each type of fatty acid chain lipolysed in the sample. Therefore using FFA units is more accurate as a FFA to fat conversion only increases the uncertainty of the measurement. Furthermore, not all of the fat is always converted to FFAs. For example, milk LPL and PPL, the enzyme used in this work are both 1,3 specific, and only convert 66% of the fat to FFA.

The selective lipolysis test is essentially the measurement of SLFA before and after an operation. The measurement of OFA is used to determine the base level of FFAs and the measurement of IFA to determine the original lipolytic activity in the sample (due to enzymes of endogenous and microbial origin), and these are not required in order to determine the amount of NMFGM damage. However, the selective lipolysis test developed in this work is still unable to give an exact absolute single FAUPF

measurement because there is no simple way of measuring directly the FAPF created during incubation.

In the early stages of this work, inconsistent FFA results obtained over half a year from industrial samples of heat treated milk measured using the LFF test of Fang (1998) indicated that the NMFGM can easily be damaged during sampling and also contaminated by microbes that tend to grow during incubation and produce a higher lipolytic activity in the sample than was originally present. These issues will be discussed in greater detail later in this section but it is important to mention them here to emphasise the need to convert UPF into FFAs as quickly as possible. One possibility of dealing with microbial contamination considered in this work was the addition of a preservative. Regrettably, it was found that the preservatives added tended to interfere with the determination of SLFA. To minimise the creation of FAPF from microbial contamination a strict protocol for hygienic handling was devised and the incubation period was shortened from 24 hours to 6 hours (Jaap Evers, personal communication, 2002). In addition, to facilitate the conversion of UPF to FFAs the incubation temperature was raised from 30°C to 37°C to ensure all the milk fat was in a liquid state so that it was as accessible as possible to the added lipolytic enzyme. The short incubation period and the use of excess amounts of exogenous lipolytic enzyme compared to the amount of lipolytic enzymes originally present in the sample results in a minimisation of FAPF. Therefore, the SLFA measured would be dominated by FAUPF and could be used as a good estimate of FAUPF in most cases (Equation 3.3).

Using measurements of IFA made it possible to estimate the impact of microbial contamination. For example, when the IFA value was small in proportion to the SLFA value it was clear that the amount of FAPF was low (as shown in Equation 3.2). However, when the IFA value was large in proportion to the SLFA value a before-and-after SLFA measurement would give a smaller value for ΔFAUPF (Equation 3.5) and also a higher percentage uncertainty. Thus, microbial contamination and more importantly the production of microbial enzymes needs to be minimised in all measurements of SLFA.

It should be clear by now that the selective lipolysis test only minimises side effects that cannot be controlled during the measurement of NMFGM damage and that there is

always room for improvement. It is not a perfect test but is nonetheless the result of a systematic analysis of the complex issues encountered and improvements developed gradually and painstakingly over a long period of time.

Figure 3.1 illustrates the amounts of different FFAs from different sources (OFA, IFA and SLFA) obtained in the analysis of a milk sample. Note that the extraction and titration processes involved in the test described in this section do not allow for clear separation of the FAUPF and FAPF. The FFAs produced most probably come from conversion of both UPF and PF.
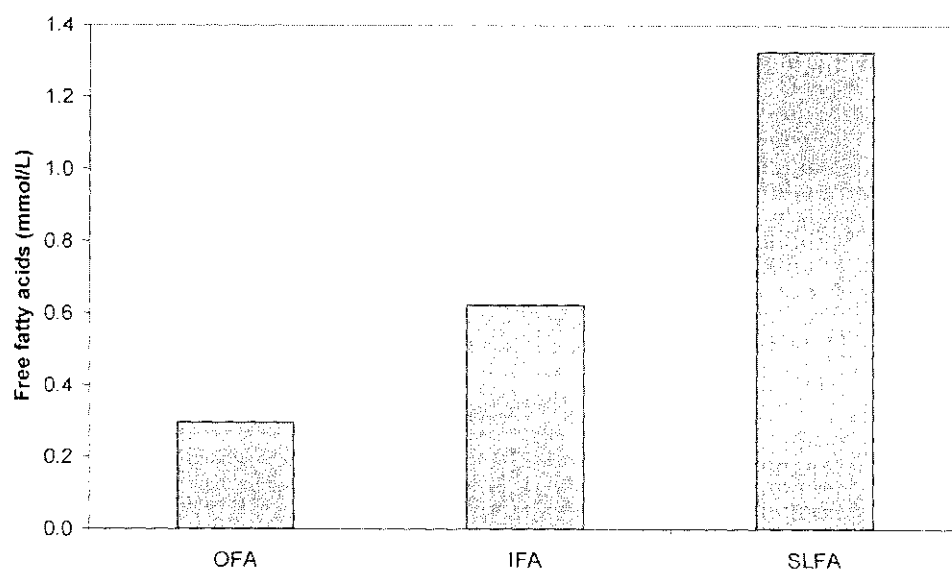
Figure 3.1: Three measurements of FFAs from a pasteurised, unhomogenised milk sample: Fresh sample (OFA), sample incubated at 37°C for 6 hours (IFA) and sample with a selective enzyme (pig pancreatic lipase, PPL) added and then incubated at 37°C for 6 hours (SLFA).

3.1.3   Protocol for measuring fatty acids from UPF

Three different tests are required for measuring the OFA, IFA and SLFA. Not all of these tests need to be applied all the time; only the measurement of SLFA is required to measure NMFGM damage. The following section describes the protocols for the OFA, IFA, SL1 and SL2 tests (the SL1 and SL2 tests both measure SLFA) used in this work. The protocol for each of these tests contains up to four steps:

1.   Sampling technique.

2.   Sample incubation technique.

3.　　Extraction of FFAs from the sample (including sub-sampling from the incubated sample).

4.　　Titration of the extracted FFAs.

The IFA, SL1 and SL2 tests each require an incubation step that is not present in the OFA test. The SL2 test (developed late in this work) contains modifications to the SL1 test to improve the sub-sampling from the incubated sample. Apart from this the protocols of each of the tests are identical.

The test procedures given in the following are written in the form of laboratory protocols. Parts of the protocols identical to each other in all of the tests will be presented first (i.e. sampling technique, FFA extraction and titration) followed by the parts that differ (i.e. incubation and sub-sampling techniques).

Sample Handling

1. Samples for all tests should be collected in sterilised containers

2. Hot samples must be cooled as rapidly as possible, by placing the containers in a temperature controlled room at 20°C. DO NOT TEMPERATURE CYCLE. Temperature cycling has been shown to increase the risk of lipolysis in raw milk (e.g. Miller & Puhan, 1986b; Puhan, 1989). Transfer the sample to an incubation bottle after it has been cooled.

3. Samples waiting to be analysed must be kept homogeneous to avoid creaming but this must be done in a gentle way to avoid further NMFGM damage. Mixing with a sterilised glass stirring rod or by a gentle rocking motion are acceptable.

Chemicals Preparation for FFA measurement

- Sterilised distilled/RO water
- α-naphtholphthalein indicator (1% m/v in ethanol)
- Bromophenol blue indicator (0.5% m/v in water)
- Phenolphthalein indicator (2% m/v in alcohol)
- Potassium hydroxide (KOH) solution (0.01 mol/L in ethanol)
- 2:1 mixture of diethyl ether and petroleum ether (40°C-60°C)
- Neutralised ethanol

- Neutralised methanol

- Standardised hydrochloric acid (HCl) (0.01 mol/L)

- Sulphuric acid (0.5 mol/L)

- Pig pancreatic lipase (PPL) – Crude (25% protein) (EC no. 3.1.1.3).
  Supplied by Biolab Industries Limited (Auckland, New Zealand).
  For the selective lipolysis test only.

All chemicals were prepared in a consistent manner. Neutralised methanol and ethanol were used as solvents. Each of these solutions was made up by adding 200mL of the relevant (Analar grade) alcohol to 1 mL of 1% α-naphtholphthalein solution per 200mL of alcohol and then enough potassium hydroxide solution to give the solution a green colour. The endpoint of the titration should be consistent with the green colour of these two alcohol solutions. Such consistency is more important than attempting to reach the same green colour in every titration.

The concentration of the potassium hydroxide (KOH) should be measured on the day by titrating against 5 mL of standardised 0.01 mol/L hydrochloric acid and 3 drops of 2% phenolphthalein indicator until a light pink colour appears in the clear solution. The process should be repeated at least twice to gain an average value and a measure of experimental uncertainty.

Free fatty acid measurement

This final protocol is based on a method from the New Zealand Ministry of Agriculture and Fisheries (New Zealand Dairy Division of Ministry of Agriculture and Fisheries, 1980) and subsequent modifications by Fang (1998). The New Zealand FFA test was itself based on original proposals by Perrin & Perrin (1958) and is now upgraded to a new test in the New Zealand Dairy Industry Chemical Methods manual (New Zealand Dairy Industry, 2000). A number of changes were gradually added on top of these protocols as an outcome of observations made in this project. The evidence that prompted these changes came from a number of experiments targeted at understanding the development and control of FFAs during the process of sampling and measuring NMFGM damage in milk. A description of these experiments is given in Section 3.1.4 and these results are reported and discussed in Chapter 4.

The following method of measuring FFAs is common to the IFA, OFA and selective lipolysis tests.

1. Pipette 10 mL of sample into a container, either a conical flask or a Mojonnier tube. Label as Container (1)

2. Pipette another 10mL of sample into a beaker. Label as Container (2)

3. Add 3 drops of 0.5% bromophenol blue indicator into Container 2. Shake the liquid in vigorously to give a uniform colour.

4. Add 0.5 mol/L sulphuric acid drop-wise to Container 2 until the colour changes from a dark blue to a greenish-yellow (pH approximately 3.0). Note the number of drops added. Perrin & Perrin (1958) showed that acidification of the mixture improved the extraction of short chain FFAs. Steps 2-4 are used simply to determine the amount of sulphuric acid required to acidify the sample to a pH of 3.0.

5. Add the same number of drops of 0.5 mol/L sulphuric acid to Container 1. Swirl gently.

6. Pipette 5 mL of neutralised ethanol into Container 1. Stopper with a rubber bung and shake vigorously for 1 minute but ensuring the liquid remains in the boot of the tube. Do not invert the container. Aqueous and solid material may adhere to the tube surface and contaminate the supernatant layer created by subsequent centrifugation. This may interfere with the final titration.

7. Pipette 15 mL of a 2:1 mixture of diethyl ether and petroleum ether to Container 1. Stopper and shake vigorously for 1 minute. Again, do not invert the container. Perrin & Perrin showed that this particular solvent mixture gave a more accurate recovery of both short and long chain FFAs than other ratios of these two solvents.

8. Place Container 1 in a centrifuge and run at 600 rpm for 5 minutes.

9. Pipette 10 mL of the clear supernatant ether layer from Container 1 after centrifugation into a 50 mL conical flask. (If Container 1 is a Mojonnier tube care should be taken as it is very difficult to pipette straight from a Mojonnier tube. In this case pour > 10 mL of the ether layer into a separate beaker, then pipette 10 mL from this layer into the conical flask).

10. Pipette 10 mL of neutralised methanol into the conical flask.

11. Immediately titrate the solution against 0.01 M potassium hydroxide (KOH) from a 10 mL burette with 0.02 mL graduations until the clear/yellow solution turns to green.

12. Prepare a blank sample using 10mL of sterilised distilled water placed into a container similar to Container 1. Bring the blank to pH 3.0 by adding 1 drop of 0.5 M sulphuric acid. Then subject this blank to steps 6-11 of the protocol.

Calculation

The procedure outlined above determines directly the number of moles of FFAs found in 10 mL of supernatant. There is a need then to calculate the FFA content of the original milk sample. Different authors have proposed different estimates of what the 10 mL of supernatant titrated represent as a fraction of the total volume of supernatant obtained from the milk sample. Perrin & Perrin (1958) quoted a ratio of 1.35 and this is used in the following equation:

$$\text{Free Fatty Acid content} = C \times 1.35 \times \frac{(T - B)}{1000} \times \frac{1000}{V} \tag{3.6}$$

where: C = actual concentration of KOH (mol/L)

     T = titrated volume of KOH (mL)

     B = blank titration volume (mL)

     V = volume of the original sample (usually 10mL)

The New Zealand Chemical Methods manual (2000) specifies a ratio of 1.5. Neither source gives a full proof of the derivation of the factor it recommends. Here, the Perrin & Perrin version has been adopted because of its long-standing acceptance by other researchers.

The subtraction of the blank term, B, is self explanatory.

The typical concentration of KOH used is 0.01 mol/L. However, the actual sample of KOH used in any particular test may differ slightly from that standard value. The concentration of all batches of KOH solution were regularly standardised as shown in the chemical preparation section above.

OFA test

The OFA test is simply the application of the above procedure for measuring FFAs to a milk sample as soon as possible after the collection of that sample. The sample is not deliberately incubated, but is stored at a temperature of 5°C. To ensure a representative sub-sample is taken, the sample container is inverted slowly 5 times immediately before a sub-sample is pipetted from the container into the conical flask or Mojonnier tube used in the FFA extraction and titration.

IFA test

In this test the samples are first incubated before the sub-sample is taken. The protocol for incubation is described in the following.

All the equipment used must be sterilised. In this work, the containers in which the samples are to be incubated, and also the distilled water containers (see below) are sterilized in an autoclave at 121°C for 15 min. Other glass equipment is sterilized by keeping it an oven at 100°C for 24 hours.

Incubation Procedure

1. Pipette 50 mL of sample into a 100 mL Duran bottle (a cylindrical glass container with a screw cap).
2. Incubate for 6 hours at 37°C.
3. Determine FFAs using the procedure described above.
4. When comparisons must be made with the results of the selective lipolysis test, add 1 mL of sterilised distilled water to the 50 mL sample before incubation, because 1 mL of lipase solution is added in the selective lipolysis test.
5. To ensure a representative sample is used in the incubation procedure the sample container is inverted slowly 5 times immediately after water/lipase solution is added to the sample.

Selective lipolysis test

The selective lipolysis test (SL1 and SL2) is identical to the IFA test except that a selective enzyme, in this case PPL, is added to the sample before incubation to ensure the complete conversion of UPF to FFAs:

1. Pipette 50 mL of sample into a 100 mL Duran bottle.
2. Add 1 mL PPL solution with a concentration of 0.05g crude lipase per 50 mL of water.
3. Incubate for 6 hours at 37°C.
4. Determine FFAs using the procedure described above.

The lipase solution is prepared by dissolving 50 mg PPL in 50 mL sterilised distilled water at room temperature.

In order to maintain its activity (shown in Appendix B1) the PPL was always stored at 2-8°C, according to the manufacturer's instructions.

SL2 test

In the SL2 test the sub-sampling inversion procedure is replaced with vigorous agitation of the sample at 10,000 rpm for 15 seconds with a Heidolph DIAX 600 Homogenising unit (Germany). This was because the inversion protocol used in the SL1 test did not succeed in mixing the fat and aqueous phases very well in those samples that created enough NMFGM damage to result in separation of phases after incubation and hence gave very scattered results (see Section 4 of Chapter 4).

3.1.4   Experiments to develop the selective lipolysis test

Results obtained over the first six months of a survey of NMFGM damage in industrial plants indicated that the lipolysable free fat (LFF) test inherited from the research of Fang (1998) was not detailed and solid enough to be applied directly in investigations in industry. The results of these runs cannot be used in the analysis of the problem studied in this work, and are therefore not reported. However, reference may be made to a few specific runs where useful observations were made that pointed to deficiencies in the test methodologies of that period.

The detailed results of the experiments used to develop the selective lipolysis test are reported in Chapter 4. All experimental runs described in this work are numbered using a one-letter prefix and a 2-3 digit number (e.g. T06). The full list can be found in Appendix D.

Sampling

Attention was directed to the sampling technique used at industrial sites in the New Zealand dairy industry. This was suspected to be further damaging the NMFGM above the level existing in process lines. Samples were taken using a Vacutainer system. The Vacutainer system (supplied by Biolab Industries Limited - Auckland, New Zealand, email: info@nzl.biolabgroup.com) consists of an evacuated 10 mL tube closed by a rubber bung (Figure 3.2). To take a sample, one end of a special two-way needle is first pushed into the rubber bung of the tube. The other end of the needle (which is sealed by a rubber sheath) is inserted into a rubber septum installed on the equipment to be sampled from. The needle pierces both the sheath and the septum, and milk flows into the container under the influence of the pressure difference that exists owing to the vacuum in the container. It was observed visually that the milk rushed into the test tube at high speed and hit the walls of the tube. Evidence in the literature indicates that agitation and splashing create significant damage to the NMFGM (e.g. Deeth & Fitz-Gerald, 1978; Kammerlehner & Kessler, 1980; Cartier & Chilliard, 1989a).
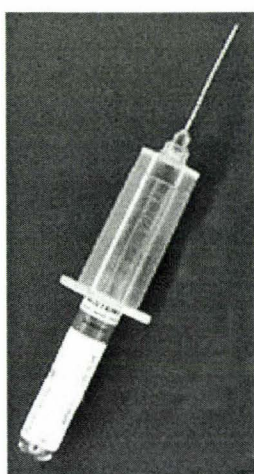
Figure 3.2: Example of Vacutainer system used in the New Zealand dairy industry.

It was suspected that the high velocity of the milk entering the Vacutainer tube was creating a significant amount of extra NMFGM damage to the sample. A set of four experimental runs was undertaken to investigate the amount of damage caused by this sampling procedure (runs T06-09). The sampling velocity of the milk was varied by adding a length of 2 mm diameter plastic tubing connecting a 300 mL disposable sampling container (not evacuated) to the sampling septum on one of the outlet pipes from the raw milk silo. This was carried out at the Waitoa dairy factory (New Zealand Dairy Group Co-operative Limited, now incorporated in the Fonterra Co-operative Group). Different sampling velocities were obtained by:

1. Connecting the sampling container (not evacuated) directly to the silo through a two-way needle.

2. Connecting through two or three metre lengths of plastic hosing.

It was thought that different lengths of tubing would give different sampling velocities due to an expected increase in pressure drop along the tubing with tube length. Unfortunately, this was not the case.

The volume of milk and the time elapsed from insertion of the needle to the end of sampling were recorded manually to allow computation of the flow rate of sampling. The calculated flow rate was found by dividing the volume of milk in the container by the time taken to fill the container to that volume. Free fat (FF) tests based on the method of Fang (1998) were carried out on the samples in duplicate. The procedure for the selective lipolysis test was not perfected at that time; in particular the method of dealing with microbial contamination had not been finalised (see below) and therefore the following discussion will be based only on FF results (Note that all further references to FF are to those measurements made using the test of Fang (1998)).

To determine whether the FF levels generated by sampling at the higher velocity were significantly different from those at the lower velocity a one-tailed t-test was applied. This was the 'two-sample assuming unequal variances' t-test in Microsoft Excel. The results, given in Section 3 of Chapter 4, indicated that indeed a significant level of damage did occur when samples were collected using too high a velocity. Therefore the following new sampling protocol was developed when taking samples from industrial sites.

A sterile disposable needle (Becton Dickinson, Singapore) was fitted to a syringe (Terumo Medical Corporation, U.S.A.), and the needle then inserted into the sampling septum on the plant. Manual pressure was applied to the syringe plunger such that milk entered the syringe slowly and gently with minimum splashing. Sterile disposable needles and syringes were used for all the experiments involving the Massey University milk processing pilot plant and for all industrial surveys carried in this work (Sections 3.4-3.5).

Microbial Contamination of Samples

Three periods of practical work were spent at industrial sites to survey the evolution of NMFGM damage during the milk powder manufacturing process. In the first period, already alluded to, the major outcome was the identification of the unreliability of the tools inherited from previous work (i.e. the LFF test) and in the second period, establishment of a new sampling protocol that avoided unwanted damage during the sampling process. However, there were also indications that even when the milk had been heated to high temperatures, e.g. those samples taken from the pasteuriser (72°C), there may have been interference with the LFF test from significant microbial contamination (note that the SL1 test had not been developed at this time). These observations were confirmed during the second practical period in industry and the problem dealt with successfully as now described.

During the second period in industry, analysis of error in the LFF test was carried out by always taking replicate samples during experiments. Because of the large number of samples in each experiment and the inadequate supply of glass tubes, plastic centrifuge tubes were used to incubate the milk samples. In many experiments where the LFF test was conducted in triplicate it was observed that one of the three results was often significantly different from the other two and the lack of repeatability of the test results became a major concern. Analysis of the results collected over a three-month period indicated that most of the anomalous results were for samples incubated in plastic containers. Significant microbial contamination of samples was suspected but had not been considered initially, because milk samples were taken at the pasteuriser, preheater and evaporator outlets, where the microbial contamination should have been minimal. A set of experimental runs, described below, were then devised to determine whether

microbial contamination of samples occurred during subsequent handling and, if it did, whether it could be stopped by the use of preservatives.

It was suspected that the inconsistent results with plastic tubes might have been the result of severe microbial contamination because of the inability to sterilise the plastic containers. Two experimental runs were conducted to check for evidence of microbial contamination in samples (T24-25). Pasteurised, unhomogenised (standardised to 4% fat) milk was purchased from a local dairy plant (Mainland Milk Products, Longburn). The milk was sealed in plastic bladders at the outlet of the plant pasteuriser, and once the milk was transported to Massey University (20 minutes away) the milk was placed in a chilled agitated tank at 4°C. In run T24 the milk was re-circulated around a pumping system (the large-volume pumping rig, described more fully in Section 3.4.3) before being passed once through a plate heat exchanger, which heated the milk to 80°C. Samples of milk were taken in duplicate from the vat (within an hour of receipt from the dairy plant), the large-volume pumping rig and the outlet of the plate heat exchanger. The equipment was first cleaned-in-place (CIP) after previous operation of the rig the day before the experimental run.

A second run was conducted (T25) with exactly the same protocol except that the equipment was first rinsed with 1% nitric acid at 60°C for 10 minutes to minimise the presence of microbes on the equipment surfaces. Each sample (diluted by a suitable amount of sterilised distilled water) was added to standard plate count agar and incubated for 24 hours at 37°C. The results, reported in Section 1 of Chapter 4, indicated that microbes were present in significantly greater quantities in samples from the pumping rig in run T24 than in run T25. There was therefore the possibility that the increased microbial population would grow substantially during the incubation step of the IFA and selective lipolysis tests, producing potentially unselective enzymes and therefore unwanted FAPF.

Prevention of microbial contamination

A new series of experiments was devised to try and nullify the effect of microbial contamination of thermally processed milk samples. In the initial industrial survey work, analysis of samples of unprocessed raw milk from tankers and silos had identified the possible interference of microbial growth with the LFF tests. Raw milk samples

were also collected after milking with the help of the Livestock Improvement Corporation Limited (Hamilton, New Zealand). These samples contained a preservative known as bronopol and the FFA levels measured from these milk samples using the LFF test were very low. This led to two experimental runs (T01-02, results not shown) designed to test the impact of bronopol on the LFF test using raw milk from the Waitoa dairy factory. Bronopol was added at different time intervals into the incubation step of the LFF test, either immediately before incubation (for a 24 or 48 hour incubation), 24 hours into the incubation period (when incubated for 48 hours) or not at all (24 hour and 48 hour incubation periods). However, the results were inconclusive.

Two years later, a set of experimental runs (T14-18, results not shown) were carried out at Massey University to investigate the effect of two preservatives on the LFF test - bronopol and sodium azide - using olive oil as a substrate. The olive oil was used because the activity of the added PPL was quoted by the manufacturer in terms of FFAs produced from olive oil, and the olive oil was likely to have a low microbial count. The results suggested problems with the use of both preservatives.

A final experimental run was carried out with bronopol and sodium azide added separately using Unhomogenised, pasteurised milk bought from a local supermarket (T32). A recent International Dairy Federation (IDF) standard for measurement of milk samples by infra-red analysis recommended the use of 0.02%-0.06% bronopol, 0.03% sodium azide or potassium dichromate (International Dairy Federation, 2000). The IDF recommends 0.02% hydrogen peroxide for storage of milk samples to be analysed for FFA content (Anderson et al., 1991). However this was not tested given the concern Miller & Puhan (1986a) had over its effect on lipase activity, a point not addressed in the IDF document.

Bronopol and sodium azide were selected for testing because of their availability. The purpose of these experiments was not primarily to test whether the preservatives were successful in inhibiting microbial growth but to see if they interfered firstly with the titration of FFAs and secondly with the action of PPL in the LFF test. Duplicate samples were measured. The experimental conditions are shown in Table 3.1 and the results reported in Section 4.1.5.

Table 3.1: Conditions of run T32 investigating the effect of two preservatives on FFA measurement of Unhomogenised, pasteurised milk. All samples were incubated at 37°C for 24 hours. Samples were taken in duplicate.

Combination	Preservative added?	Lipase added?
1	no	no
2	no	yes
3	0.02% Bronopol	no
4	0.02% Bronopol	yes
5	0.03% Sodium Azide	no
6	0.03% Sodium Azide	yes

The results showed that both preservatives affected the measurement of FFAs by either increasing or decreasing the FFA value from the control values. This information, coupled with work by other authors (e.g. Miller & Puhan, 1986a; Imhof & Bosset, 1995) led us to question the suitability of a preservative in a FFA test. Therefore it was decided not to use preservatives to reduce microbial growth in the selective lipolysis test.

In the final version of the SL1 test the milk sample is incubated at 37°C for 6 hours. A fellow post graduate candidate, Jaap Evers (personal communication) suggested that within this time frame microbial contamination was minimised. An experimental run was performed to verify that this reduced incubation time could control microbial contamination, and more importantly, could minimise any increase in the level of IFA. A number of Unhomogenised, pasteurised milk samples from the same batch of milk sourced from a local supermarket were incubated at 37°C for different periods (run T31), as shown in Table 3.2. The FFA content was monitored without adding any PPL. The microbial population was monitored by a plate count using skim milk agar and incubating plated samples for 48 hours. The results are reported in Section 1 of Chapter 4.

Table 3.2: Conditions of run T31 investigating the effect of incubation time on FFA measurement.

Incubation time (hrs)	Incubation temperature (°C)
0	37
6	37
24	37

3.2   Measurement of the physical consequences of NMFGM damage

3.2.1   Background

It has long been known that damage to the NMFGM can result in changes to the particle size distribution (PSD) of the fat globules in milk. For example, Walstra (1983) reported that 95 to 98% of the total mass of fat is found in the range of particle sizes from 1 to 10 μm. Walstra argued that a moderate amount of damage results in either coalescence or flocculation of fat globules. This in turn results in an increase in the number of large globules and possibly of sizes greater than occur naturally (i.e. >10 μm). Fang (1998), for example, showed that passing milk through a cavitating pump network results in a new peak, in the upper region of sizes, in the PSD of fat globules. Under severe conditions, disruption of the fat globules can occur resulting in an increase in smaller fat globules or even globules of sizes smaller than found naturally. Michalski et al. (2002a) using laser light scattering and dynamic light scattering found that severe damage (in this case homogenisation) to fat globules resulted in a significant increase in the numbers of submicronic fat globules (of 400 nm or less).

The change in PSD of the fat is a physical consequence of NMFGM damage that can be assessed separately from the susceptibility of the fat to lipolysis (which is a chemical consequence of NMFGM damage). These two approaches together give a better overall measure of NMFGM damage than the results of either test on its own and in fact a combination of both is necessary to give a reasonably comprehensive picture of NMFGM damage.

However, changes in the PSD of milk fat globules are difficult to assess. It is very rare to find the creation by damage of an entirely new peak. Fang (1998) showed one such case but only at the highest level of damage (of five) he tested. More often the changes are less dramatic. Most workers in the field describe the PSD by comparing graphical representations (e.g. Fang, 1998) or by comparing the average particle size diameter of PSDs (e.g. Ye, 2003; Wiking et al., 2003; Kaw, 1998) or the specific surface area (e.g. Michalski et al., 2002a). Kaw (1998) described the qualitative shift in PSDs of milks by using volume to surface average particle diameter, weight-average particle diameter, and by labelling particular size ranges. However, no authors are known to have described the *quantitative* shift of particular size ranges within a PSD, as a result of NMFGM damage.

Walstra (1969b) divided the PSD of milk fat globules into three zones (Walstra called these zones "sub-distributions"): 1 to 10 µm, <1 µm, and >10 µm. By calculating the number of fat globules in each zone he was able to give a quantitative estimate of changes in the PSD of milk fat globules. Surprisingly, the approach used by Walstra has not until now been taken up by other workers in the field of NMFGM damage.

The PSD of milk received at dairy plants in New Zealand, and the PSD of most of the Unhomogenised, pasteurised milk supplied for the present work shows two distinct peaks (Figure 3.3). Most of the globules range in size from 1 to 10 µm. The first peak from the left represents approximately 30% of the fat by volume and the second peak represents approximately 70%. When NMFGM damage occurs the PSD changes. An example of this change is shown by the dotted line in Figure 3.3. Extreme flocculation or coalescence can easily be detected as an increase in the proportion of fat found in the particle size range above the second peak. Disruption, on the other hand, is detected as an increase in the proportion of fat found in the size range below the second peak.

While the PSDs of fresh samples obtained during this work from the dairy industry over a period of three years were remarkably consistent, there were nonetheless small but perceptible variations in the positions and shapes of the two peaks as well as in their relative proportions. Thus it was not possible to have absolutely fixed particle size boundaries in the analysis of the PSDs of milk fat globules. The problem was to define

boundaries in a way that allowed for natural variation. This was done as described in the following, and led to the development of the 'particle size zoning (PSZ) test'.

The range 1 to 10 μm of the PSD is called here the primary region. Figure 3.3 shows that the area under the second peak, which represents the zone of the dominant fat globule sizes, does not extend to the edges of the primary region. As changes to the PSD within the primary region owing to either disruption or coalescence can be substantial, the scheme of Walstra (1969b) is not a highly sensitive way of assessing damage.
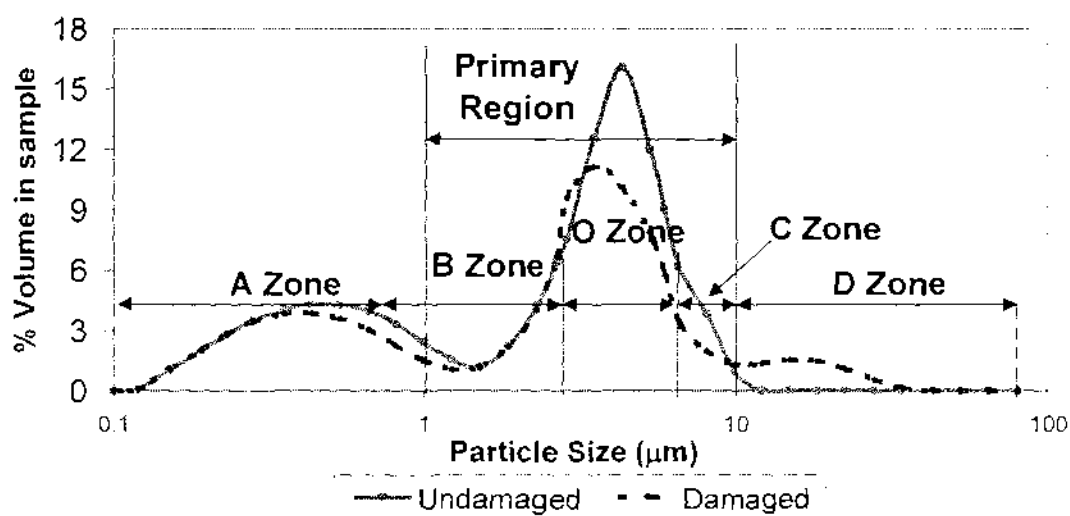
Figure 3.3: Typical particle size distribution for pasteurised, unhomogenised standardised 4% fat milk (undamaged), and a distribution showing evidence of native milk fat globule membrane damage (damaged). The Malvern Mastersizer E, particle sizing machine was used to obtain this data.

Instead, the PSD (of unhomogenised milk) is divided into 5 zones, as shown in Figure 3.3:

1. A Zone: all sizes below 1 μm.

2. D Zone: all sizes above 10 μm.

3. B Zone: defined as the 25% of the area of the primary region to the right of the 1 μm boundary. The right hand boundary of zone B is thus the upper boundary of the lower quartile of the primary region.

4. C zone: defined as the 25% of the area of the primary region to the left of the 10μm boundary. The left hand boundary of C Zone is thus the lower boundary of the upper quartile of the primary region.

5. O Zone: defined as the 50% of the area of the primary region found between the upper boundary of the B Zone and the lower boundary of the C Zone. This zone contains the most dominant milk fat globule sizes (Figure 3.3).

The purpose of defining the B and C zone is to monitor relatively small amounts of physical damage that could not be identified in Walstra's scheme. However the characteristic particle sizes needed to set the boundaries between the B, C and O zones were not known. The use of two regions of 25% and one of 50% to describe the primary region was to have a simple method to define these three regions: by using the statistical upper and lower quartiles.

3.2.2   Protocol of the PSZ test

The PSZ test is a before-and-after NMFGM damage measurement test, like the selective lipolysis test, and therefore requires two samples (one as a control).

The protocol for the PSZ test consists of three parts, consisting of:
1. Correct handling of a milk sample.
2. Measurement of the PSD of a sample.
3. Data analysis.

Particle Size Distribution Measurement

A Malvern Mastersizer E particle sizing machine (Malvern Instruments Ltd., U.K.) was used in this work. The machine was set up to measure particle sizes in the range 0.1-80 µm. The protocol used was that included in the instrument manual, with the addition of the following steps:

1. If samples cannot be processed immediately they should be stored in a chiller at 5°C. Storage of pasteurised milk for up to 24 hours will not affect the PSD of fat globules (see Section 2 of Chapter 4).

2. Ensure the bulk sample from which a sample will be added to the Mastersizer is homogeneous. This can be done by gentle swirling with a glass rod or inverting the container slowly, five times.

3. About 5 mL of milk is needed for this procedure. This will depend on its fat content. The higher the fat content, the less sample that is required.

4. 1 mL of sample is added to a container followed by 9 mL of Walstra's Solution A (Appendix B4). This mixture is then gently agitated by swirling and presented to the Mastersizer as per the instructions in the instrument manual.

5. The Mastersizer E uses a presentation unit for small samples. This chamber allows the sample to be re-circulated through the optical components of the Mastersizer and returned to the presentation unit so that the sample is analysed uniformly. Samples are dispersed in reverse-osmosis water.

6. Air is removed from the water in the presentation unit before the sample is added by stopping the agitator on the unit and allowing the air bubbles to rise to the surface. Air is removed from the presentation unit because air inclusion particularly accompanied by agitation can cause NMFGM damage.

The data output from the Malvern Mastersizer was processed using a computer spreadsheet. The output from the instrument was, for each sample, a table of the percentage of fat volume ("% volume") against different particle size ranges (Appendix C6). The data are illustrated graphically in Figure 3.3. These data were used to calculate the proportion of the fat volume lying in each zone, for each of the samples.

Determination of the boundaries of the five PSD zones

An example calculation is given in Appendix C7-8.

1. The upper boundary of the A Zone is 1 μm. The smallest particle size the Mastersizer E can measure is 0.1 μm. This was the lower boundary.

2. The lower boundary of the D Zone is 10 μm or the nearest particle diameter reported by the instrument. With the Mastersizer E it is 9.91 μm. The upper boundary of the D Zone is the highest particle size measured by the particle sizing machine (80 μm for the Mastersizer E).

3. Calculate the area under the PSD curve for the primary region, P.R. (shown in Figure 3.3). For the Mastersizer E this was the particle sizes between 1 μm and 9.91 μm. Any numerical method of integration can be used. In this case the trapezoidal rule was used because the Mastersizer E output already lists percentage volume of particles between regular logarithmic intervals of particle size.

4. As previously explained, the lower boundary of the B Zone is defined as 1 μm and is fixed. The upper boundary of the B Zone varies depending on the size of the P.R. To determine this boundary, the P.R. PSD is converted into a cumulative distribution, as shown in Figure 3.4 (Line A). 25% of the P.R. (in volume %) is calculated and the upper B Zone boundary is defined as the particle size where the 25% x P.R. value intersects with the cumulative PSD.

5. As previously explained, the upper boundary of the C Zone is 9.91 μm and is fixed. The lower boundary of the C Zone varies depending on the size of the P.R. To determine this boundary, the P.R. PSD is converted into a cumulative distribution, starting from 9.91 μm, as shown in Figure 3.4 (Line B). 25% of the P.R. (in volume %) is calculated and the lower C Zone boundary is defined as the particle size where the 25% x P.R. value intersects with the cumulative PSD.

6. The O Zone is therefore the remaining middle 50% of the primary region, and is automatically defined by the boundaries of the B and C Zones as illustrated in Figure 3.4.
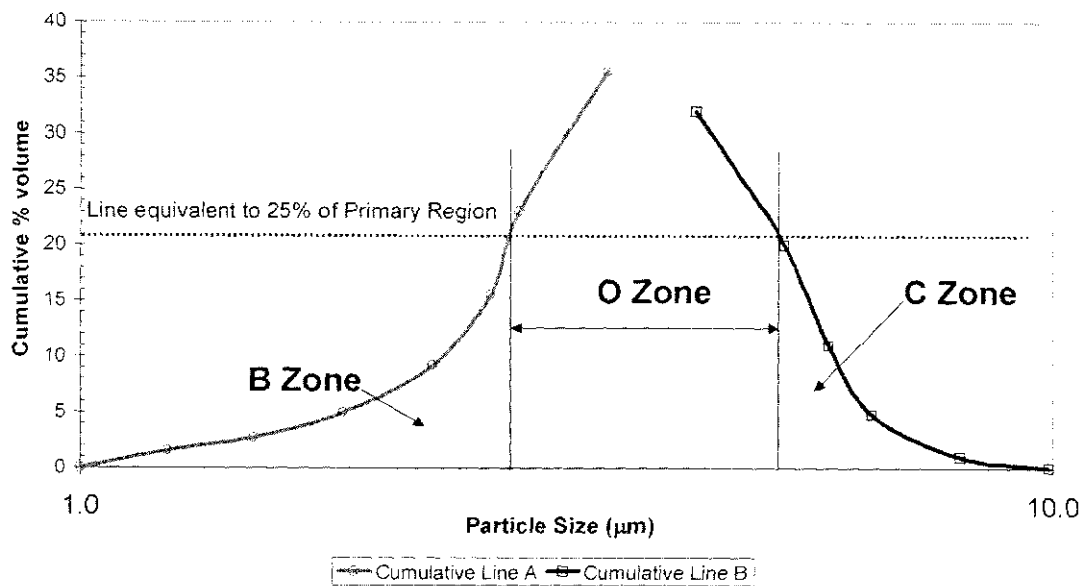
Figure 3.4: Graph showing the primary region portion of Figure 3.3 converted into a cumulative distribution, illustrating how the B-O and O-C Zone boundaries are defined.

In standard practice the undamaged sample (first sample), or for a unit operation the sample taken directly before the unit operation, is defined as the control and is analysed as described above. Subsequent samples using the same batch of milk are analysed using the same particle size boundaries for the B, C, and O Zones. This is preferable to

defining a new set of boundaries for each sample because it allows easier comparison between samples from the same batch of milk. Essentially this means that the zone boundaries for a particular batch of milk are fixed but they can vary between batches of milk. This procedure typically gives a B-O boundary between 2.7 and 3.0 μm and a C-O boundary between 5.1 and 4.6 μm for Unhomogenised, pasteurised milk.

Determination of the % volume of sample within each zone

Once the particle size boundaries are defined for each control and subsequent sample, the procedure for calculating the % volume of sample within each zone is relatively straightforward. Essentially, the area under the PSD curve within the upper and lower boundary of each zone is calculated and gives a number between 0-100% of the sample volume. The sum of the five zones should equal 100%.

Interpretation of changes in particle size zones

Increases to the left of the O Zone can be related to some level of disruption of the dominant sizes. An increase in the A Zone as a result of processing is almost a certain indication of substantial disruption of the fat globules. Interpretation of increases in the B Zone is more difficult and requires study of the other zones, because such increases can result from disruption of larger globules or flocculates originally in zones O, C and D, and also conceivably from coalescence or flocculation of particles from the A zone. However, in high shear processes flocculation and coalescence is unlikely as they normally occur under mild agitation conditions (Walstra, 1983). In these cases where disruption is the vastly dominating process, changes in the B Zone can be interpreted clearly since the direction of particle size change will be largely in one direction. A mixed effect of flocculation/coalescence and disruption on the B Zone occurs only in processes that generate a relatively narrow range of middle level shear stresses.

Increases to the right of the O Zone are the result of globule size increases upon handling of the milk. Such increases must be the result of coalescence and/or flocculation. Flocculation is often due to proteins bonding fat globules together. In some cases protein binding can have very little to do with NMFGM damage. These clusters of fat and protein can be minimised in particle size measurement with the use of Walstra's solution A (Walstra, 1965). Kaw (1998) used a similar technique. Flocculation of fat globules owing to protein interaction is likely to be minimised but not flocculation due

to fat-fat contact. Walstra's solution A causes the dissolution of casein within the milk and therefore minimises the protein-fat clusters in the milk emulsion, thereby reducing turbidity created by these clusters and improving accuracy of particle size measurements of fat globules (Walstra, 1965). It contains EDTA and Tween-20, and is adjusted to pH 10 (see Appendix B2). The effect of Walstra's Solution A is shown in Figure 3.5. Protein particles (essentially casein) less than 1 μm in diameter are removed, causing a decrease in the proportion of particles below 1 μm. This causes an increase in the proportion of particles larger than 1 μm in diameter. If there had been significant flocculation of protein with fat at larger particle sizes then this would have caused a reduction in the proportion of particles at the larger sizes.
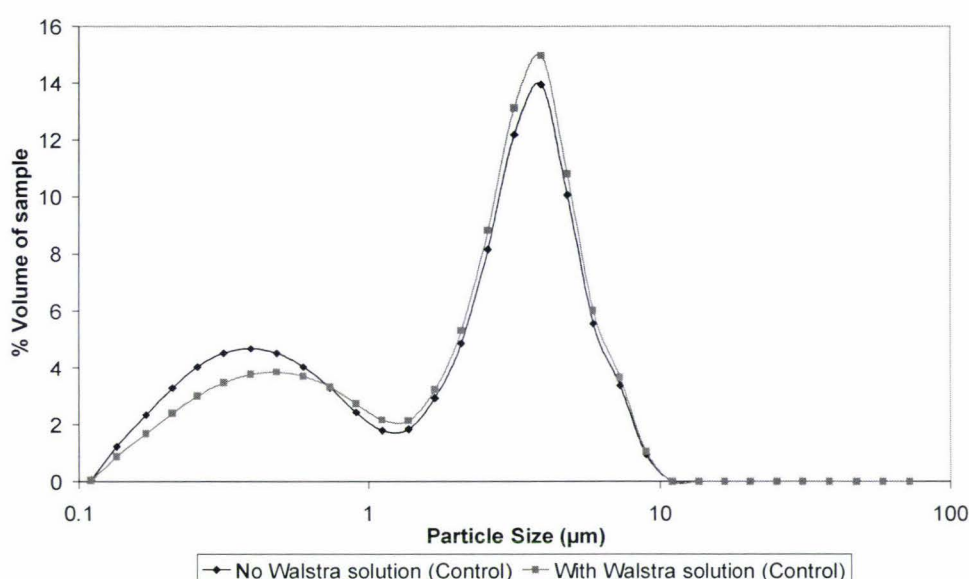
Figure 3.5: Effect of Walstra's solution A on a particle size distribution of Unhomogenised, pasteurised milk. Samples were measured using the Malvern Mastersizer E particle sizing machine.

An increase in the C Zone can result from coalescence of smaller globules mostly from the B and O zones when the NMFGM damage is moderate. However, it can also result from disruption of larger fat globules in the D Zone when shear forces in the milk are increased. A clear example of this phenomenon is discussed in the agitation experiments reported in Section 3 of Chapter 5. As alluded to in discussing fat globule sizes above 10 μm, new peaks are rare, but in the PSZ test can be identified by an increase in the D Zone. Any increase in the D Zone reflects the coalescence or flocculation of smaller sized globules from the other zones.

3.2.3 Experiment on sample handling in the PSZ test

In many cases the NMFGM damaging operation being investigated, and hence the location of sampling, can be quite remote from the nearest particle size measuring equipment available. Because particle size analysis can be made easily and efficiently large numbers of samples are often taken, and these samples must sometimes be kept for long periods before PSD measurement.

An experiment consisting of four runs (T19-22) was designed to investigate the effect of storage temperatures and times on the measured PSD. Unhomogenised, pasteurised milk was damaged at four levels by stirring at 5°C at 2000 rpm for periods of 0, 1, 2 and 4 minutes, then each damage level stored for periods of 1, 4, 24 and 48 hours at 5°C and 20°C. The experimental run conditions are shown in Table 3.3. The PSD of all these samples was measured in duplicate according to the protocol described previously and compared. The data showed that room temperature storage was not acceptable (shown in Section 3 of Chapter 4) but that 5°C storage gave consistent results for at least 24 hours before the particle size distribution of the samples deteriorated.

Table 3.3: Conditions of runs T19-22 investigating the effect of storage time and temperature on the PSZ test. Samples were taken in duplicate.

Run #	Agitation time (minutes)	Storage time of samples at 5°C (hours)	Storage time of samples at 20°C (hours)
T19	0	0, 4, 24, 48	0, 4, 24, 48
T20	1	0, 4, 24, 48	0, 4, 24, 48
T21	2	0, 4, 24, 48	0, 4, 24, 48
T22	4	0, 4, 24, 48	0, 4, 24, 48

3.3 Validation of the selective lipolysis and PSZ tests

It was clearly apparent from Chapter 2 that there was no currently available direct measurement of NMFGM damage and therefore there was no independent standard against which a proposed NMFGM damage measurement technique could be compared formally. In developing experiments to validate the applicability of the two proposed methods from this work it was decided to rely on trends set experimentally (e.g. a

damage treatment repeated over different time intervals). These trends were set by processing the milk in a re-circulation rig called the large-volume pumping rig (described in Section 3.4.3) and taking samples at regular intervals of time. Damage to the NMFGM increases with re-circulation time in a process operation (e.g. Deeth & Fitz-Gerald, 1978). Thus the system was designed to give a clear trend from samples damaged for a short to progressively longer times.

Three runs on the large-volume pumping rig (T28-30) were performed with the conditions summarised in Table 3.4. All samples were taken in duplicate. SL1, IFA, OFA, and PSZ tests were applied to all samples. In addition, solvent-extraction FF was measured using the method of Fang (1998). The results of the different tests were analysed in duplicate to see whether they could reliably follow the trend preset experimentally. Exact replicate runs were not possible because the original NMFGM damage in each new batch of Unhomogenised, pasteurised milk varied from day to day. In addition, the number of samples required to describe the changes in NMFGM damage between 15 and 40 minutes of pumping were too many to be completed in one day. Hence the three runs were designed so that the re-circulation times within each run over-lapped the other two in order to attempt to give an idea of the scatter encountered in industrial measurements where the raw material cannot be controlled. The results are shown in Section 4 of Chapter 4.

Table 3.4: Description of experimental settings designed to assess the OFA, IFA SL1, PSZ and solvent-extraction FF tests (runs T28-30).

Time re-circulated in large-volume pumping rig (minutes)	Experimental runs where a sample was taken at that time interval.
0	T28, 29, 30
15	T28, 30
20	T28, 29
25	T28, 29
30	T28
35	T29
40	T29, 30

3.4 Assessment of mechanical factors affecting NMFGM damage

The process of milk powder manufacture involves a number of unit operations that handle the milk physically as summarised in Figure 3.6. Since this work is about NMFGM damage rather than the art of producing good quality milk powders the detail of the complex issues involved in the design of milk powder processes will not be discussed.

The operations in a milk powder plant can be roughly categorised as thermal processing, water removal and fluid handling. The equipment involved in these processes can be operated under different conditions, e.g. different shear rates or temperatures. Some conditions are specific to particular types of operation such as speed of agitator, or pump impeller. Others. such as residence time and air inclusion are dictated by equipment design. Clearly there is a need to separate a survey of NMFGM damage in existing milk powder manufacturing plants with their existing design and operation conditions from the more fundamental analysis of mechanical factors that underline these operations. A number of controlled experiments were conducted in the milk processing pilot plant at Massey University to provide a scientific platform for the interpretation of the results of the industrial survey to follow.

The factors analysed were:
- Pumping speed
- Air inclusion
- Splashing
- Agitation speed
- Time
- Shear rate through a pipe
- Direct steam injection (DSI)

This is not an exhaustive list of factors that could be studied, but these were the most commonly encountered not only in milk powder processes but in other dairy processes as well.

Reception

Pasteurisation   Separation   Separation

Separation   Standardisation   Pasteurisation

Standardisation   Pasteurisation   Standardisation

Intermediate storage

Preheating

Evaporation

Concentrate heating

Homogenisation

Atomisation

Drying & Agglomeration

Secondary drying

Lecithination

Sifting

Packing
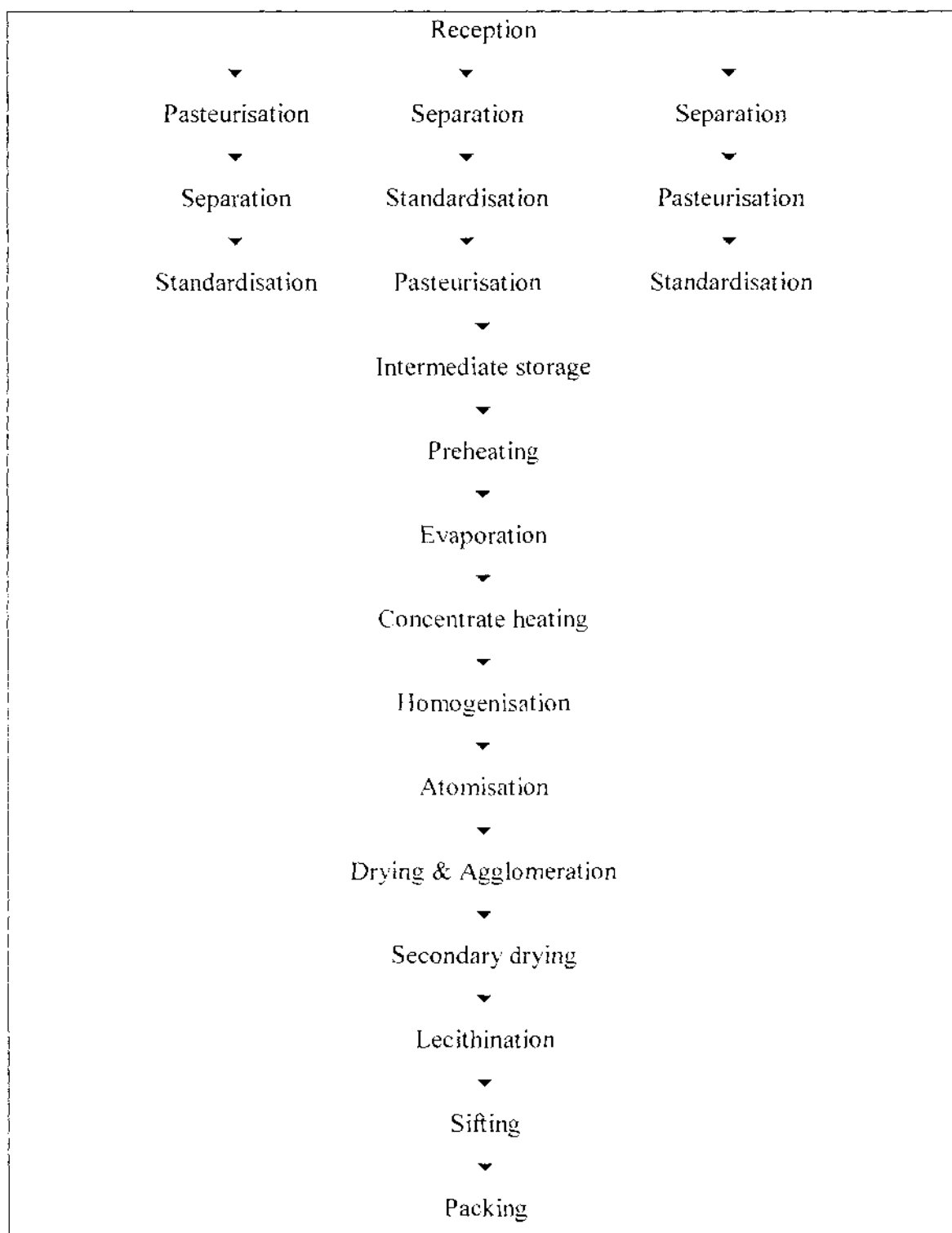
Figure 3.6: Flowchart showing unit operations involved in the milk powder manufacturing process.

In Sections 3.4.2 to 3.4.7 each individual rig is described, followed by the methodology for the experiment. Four different pumping rigs were used in this work. The design of each rig was dictated by the need for independent control of the particular variable

studied. For example, the large-volume rig (Section 3.4.3) required facilities for air inclusion and splashing. The single-pass rig (Section 3.4.4) was designed for studying the amount of damage created by a centrifugal pump and therefore did not include valves or other flow control devices. What many previous workers reported as NMFGM damage by pumps actually also involved valves (Kammerlehner & Kessler, 1980; Fang, 1998). The effect of high shear rates in pipelines (up to 41,400 s^{-1}) on NMFGM damage required a pump developing high pressure such as the monopump used in the BT1000 recirculation rig (Section 3.4.5). The effect of valve cavitation was monitored in a small-volume rig (Section 3.4.6) where a valve cavitation monitoring system was designed and constructed according to the standards of the Instrument Society of America (1995). Thus the use of pumps of different designs in different rigs was dictated by specific needs, not by the wish to study the effect of pump design. That has already been dealt with by previous authors (Kammerlehner & Kessler, 1980).

Most measurements were carried out in duplicate although three or more replicates were taken occasionally. The number of replicate samples was dictated by the number of experimental points sought and the limitations of resources, essentially the time within the day (up to 18 hours in a few cases) or the number of Mojonnier tubes available. Uncertainty was calculated as the half-range of the replicates. In cases where more than three replicates were taken (see Sections 3.4.4-5), 95% confidence intervals were calculated using the 'repeated measures analysis of variance' within the SAS System for Windows, version 8. The results and discussion for all of these experiments are given in Chapter 5. An example of the SAS analysis can be found in Appendix C9.

3.4.1 Raw material

The raw material was sourced from a local company, the Mainland Milk Products factory at Longburn. The unhomogenised milk, described as 'Farmhouse milk' by the company, was ordered a day in advance and withdrawn from the processing line after pasteurisation and standardisation (4.0% fat, 3.1% protein). The milk could either be packed in disposable 10 litre bags that were sealed on site or pumped into a 1000 litre capacity plastic bag fitted with a tap. The bag itself was put inside a pallecon, essentially a rigid frame situated on a truck for ease of transportation. Upon arrival at Massey University, after roughly a half an hour by road, the smaller bags were put into

cold storage in chilled rooms at 4°C and milk from the pallecon was pumped using a centrifugal pump and plastic hose attachment into a stirred milk vat of the milk processing pilot plant. Smaller volumes of milk of less than 10 litres were sourced in one or two litre plastic containers of Farmhouse milk from a local supermarket in Palmerston North.

Use of milk supplied by one of Massey University's experimental farms was briefly considered but rejected because the milk had large variations in fat and protein content, thereby making comparisons between experiments very difficult. In addition, the equipment required to standardise and pasteurise the milk was not available. Since uncontrolled microbial contamination leads to the development of lipases in milk, which have a direct impact on the analysis of FFAs in the study of NMFGM damage, commercially pasteurised milk was used.

3.4.2 Agitation rig

Historically, agitation has been the process of choice to assess NMFGM damage or lipolysis in milk (e.g. Krukovsky & Sharp, 1938; Hlynka et al., 1944; Tarassuk & Frankel, 1955; Kitchen & Aston, 1970; Fitz-Gerald, 1974; Deeth & Fitz-Gerald, 1977; Deeth & Fitz-Gerald, 1978; Kammerlehner & Kessler, 1980; Miller, 1981; Miller & Puhan, 1986b). In this project a small stirred tank was used to create NMFGM damage.

A baffled two litre stainless steel cylindrical tank was chosen, fitted with a Ruston Turbine type impeller (as shown in Figure 3.7 and 3.8) connected to a Heidolph (Germany) RZR2050, 40-2000 rpm motor unit. The agitator was always located in the same position, 10 mm above the bottom of the tank, which was filled with either 300 or 400 mL of milk. The amount of milk added to the tank was selected to ensure an adequate number of samples for the selective lipolysis and PSZ tests. Using the minimum volume required instead of filling the tank completely enabled short agitation times within which measurable levels of NMFGM damage could be reached. Air inclusion was minimised by the presence of baffles attached to the inside walls of the tank (Figure 3.7) which prevented the formation of a vortex at the centre of the tank.

Figure 3.7: Agitated baffled two litre tank used in this project. Milk was damaged using a Ruston Turbine type impeller.
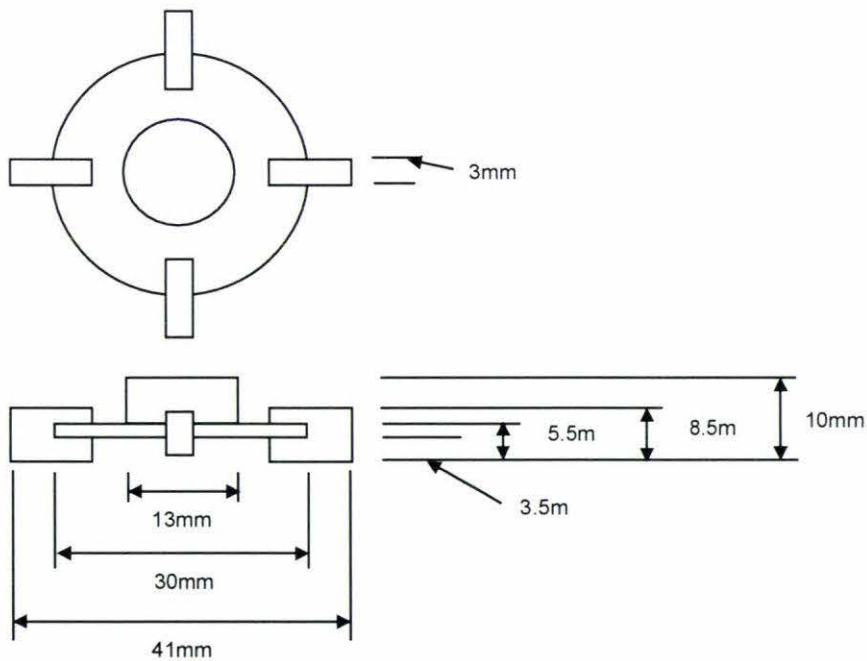
Figure 3.8: Schematic of Ruston Turbine type impeller used with the agitated tank

Samples of milk from this rig were withdrawn using a sterile 30 mL syringe without a needle or a 50 mL pipette.

In all cases the agitation rig was cleaned after each batch of milk was given a damage run using a five-cycle process. Each treatment was agitated for 5 minutes in this order:

- Hot water rinse

- Rinse with a 1% caustic solution

- Hot water rinse

- Rinse with a 1% nitric acid solution

- Cold water rinse

This rig was used to create damage in the test development and validation experiments for the SL1 and PSZ tests described in Sections 3.1, 3.2 and 3.3. The rig was also used to analyse the effect of agitation time, speed and temperature on NMFGM damage in two additional series of experiments.

In the first series of experiments the agitation rig was used in two sets of experimental runs to test the effect of agitation speed, temperature and time.

In the first set of runs (C39-44) the agitation rig was placed in a 25°C water bath. Farmhouse milk was agitated for 5 minutes at six different agitator speeds, from 300-2000 rpm, as shown in Table 3.5. Because of time constraints the work was carried out over two days at four runs per day, using a different batch of milk from a local supermarket each day. Thus on each day a control sample (0 rpm, 25°C) was also included. Each sample was measured in triplicate. The measurement of NMFGM damage was made with the PSZ test because at that time the selective lipolysis test had not been perfected. There were a number of LFF test measurements but these were only useful in identifying the deficiencies in the test procedures inherited from previous work.

Table 3.5: Description of experimental settings to assess the affect of agitation speed on NMFGM damage using the agitation rig (runs C39-44). Control samples were included on both days.

Run #	Speed of agitator (rpm)	Day of run
C39	500	1
C40	1000	1
C41	2000	1
C42	300	2
C43	800	2
C44	1300	2

In a second and third set of runs (C50-52 and C53-56), both the milk temperature and duration of agitation were varied. The experimental conditions are shown in Table 3.6. Runs C53-56 were carried out with a new batch of milk five days after runs C50-52. Samples were taken in duplicate and analysed using the PSZ test.

Table 3.6: Description of experimental settings to assess the effect of agitation temperature and time on NMFGM damage using the agitation rig (runs C50-56). All samples were agitated at 2000 rpm. Samples were measured in duplicate.

Run #	Temperature of agitation (°C)	Duration of agitation (minutes)	Day of run
C50	20	4	1
C51	5	4	1
C52	45	4	1
C53	20	4	6
C54	45	4	6
C55	45	2.5	6
C56	45	1	6

In the second series of experiments the effect of agitation speed and temperature on NMFGM damage was again assessed, but this time using the SL1 or SL2 tests as well as the PSZ test. In addition, the number of experimental points (i.e. agitation temperatures or agitation speeds) was extended, as shown in Tables 3.7 and 3.8.

In a number of these runs both liquid milk and churned fat/foam samples were taken. The SLFA content of the liquid milk, foam and churned fat was determined where possible, but only the PSD of the liquid milk samples was analysed since analysis of the churned fat proved very difficult as the samples would not disperse in the water used in the presentation unit of the Mastersizer E. In addition, the mass of the foam and churned fat phase was measured using a balance.

Table 3.7: Description of experimental settings to assess the effect of agitation temperature on NMFGM damage using the agitation rig (runs C85-90, C97-102). All samples were agitated at 2000 rpm for 4 minutes. Samples in runs C85-90 were taken in duplicate, those in runs C97-102 only once.

Run # (PSZ & SL1 tests)	Run # (PSZ & SL2 tests)	Temperature of agitation (°C)
C85*	C100*	5
C86	C101*	15
C87	C99*	45
C88	C97*	10
C89*	C98*	20
C90	C102*	25

* Indicates that a churned fat/foam sample was taken in addition to a liquid sample.

Table 3.8: Description of experimental settings to assess the effect of agitation speed on NMFGM damage using the agitation rig (runs C91-96). All samples were agitated for 5 minutes at 25°C. Both liquid and churned fat/foam samples were measured in duplicate.

Run #	Agitation speed (rpm)
C91	300
C92*	1700
C93*	2000
C94	1000
C95	1300
C96	800

* Indicates that a churned fat/foam sample was taken in addition to a liquid sample.

In order to assess the impact of foam and churned fat, a mass balance of the fat for all the phases had to be determined. This required considerable extra effort. Therefore a

final run (C107) was carried out specifically to analyse the typical amount of fat in each phase. The operating conditions were identical to those used in runs C97-99, previously described. The results can be found in Section 3 of Chapter 4.

3.4.3   Large-volume pumping rig

Another rig was built to assess the damage created by pumps in milk handling. It was much more convenient to test a number of related factors such as splashing and air inclusion in a pumping rig than in the agitation rig. Variations of this rig were made specifically to test the effect of a number of key components of pipeline systems. These are described separately in Sections 3.4.4 to 3.4.6. The large-volume pumping rig is shown schematically in Figure 3.9.

The large-volume pumping rig consisted of a 500 litre baffled stainless steel tank connected to a 2800 rpm, 1.1 kW, Fristam type FP712KF centrifugal pump. The pump was connected to a variable speed drive and could be run at a wide range of flow rates from 1 to 3 m^3/hr. The piping network consisted of stainless steel piping. A 1 inch diameter outlet connected the bottom of the tank to the inlet of the pump. The outlet of the pump was fitted with ¾ inch piping to take the milk to the top of the tank or connect this rig with other parts of the Massey University milk processing pilot plant (described in Section 3.4.7) if required. There were separate lines to the CIP system and to the drain, as shown in Figure 3.9.

The end of the ¾ inch exit pipe was situated 1 metre above the liquid level of milk in the tank. If splashing was not required a plastic hose was attached to the ¾ inch pipe outlet to lead the milk to the bottom of the tank. Air was added to the system using a compressed air line connected to a T-junction placed 130 mm from the inlet of the pump (as shown in Figure 3.10). Care was taken to ensure the airflow did not interfere significantly with the flow rate generated by the pump. This was done by ensuring the air line was placed perpendicular to the direction of flow and that the airflow was not too large. The airflow rate was set at 0.5 L/min for all experiments and was measured by a rotameter (Aaalborg instruments, 65mm scale, tube number 0365ST). The air line was controlled by a needle valve. The large-volume pumping rig was set up by using a pump

and tank already present in the milk processing pilot plant (shown schematically in Figure 3.15 of Section 3.4.7), for convenience.
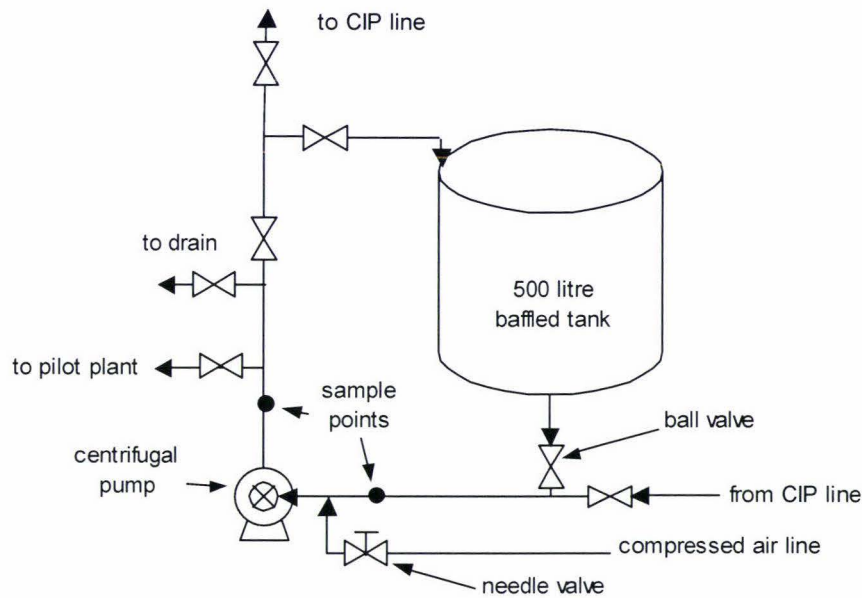
Figure 3.9: Schematic diagram of large-volume pumping rig used to damage milk by using a combination of pumping speed, splashing from 1 metre above the liquid level in the tank or air inclusion through a compressed air line.

One major advantage of the large-volume pumping rig was that it was connected to a PLC (programmable logic controller). Temperatures, flow rates and pressures of process lines could be controlled from a central computer that was also connected to a data logger.
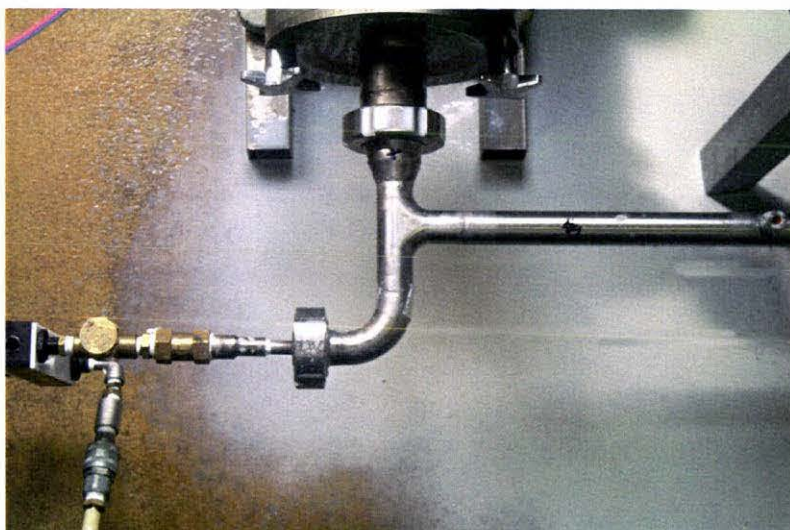
Figure 3.10: Photo of system used to add air via a compressed air line to the large-volume pumping rig. A rotameter (far left) was used to measure the flow rate of air and a needle valve was used to isolate the air line when air inclusion was not required. The direction of flow is toward the top of the picture.

Two sample points each consisting of a rubber septum restrained by a small nut (approximately 1 cm in diameter) were situated before and after the pump. An example of a sampling point is shown in Figure 3.11. Samples were taken with a disposable sterile 30 mL syringe with a 0.9 mm diameter by 25 mm long needle.

Figure 3.11: Sampling point design used in large-volume pumping rig and throughout the Massey University milk processing pilot plant.

Cleaning was carried out in a similar way to the agitation rig, except cycles were 10 minutes in duration. CIP solutions were re-circulated around the entire rig using the centrifugal pump.

Pump Speed, Air Inclusion and Splashing Experiments

The large-volume pumping rig was used to test the effect of pump speed, air inclusion and splashing on NMFGM damage using measurements from the PSZ test and, very late in this work, the SL2 test. For each run, 50 litres of milk initially at 4°C was transferred to the rig from the milk processing pilot plant's milk vat. The details are shown in Table 3.9. Milk was re-circulated around the large-volume pumping rig for 20 minutes. Samples were taken by the method described previously at intervals of 5, 12 and 20 minutes into the run. At the beginning of each run the speed of the pump was set by altering the current to the pump to give frequencies of 35 and 50 Hz (pump speeds of 1960 and 2800 rpm respectively). Eight runs (C63-C70) were carried out.

Table 3.9: Settings for re-circulation experiment to test the effect of combinations of pump speed, air inclusion and splashing on NMFGM damage, using the large-volume pumping rig.

Run #	Pump speed (rpm)	Splashing?	Added air?
C63	1960	no	yes
C64	2800	no	yes
C65	2800	yes	yes
C66	1960	yes	yes
C67	1960	yes	no
C68	2800	yes	no
C69	1960	no	no
C70	2800	no	no

The development of the selective lipolysis test made it possible to investigate the effect of pumping, air inclusion and splashing on NMFGM damage using this test. However, time constraints meant only one experiment was possible. An experiment was designed to study the impact of pumping temperature as well as air inclusion on NMFGM damage in milk passed through the large-volume pumping rig. Conditions from runs

C64 and C70 (Table 3.9) were replicated at 10°C and 20°C. To achieve this, the large-volume pumping rig was connected to an Alfa Laval M3 FGC plate heat exchanger so that the milk temperature could be controlled. The conditions of this experiment (runs C103-106) are shown in Table 3.10.

The liquid milk was analysed using the PSZ and SL2 tests. In addition the foam and churned fat was recovered from the rig at the end of the run, weighed and analysed for SLFA.

Table 3.10: Settings for re-circulation experiment to test the effect of pumping temperature and air inclusion on NMFGM damage, using the large-volume pumping rig.

Run #	Milk Temperature (°C)	Air Inclusion ?
C103	10	no
C104	20	yes
C105	20	no
C106	10	yes

Replicate runs using the PSZ test were carried out to test the repeatability of the large-volume pumping rig. To ensure that only the operation of the rig was a variable, the milk for the two runs was taken from one single batch and the experiments carried out on the same day. In that way the composition and quality of the milk was kept reasonably constant. The conditions of the experiment were replicate settings from run C68 (Table 3.9). Samples were taken after re-circulation time intervals of 0, 10, 15, 20 and 25 minutes. The runs (T26-27) are described in Table 3.11 and results reported in Section 1.1 of Chapter 5.

Table 3.11: Conditions for a set of two runs designed to test the repeatability of the large-volume pumping rig using Farmhouse milk.

Run #	Pump Speed	Splashing	Time of Sampling (minutes)
T26-27	2800	Yes	0, 10, 15, 20, 25

A number of runs were performed during the commissioning of this pumping rig to assess the volumes of milk and the circulating times required to create measurable damage. Because of their exploratory nature they are not reported.

The large-volume pumping rig was also used for combined analysis of samples by the SL1, PSZ and FF tests (runs T28-30). Runs T28-30 are described separately in Section 3.3.

3.4.4   Contribution of the pump to NMFGM damage

In the following three sections variations on the basic design of the large-volume pumping rig are described that were used to allow the analysis of specific issues encountered during handling of milk in a milk processing plant.

The piping in the large-volume pumping rig consisted of a number of elements and the damage created could have come from any and probably all of the components in that rig. Work was done to separate the effects of the different components.

The results of operation of the large-volume pumping rig, as reported in Chapter 5, indicated that all the mechanical factors investigated contributed in some sense to damage of the NMFGM. However, upon further analysis it was suspected that each component of the rig, such as the pump, the valves and the piping had intrinsically contributed to that value of NMFGM damage, and that the contributions could not be separated.

A single-pass pumping rig was constructed by a postgraduate student, Mr Jun Ji, under the supervision of this PhD project. This pumping rig consisted of a 1.1 kW, 1450 rpm, Anema-Sneek 3843/130 CCRM (Holland) centrifugal pump connecting two stainless steel tanks through 1 inch diameter piping. The system included no valves to control flow rate and the piping diameter was selected to be large enough to have a low shear rate. The rig was set up as shown in Figure 3.12. Ten litres of milk at approximately 10°C was pumped from one tank to another. The polarity of the pump was reversed and the milk pumped back, and so on. In that way samples could be taken after each individual pass through the pump. Preliminary trials (run J01, shown in Section 1 of

Chapter 4) showed that NMFGM damage after five passes allowed reliable measurement within the accuracy of SL1 and PSZ tests. On and off valves were selected and left in the open position. Their contribution to damage was assumed to be minimal since no cavitation was possible within them and they were of a simple design without any narrow channels.

Samples were taken from the tanks using a disposable syringe.

An experimental run (J02) was conducted in this rig using five different pumping speeds and the flow rate measured for each motor speed setting.
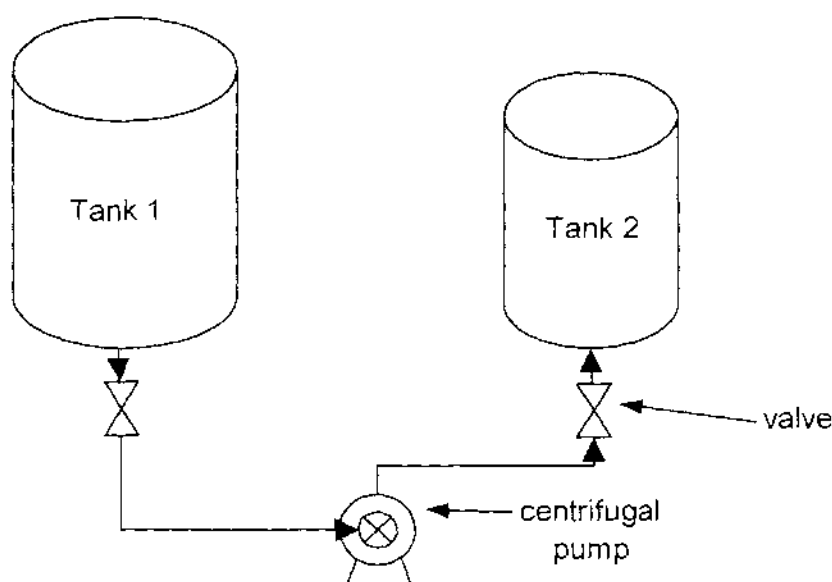
Figure 3.12: Schematic of single-pass pumping rig used to measure the effect of pumping speed on NMFGM damage.

Speeds of 725, 870, 1015, 1160 and 1305 rpm were selected giving flow rates of 325, 1070, 1870, 2540 and 3460 L/hr. The results are presented in Section 1 of Chapter 5.

Another set of experiments was conducted to assess reproducibility of single-pass pumping rig results (runs J03-06). Exact replicate runs were carried out four times at pump speeds of 870 rpm and 1305 rpm through the single-pass pumping rig. In addition, there were duplicate titrations on single samples. The 95% confidence interval of the average SLFA value for the four runs at each speed was calculated, and used as a measure of uncertainty of the data obtainable with the pumping rig. The details of this

experiment are given in Table 3.12. A full analysis of the results, and the error analysis, are given in Section 1 of Chapter 5.

Table 3.12: Settings of runs J03-06 designed to test the reproducibility of the SL1 test using a pumping rig. Farmhouse milk was passed from one tank to another 5 times via an Anema-Sneek centrifugal pump. The same milk was used for the run at a second speed. Samples were measured in triplicate.

Pump Speed (rpm)	No. passes through pump before sample taken	Total passes through pump for batch of milk
0	0	0
870	5	5
1305	5	10

The cleaning protocol for this rig was the same as that described for the large-volume pumping rig (Section 3.4.3).

3.4.5   Contribution of the pipeline to NMFGM damage

In addition to the pump, there was evidence that the pipeline itself might have been causing NMFGM damage. Hinrichs (1994) found that a pipeline alone could create significant NMFGM damage once a critical shear rate is exceeded. Hinrichs used a centrifugal FF test to measure NMFGM damage. In order to test how significant this effect might be using the measurement techniques developed in this work a pumping rig was required that could deliver different levels of pipeline shear rate. Such a rig (Figure 3.13) was already available at Massey University and used without modification.

The BT1000 as it is called by its designer (Trinh et al., 2002) consisted of a 5 litre jacketed baffled tank attached to a 0.37kW, 2650rpm, Dressler SLF2022105 monopump that re-circulated the test fluid through a ½ inch piping network. A special test section was incorporated in parallel to the main re-circulation piping to allow flow under different wall shear rates. The test section consisted of interchangeable capillaries of different internal diameters that could be easily put in place through tri-clover attachments. The diameters of the capillaries were much smaller than the pipe diameter in the rest of the BT1000 rig to ensure that the test section gave the highest shear rate in

the circuit. A number of connections were available to send the milk to a plate heat exchanger or to drain as shown in Figure 3.13.
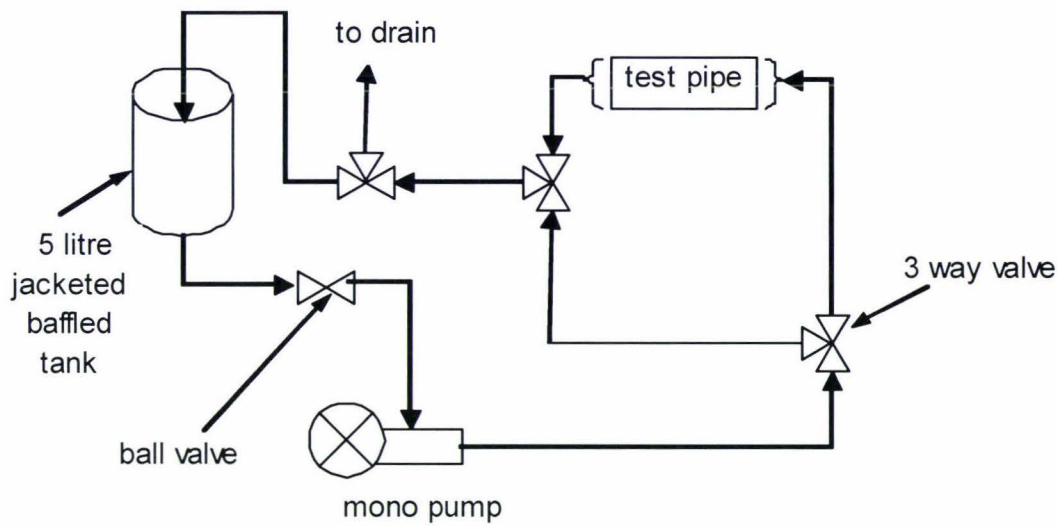
Figure 3.13: Schematic of BT1000 rig used to damage the NMFGM by shear at a pipe wall. A mono pump was used to re-circulate the milk around the rig.

The pump was operated in all runs at 2650 rpm giving a constant flow rate of 80 L/hr. The shear rate in the test section was varied between 550 s^{-1} and 41,400 s^{-1} by the use of five 50 cm long stainless steel capillaries of internal diameters ranging from 7.42 mm to 1.76 mm in diameter. Three litres of Farmhouse milk was re-circulated for 23 minutes in each run. Samples were analysed in triplicate. The details of these runs are summarised in Table 3.13.

Table 3.13: Experimental runs used with BT1000 rig. The mono pump was run at 2650 rpm giving a flow rate of 80 L/hr. Farmhouse milk was re-circulated at 10°C around the rig for a constant time period (runs J08-12). Samples were analysed using the SL1 test (in triplicate) and the PSZ test (unreplicated).

Run #	Pipe internal diameter (mm)	Velocity through test section (m/s)	Shear rate (s^{-1})
J08	7.42	0.51	550
J09	6.00	0.79	1040
J10	3.62	2.16	4700
J11	2.82	3.56	10,100
J12	1.76	9.13	41,400

Another set of experiments was conducted to assess reproducibility of BT1000 pumping rig results (runs J13-20). Four replicates each of run J09 and run J12 were carried out through the BT1000 rig (Table 3.13). In addition, there were triplicate titrations on single samples. The 95% confidence interval of the average SLFA value from the four runs at each shear rate was calculated, and used as a measure of uncertainty of the data obtainable from the pumping rig. A full analysis of the results, and the error analysis, is given in Section 1 of Chapter 5.

Sampling and cleaning were carried out in a manner similar to that for the large-volume pumping rig described in Section 3.4.3.

3.4.6   Contribution of valves to NMFGM damage

Valves are known to cavitate under extreme conditions of flow (Instrument Society of America, 1995). Fang (1998) reported that in his pumping rig, damage to the NMFGM in each pass through the pump was achieved by cavitation but there was no measurement to identify whether cavitation occurred mainly at the pump or at the valve. A small-volume re-circulation pumping rig was set up to analyse the contribution of the valve to NMFGM damage. Another postgraduate diploma student, Mr Preyas Anantpure, performed this work, again under the supervision of this PhD project.

The small-volume pumping rig, shown in Figure 3.14, consisted of three major components:

1. A 2800 rpm, 1.1 kW, Fristam type FP712KF centrifugal pump connected to a 90 litre stainless steel tank in a ¾ inch piping re-circulation configuration. An Endress+Hauser Promag-F electromagnetic flow meter was placed after the pump. Flow rate measurements could be recorded onto a computer using the Massey University milk processing pilot plant data logger.

2. Each valve had two analogue pressure sensors (-100 to 150 kPa) installed, one before and one after the test valve along a 50 cm length of straight stainless steel piping to provide data for the calculation of a valve cavitation index. Each valve had its own 50 cm length of piping to allow for variations in the size of each valve. The placement of the pressure sensors and the calculation of cavitation

were carried out according to standards issued by the Instrument Society of America (1995).

3. A cooling system to keep the milk at a constant temperature. An Alfa Laval M3 FGC plate heat exchanger was connected to a tank of water at approximately 5°C and water re-circulated using an Anema-Sneek, 1.1kW centrifugal pump.
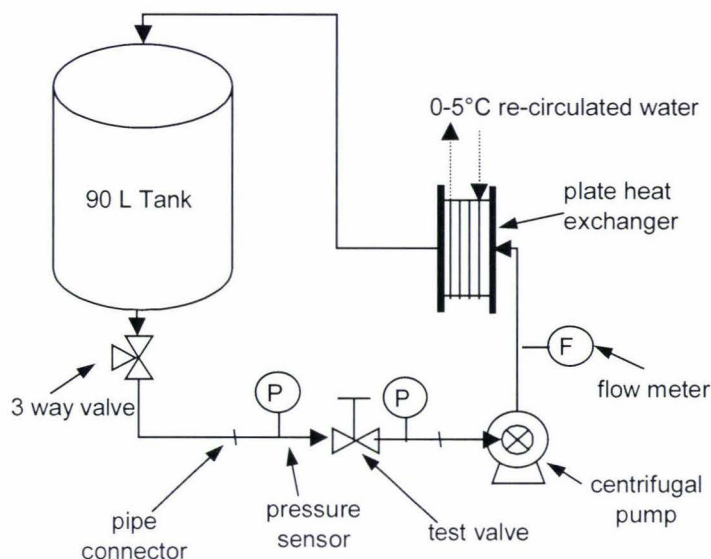
Figure 3.14: Schematic of small-volume re-circulation rig used to test the effect of valve cavitation on NMFGM damage.

In this work three different valves were tested: a 1 inch globe valve, 1 inch gate valve and a ½ inch needle valve. Each valve, in turn, was placed at the appropriate distance before the pump. This was done because it made valve cavitation more likely than if the valve was placed downstream of the pump. A number of runs were conducted using water instead of milk to establish the conditions required for cavitation. The speed of the pump and the percentage valve opening were changed in order to give varying conditions of cavitation as shown in Section 1 of Chapter 5. Run details are summarised in Table 3.14.

To test the effect of a cavitating valve on NMFGM damage, a second experiment was carried out on Farmhouse milk by running the milk first through a pump under extreme cavitation conditions or under non-cavitating conditions. The run details are summarised in Table 3.15.

Cleaning and sampling were carried out the same as for the large-volume pumping rig (Section 3.4.3).

Table 3.14: Water runs used to monitor the variables associated with cavitation in valves with the small-volume pumping rig.

Valve type	Valve Position	Pump Speed (rpm)	Cavitation Expected?
Needle	10% open	1960	no
	50% open	1120	yes
Globe	5% open	1960	no
	5% open	1120	yes
Gate	4% open	1960	no
	5% open	1120	yes

Table 3.15: Settings of runs P01-04 on designed to test the effect of cavitation in valves on Farmhouse milk used with the small-volume pumping rig.

Run #	Valve type	Valve Position	Pump speed (rpm)	Cavitation Expected?
P01	Needle	100% open	1120	no
P02	Globe	100% open	1120	no
P03	Needle	40% open	2800	yes
P04	Globe	40% open	2800	yes

3.4.7 Contribution of direct and indirect heaters to NMFGM damage

Milk powder manufacture involves three major processes: fluid handling (which includes homogenisation and separation besides just pumping), water removal (in evaporators and spray dryers, and thermal processing (to condition the microbial and functional properties of the product). Thermal processing is carried out in direct and indirect heaters. Indirect heaters include plate heat exchangers (PHEs) and shell and tubular heat exchangers. Direct heat exchangers include direct steam injection (DSI) units and direct contact heaters.

During preliminary surveys of NMFGM damage at the Waitoa dairy factory of the then New Zealand Dairy Group Co-operative Limited it was observed that the measurement of NMFGM damage in heaters was complicated by the effect of thermal processes on the measurement of FFAs. The LFF test of Fang (1998) applied at that time showed unusual results. Besides promoting the complete redesign of testing of the measurement of NMFGM damage described in the previous sections of this chapter (Section 3.1) it showed the need to carry out a set of experiments specially designed for the analysis of NMFGM damage through thermal processing equipment.

Four sets of experiments were conducted using the preheating section of the Massey University milk processing pilot plant. Incoming Farmhouse milk was kept in an 850 litre milk vat agitated with a propeller agitator (NDA Engineering Limited) and chilled by a 1.1kW refrigeration unit (NDA Engineering Limited). The milk was then pumped through a centrifugal pump (2760 rpm, 0.37kW, CEG type CDX/A70/05), through a plate heat exchanger (Alfa Laval, type U-265-R) and a first DSI unit before it entered a special fouling and thermophile contamination rig specifically designed for research at Massey University. The expansion of this heating section beyond the configuration normally found in industry allowed better control of temperature at various stages of the fluid flow. In order to confine the growth of thermophiles to the special test section which included a battery of tubular heat exchangers operated at between 50°C and 60°C the PHE was operated below 40°C to prevent thermophile contamination in the early part of the plant and the first DSI unit allowed an almost instantaneous jump to the required 50-60°C range. After the fouling and microbial section the milk continued to a second DSI unit used for preheating that brought the milk to temperatures as high as 100°C. The design of this plant was not made specifically for this project but simultaneously for six PhD projects, hence its flexible and complicated configuration. The microbial and fouling rig could be bypassed when required but it was not worth making large reconfigurations every time a run was made for this project since the thermal processing work in this project involved the PHE and both DSI units, as shown in Figure 3.15. The PHE was supplied with hot water, created by mixing steam and cold water, which flowed counter-currently to the milk.
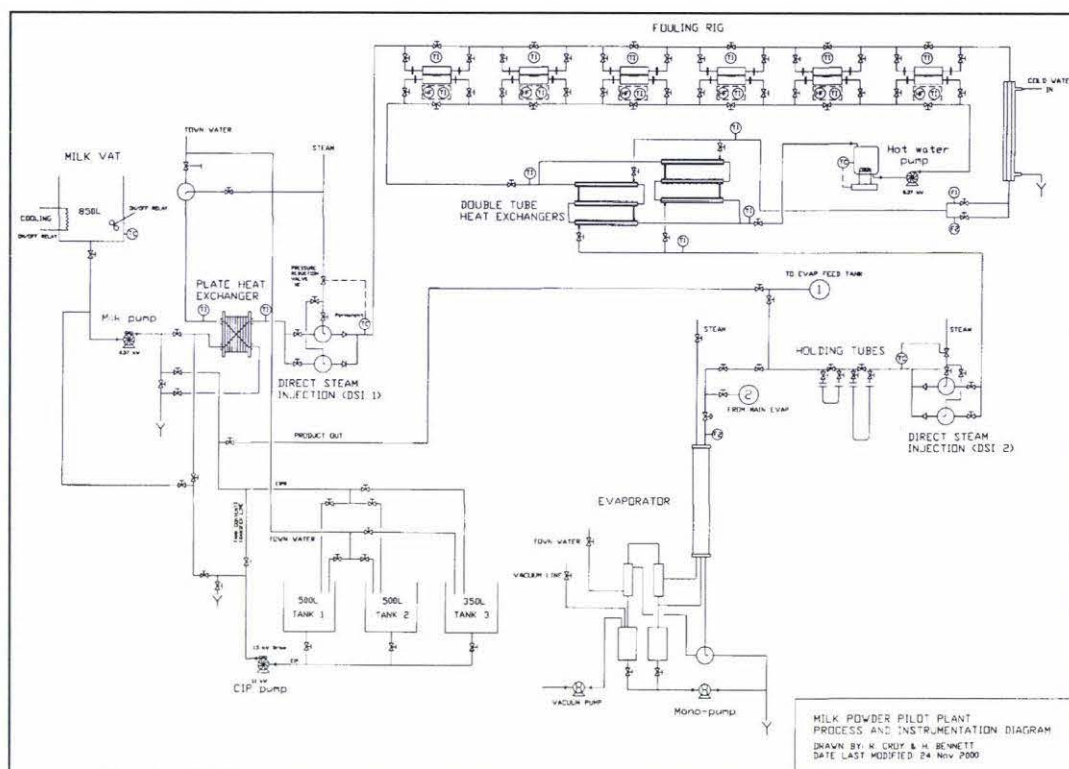
Figure 3.15: The Massey University milk processing pilot plant. (Bennett, 2000; Croy, 2000).

Steam was fed through each DSI unit through control valves. All variables (flow rate and temperatures) were computer controlled through a PLC (Allen Bradley, CPU model SLC 5/03) and the variables recorded through a data acquisition programme (Intellution FIX DMACS 7.0). The temperatures were monitored using copper-constantan thermocouples or stainless steel resistance temperature detectors (RTDs). Pressures on the milk and steam sides were measured with inline sensors (Data Instruments, XPRO 25 or 50 psig) and flow rates were measured with an Endress-Hauser Picomag 11 PM16533 electromagnetic flow meter.

The two DSI units were custom-made at Massey University and each consisted of 35 mm diameter stainless steel casing with a Teflon insert. A venturi was machined inside the plastic insert to create a 5 mm diameter channel. Three 0.75 mm diameter holes were drilled at the venturi at an angle of 30° to lead from the steam side to the milk side. Steam entered from a 12.7 mm tee section into the outer chamber of the Teflon insert. A drawing of the design is given in Appendix B5.

Sampling and cleaning were carried out in a similar manner to the large-volume pumping rig (Section 3.4.3). In some cases it was necessary to extract air entrapped in

the system first using a separate syringe before any samples were taken. Samples were immediately placed in a 4°C chiller before being tested.

Milk flow rate for the first two sets of experimental runs (C07-11 and C21-38) was kept at 45 L/hr. In the third experiment the milk flow rate was 30 L/hr to allow temperatures of up to 85°C to be reached using the restricted steam available. 45 L/hr was attempted but it was found the amount of steam injected was not sufficient to bring the milk to the highest required temperatures. In all cases the milk in the vat was sampled to make a basis for comparison between experiments.

In the first set of experimental runs (C07-11) the effect of thermal processing on the LFF test of Fang (1998) previously observed at the Waitoa milk powder plant were examined more carefully and systematically under controlled conditions. Analysis of particle size distributions (PSDs) of milk fat globules was also made. In this set of five runs, milk was heated from 4°C to a number of temperatures ranging from 16 to 95°C as listed in Table 3.16. The temperature of the milk at the three heaters (plate heat exchanger and two DSI units) is also shown. In this set of runs samples were analysed using both the LFF and PSZ tests.

The results of this first set of experiments, discussed in detail in Section 2 of Chapter 5, showed that the temperature to which the milk was heated had a dramatic and consistent effect on results as measured with the LFF test but these were independent of the heater used. However, the effect on PSD of fat globules was completely different for the three heaters. Thus clearly the effects of thermal processing equipment with respect to chemical and physical damage to the NMFGM are distinct and follow different patterns and must be studied separately.

Table 3.16: Heat treatments given to whole milk for runs C07-11.

Run #	PHE outlet temperature (°C)	DSI 1 outlet temperature (°C)	DSI 2 outlet temperature (°C)
C07	40	60	75
C08	50	75	95
C09	55	80	95
C10	16	38	55
C11	39	56	75

Because the PSD of samples taken from DSI 1 and DSI 2 (which were identical in design and manufacture) were very different in the first set of experiments, a second set of experiments (C21-38) was performed (Table 3.17). It was hypothesised that the different amounts of disruption to the fat globules created must have been due to the pressure differential between the steam and the milk flow. DSI 1 was located earlier in the plant and at a lower physical level (approximately two metres below DSI 2). To avoid any interaction between the two DSI units in this set of experiments nine runs were operated with DSI 1 operating only and another nine runs were operated with DSI 2 only. The temperatures exiting the DSI units were also dropped to target a range from only 15°C to 55°C. Thus the experiment was designed to avoid complicating factors due to heating the milk above critical temperatures and target only the physical effect of injecting high speed steam into milk. The results of this set of experiments can be found in Section 2 of Chapter 5.

Table 3.17: Heat treatments given to whole milk for runs C21-38.

Run #	PHE outlet temperature (°C)	DSI 1 outlet temperature (°C)		Run #	PHE outlet temperature (°C)	DSI 2 outlet temperature (°C)
C21	10	15		C30	9	14
C22	10	25		C31	9	24
C23	10	35		C32	10	35
C24	30	55		C33	30	55
C25	30	45		C34	30	45
C26	30	35		C35	30	35
C27	20	25		C36	20	26
C28	20	35		C37	20	32
C29	20	45		C38	20	45

A third experiment consisting of ten runs (C75-84) was conducted once the SL1 test was developed, since it was much more reliable than the LFF test for measuring the chemical consequences of NMFGM damage. In this experiment only the PHE and DSI 1 were used, sometimes running co-currently, sometimes separately, as shown in Table 3.18. The purpose of the test was to gain a combined picture of chemical and physical consequences of NMFGM damage. Samples were analysed with the PSZ test and selective lipolysis test. Results of the experiment are given in Section 2 of Chapter 5.

Table 3.18: Heat treatments given to whole milk for runs C75-84.

Run #	Trial Day	PHE or DSI tested?	PHE outlet temperature (°C)	DSI 1 outlet temperature (°C)
C75	1	PHE	65	NA
C76	1	DSI	20	40
C77	1	PHE	85	NA
C78	1	DSI	55	75
C79	1	PHE	40	NA
C80	2	PHE	80	NA
C81	2	DSI	65	85
C82	2	DSI	45	65
C83	2	DSI	60	80
C84	2	PHE	75	NA

3.4.8   Contribution of evaporation to NMFGM damage

Water removal processes in the milk powder manufacturing process include both evaporation and spray drying. The Massey University milk processing pilot plant also contained a 3-effect falling film evaporator (NDA Engineering, New Zealand) shown schematically in Figure 3.16. Each effect had a single 2.9 metre long evaporation tube of 38 mm in diameter, in effects 1 and 2, and 25 mm in diameter, in effect 3. Milk was recycled to give adequate coverage of the tube walls. The re-circulation flow rates were 250 l/hr in the first two effects and 150 l/hr in effect 3. A proportion of the recycled flow in each effect was continuously bled off into the next effect at the flow rate required to maintain a constant hold-up of 8 litres in each effect. At bleed flow rates of 40 L/hr this gave an equivalent height of 18 to 25 m for each tube, similar to an industrial evaporator. Steam entered the shell side of the first effect with each subsequent stage heated by the vapour from the previous effect.

The evaporator was connected to the PLC of the pilot plant so that temperatures, liquid levels and flow rates could be controlled and recorded on computer.
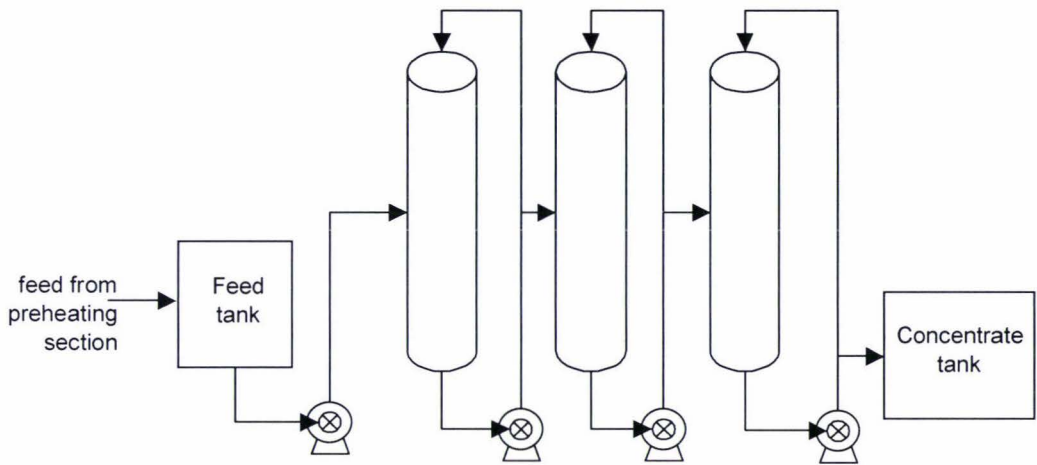
Figure 3.16: Schematic of Massey University 3-effect falling film evaporator used in runs C71-73.

A set of three runs (C71-73) was carried out using the pilot plant evaporator to test how NMFGM damage in Farmhouse milk passed through it occurred. Milk was pumped at 30 L/hr from the milk vat (4°C) of the Massey University milk processing pilot plant through either the PHE or the PHE and DSI unit of the preheating section (75°C), shown in Figure 3.15 (described in Section 3.4.7). Milk then entered the feed tank of the evaporator and was then passed through each effect as described above.

Samples were taken at taps located after each evaporator pump. The conditions of each run are shown in Table 3.19. CIP of the evaporator was similar to that for the pilot plant (Section 3.4.7) but was longer in duration owing to the more viscous nature of deposits.

Table 3.19: Conditions of runs (C71-73) conducted at Massey University on a pilot plant falling film evaporator run at 30 L/hr with preheating at 75°C.

Run #	Preheating type	Tests
C71	PHE +DSI	SL1, PSZ
C72	PHE+DSI	PSZ
C73	PHE	SL1, PSZ

3.5   Industrial survey

Three surveys of NMFGM damage in milk powder manufacturing plants were made, each over a period of six months. The first two surveys at Waitoa undertaken in the months of November 1999 to April 2000 and February 2001 to April 2001 were a good

introduction to the operation and organisation of milk powder manufacturing plants but the measurement techniques available at that time were not reliable enough to give usable results. Further, PSD analysis equipment was unavailable. The major outcome of these surveys was in fact the identification of drawbacks in techniques for monitoring NMFGM damage available both from previous work at Massey University and from the literature. These observations spurred a number of trials as described in Sections 3.1 and 3.2 above and allowed the author to revise existing literature in a new light. The third survey was performed at the Pahiatua site of the Fonterra Co-operative Group because of convenience of travel and also because there were two separate powder plants there.

The survey techniques reported here are based on the third industrial survey, made in 2003 (runs S10-S12).

The Pahiatua site

All milk powder manufacturing plants in New Zealand follow the generic flow diagram shown in Figure 3.6. The main differences exist in the configuration of the preheater, evaporator, concentrate heater, and the spray drier. The two plants at Pahiatua, called Powder 1 and Powder 2, were fitted with two different types of evaporator. The evaporator in Powder 1 was of a GEA Wiegand (Germany) manufacture and based on a 5-effect thermal vapour recompression (TVR) evaporator design. This is shown diagrammatically in Figure 3.17. The evaporator in Powder 2 was of a 2-effect mechanical vapour recompression (MVR) evaporator design. This is shown diagrammatically in Figure 3.18.

A number of rubber septa sampling points were situated in-line as shown in Figure 3.17 and 3.18. Essentially points beyond each effect and along strategic positions in the milk powder plant. The descriptions of the sampling points for each evaporator and processing plant are listed in Table 3.20.

Sample Handling

Because these were commercial plants the composition and process specification of milk powders were determined by a production schedule outside the control of the author. In addition, the plants could alternately make skim or whole milk powder and for comparison between the two plants only whole milk runs were useful in this work

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because of the very low fat content of skim milk powders. Thus it was necessary to work intermittently at the plant. Within each particular run the number of samples to be taken as shown in Table 3.20 was fairly large but duplicate samples were taken when possible.

Since the analytical facilities were located at several locations at Massey University, an hour away from the plant, the issue of sample handling became important. In particular it is well known that concentrated solutions of whole milk (especially above 40% total solids) tend to thicken with holding time at high temperature, giving a threat of inaccurate measurement of in-line PSD of fat globules from measurements performed at the end of the day on samples taken back to Massey University. Thus it was necessary to dilute samples at higher than 40% total solids with known proportions of sterilised distilled water to obtain a dilution of approximately 100%. Samples collected were immediately stored in a "chilli bin" (a storage box made of polystyrene) filled with ice.

All equipment used in the SL1 test including sampling bottles and syringes was cleaned and sterilised the day before a survey trip. SL1 test equipment was described in Section 3.1.3. Sampling equipment taken for the day consisted of:

- Disposable syringes.
- 500 ml distilled water.
- 250 mL sterilised glass bottles with glass stoppers.
- A chilli bin.

On the day of a survey, sampling started with departure from Massey University at 8 am. After a 1 hour trip, half an hour was used to set up the equipment on site in the plant. Sampling would be completed by 11 am with return to Massey at 12 pm. Samples used in the SL1 test would be transferred to Duran bottles by 1 pm. This left the rest of the afternoon to complete analysis of total fat and PSD for each sample.

An exploratory experiment was performed by running the SL1 test with concentrated samples taken from the line and the same sample after dilution with sterilised reverse-osmosis water to 12% total solids, the same as Farmhouse milk. The test revealed that the diethyl ether/petroleum ether mixture added to the incubated sample in the

Mojonnier tube could not be mixed effectively by the method described in Section 3.1.3, raising significant doubts about the results of the extraction process for FFAs. The author believes that the problems lie with the viscosity of the concentrated samples that make mixing by simple inversion ineffective.

Thus all samples taken during surveys were diluted to Farmhouse milk total solids content (12%). Because of standardisation of fat to solids non-fat the dilution process could be conveniently monitored by measuring the fat content of the final diluted samples since this measurement was also needed in the selective lipolysis test. The fat content of the samples was measured using the AACC 30-10 method, with the rotary evaporator replaced with a steam bath (Appendix A1).

Otherwise, the PSZ and SL1 tests were conducted on survey samples according to the protocols described in Sections 3.1.3 and 3.2.2 for the SL1 and PSZ tests, respectively. Three surveys (S10-12) were conducted at the Pahiatua site. The conditions of each survey are described in Table 3.21.
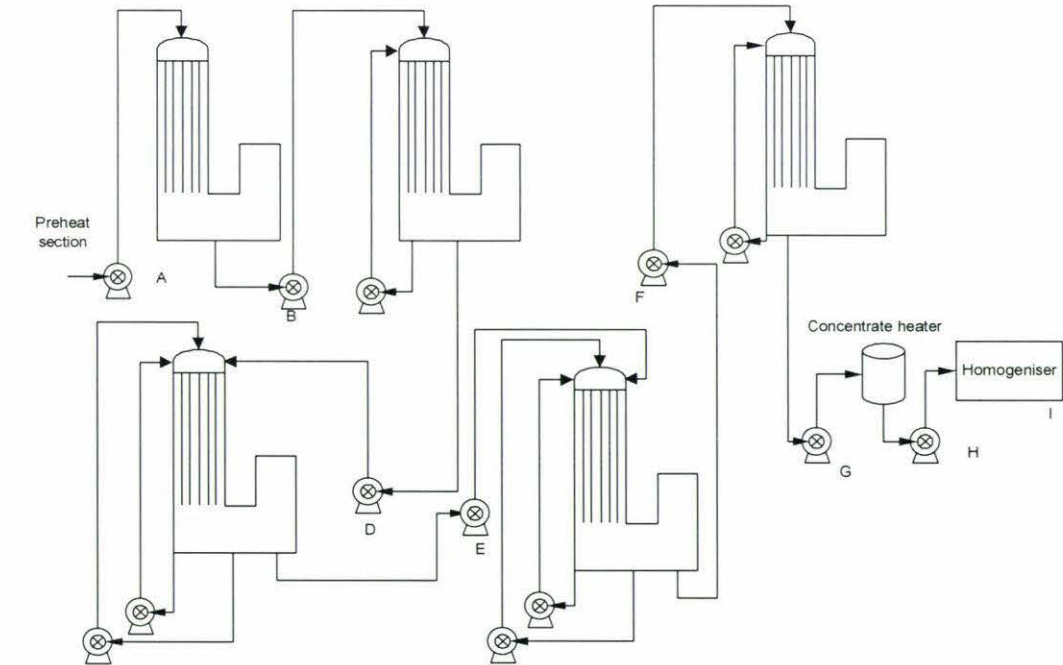
Figure 3.17: Schematic of evaporator in Powder 1 at the Pahiatua dairy factory, a 5-effect thermal vapour recompression evaporator. Each letter refers to a sample point used in this work (Table 3.21).
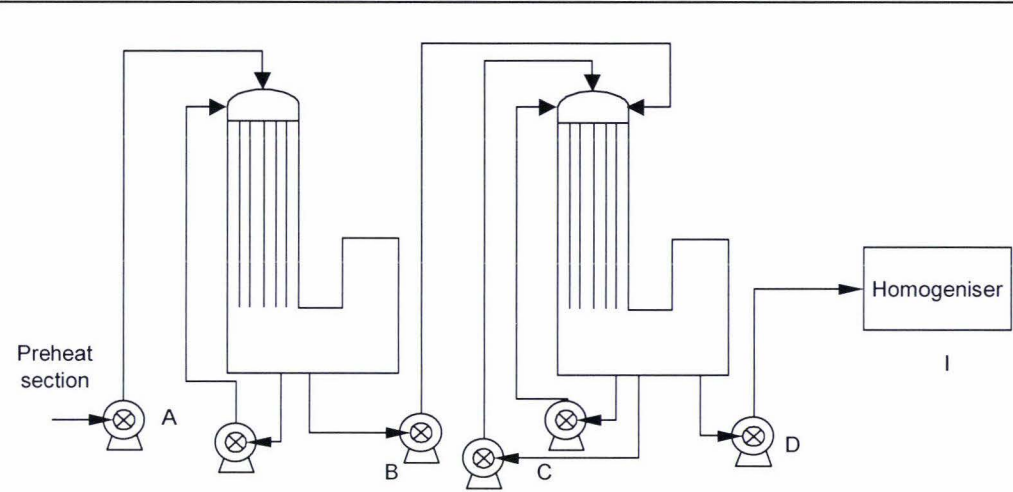
Figure 3.18: Schematic of evaporator in Powder 2 at the Pahiatua dairy factory, a 2-effect mechanical vapour recompression evaporator. Each letter refers to a sample point used in this work (Table 3.21).

Milk was sampled using the same procedure as in Section 3.4.7 at points around the plant where rubber septa were located. Milk from the factory tended to vary depending on the specifications, which are set from day to day.

Table 3.20: Physical locations of samples taken from the evaporators of Powder 1 and 2 at the Pahiatua dairy factory. Reference numbers are located on Figures 3.17 (Powder 1) and 3.18 (Powder 2). Sample points were always located after the pump.

Location	Pump Reference #	Powder 1*	Powder 2*
Evaporator balance tank		0	0
Indirect preheat outlet		3	7
Total preheat outlet	A	4	7
Effect 1 outlet	B	5	9
Effect 2	C	na	10
Effect 2 outlet	D	7	12
Effect 3 outlet	E	10	na
Effect 4 outlet	F	13	na
Effect 5 outlet	G	15	na
Concentrate Heater	H	16	na
Homogeniser	I	16	13

*Refers to the number of pumps the milk had passed through starting from the evaporator balance tank.

Table 3.21: Conditions within Powder 1 or Powder 2 for each survey conducted in this work. Sample points refer to Table 3.20.

Run #	Plant	Preheat temperature (°C)	Holding time (seconds)	Sample points used
S10	Powder 1	92	45	A, B, D, E, F, G, H
S11	Powder 1	98	15	A, B, E, G, I
S12	Powder 2	94	0	A, B, C, D, I

Chapter 4
Measurement of NMFGM Damage

This chapter reports the results of work to develop two new techniques of measurement, the selective lipolysis tests (SL1 and SL2) and the particle size zoning (PSZ) test that assess respectively a chemical and a physical consequence of NMFGM damage.

Section 4.1 describes the SL1 test with typical examples of applications, and an uncertainty analysis.

Section 4.2 describes the PSZ test, again with typical examples and an uncertainty analysis.

Section 4.3 discusses issues in sampling and sample handling.

Section 4.4 discusses improvements to the SL2 test, which was developed to obtain a more representative extraction of FFAs produced by incubation.

Finally, Section 4.5 shows how the SL1 and PSZ tests combine to give a more comprehensive picture of NMFGM damage than has been available with previous analyses.

4.1   Selective lipolysis test

4.1.1   Variables measured

The main result of the selective lipolysis test is the amount of selective lipolysis free fatty acids (SLFA) developed after incubation of a sample enriched with approximately 20 milligrams of pig pancreatic lipase (PPL) per litre, and incubated for 6 hours at 37°C. An example of the change in SLFA in 4% fat, pasteurised, unhomogenised (Farmhouse) milk with re-circulation time at 10°C in a pumping rig operating at 2800 rpm is shown in Figure 4.1.

The test itself does not measure directly the amount of damage created in the rig over time because the original milk already contains a certain amount of free fatty acids (FFAs) that can be measured by traditional FFA tests. In the present nomenclature (see Section 1 of Chapter 3) this is designated as original fatty acids (OFA).
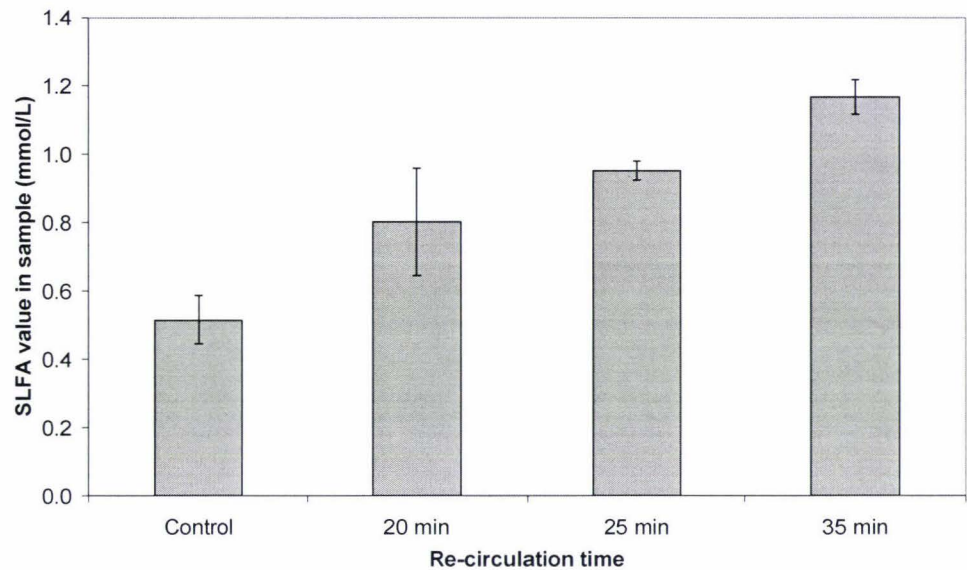
Figure 4.1: Example of typical FFA values obtained using the SL1 test. Run (T29) with commercially pasteurised, unhomogenised, standardised 4% fat (Farmhouse) milk re-circulated around a pumping rig with a 2800 rpm Fristam centrifugal pump. Error bars indicate the uncertainty in duplicate samples.

The addition of the pig pancreatic lipase (PPL) is essential. Normally the amount of lipolytic enzymes available in the sample is insufficient to convert all fat with damaged NMFGM (unprotected fat, UPF, in the nomenclature used here) to FFAs. A sample incubated without addition of exogenous enzymes for 6 hours at 37°C is called incubation fatty acids (IFA). Typical values of different FFAs after 0 minutes and 25 minutes of pumping are shown in Figure 4.2 to give an idea of the relative magnitudes of OFA, IFA and SLFA. Note that the OFA and IFA values of samples before and after pumping are very similar but the values of SLFA have changed significantly.
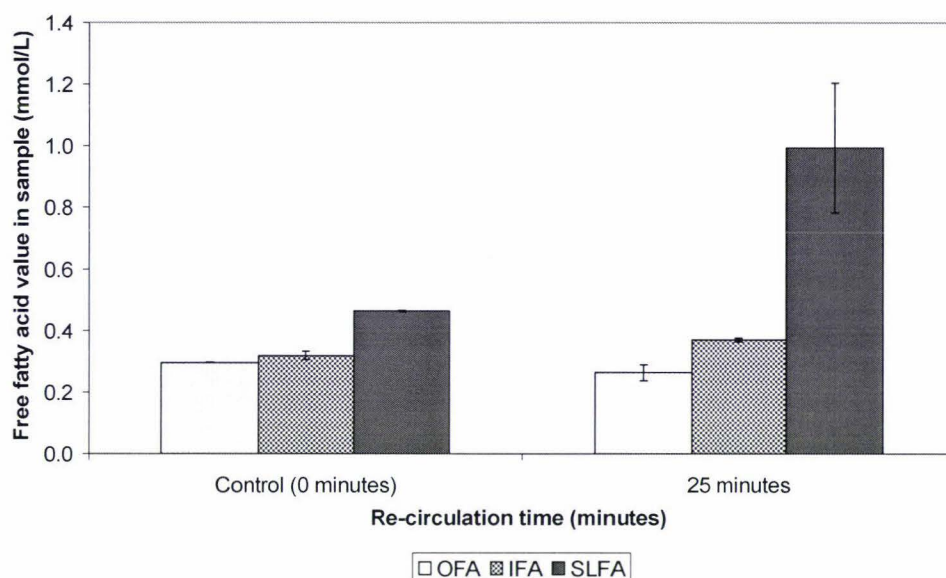
Figure 4.2: Example of typical original fatty acid (OFA), incubation fatty acid (IFA) and selective lipolysis fatty acid (SLFA) values. Run (T28) with the same experimental conditions as described in Figure 4.1.

4.1.2   Uncertainties of measurement

Most of the tests carried out in this work involved duplicate or triplicate samples and titrations. The uncertainties in the results of the SL1 test and other fatty acid tests (i.e. measures of OFA and IFA) can be attributed to several sources:

1.   Sampling technique
2.   Sample incubation
3.   Extraction of FFAs from the sample
4.   Titration of the extracted FFAs.

A summary of the average and median half-range uncertainties of SLFA, IFA and OFA based on a population of over 200 duplicate samples is shown in Figure 4.3. Also included in the figure is the average and median uncertainty of the lipolysable free fat (LFF) test of Fang (1998), which was an earlier version of the present SL1 test. The main difference between the two tests is that the samples in the LFF test are incubated over 24 hours at 30°C with 5 µg of PPL per mL of sample whereas the SL1 and SL2 tests are conducted over 6 hours at 37°C with a PPL concentration of 20 µg/mL.

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It is clear that the uncertainty of the OFA test is the lowest at 0.01 mmol of FFAs per litre of sample. The OFA test simply applies a slightly modified version of the test used by Fang (1998) as a measurement of FFAs in milk samples. The uncertainty in the IFA test was 0.03 mmol/L. Clearly the added uncertainty is related to the extra incubation step in the IFA test. One of the major variations in IFA duplicate samples results from the fact that the lipolytic enzymes present in the milk, either from endogenous or microbial sources, are likely to vary considerably between runs, both in types and quantities. The results in Figure 4.3 do not address these large variations but only reflect the uncertainties linked with the incubation and titration process for duplicate samples from the same batch of milk.

As discussed in Section 4.1.1, neither the IFA nor OFA values could reflect the full extent of NMFGM damage in the sample. The addition of PPL in the SL1 test has increased both the value of FFAs measured, and the median of the uncertainty to 0.08 mmol/L. One source of uncertainty can be attributed to difficulties in dosage of the PPL. Ideally the same amount of enzyme activity would be added to each sample. This requires that the exact activity of the PPL added is known. While the enzyme activity of commercial supplies was tested to ensure that they had not changed with storage time, the testing procedure itself added a certain amount of uncertainty to the results. In addition there will be uncertainties in measuring the quantities of both enzyme and milk samples.

The median uncertainty measured with the LFF test of Fang was 0.21 mmol/L, which is more than twice the uncertainty in the selective lipolysis tests and vindicates the improvements introduced by the present selective lipolysis tests. In Section 4.1.6 the reasons for this difference in uncertainties between the SL1 and LFF tests - which can be attributed largely to microbial contamination - will be discussed.
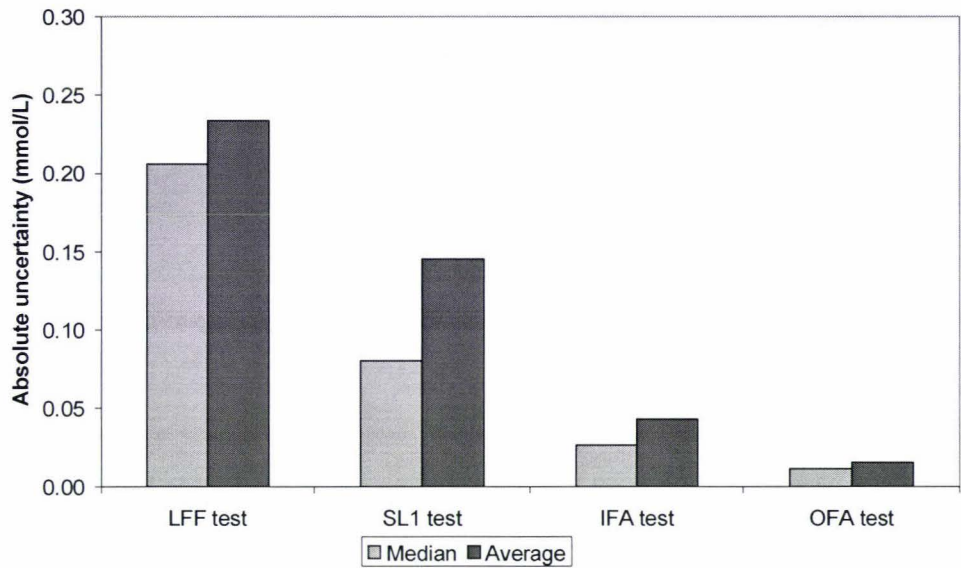
Figure 4.3: Reproducibility of FFA tests used in this work. Columns show the median and average absolute uncertainty in the measurement. Results were calculated from sample populations (from left to right) of 92 (LFF), 73 (SL1), 23 (IFA) and 18 (OFA) averages of duplicate samples.

A comparison of average uncertainties between the LFF and SL1 tests, also shown in Figure 4.3 indicates that the improved precision is only about 35%. This is because the distribution of uncertainties in the sample population is skewed as shown in Figure 4.4, which plots uncertainty in FFA values against percentile of the distribution. Clearly 50% of the tests made showed a two to one improvement in accuracy of the SL1 test over the LFF test but when very large or very small uncertainties are involved then the relative improvement is diminished or even reversed. It is possible that the large errors in the SL1 test were due to poor experience in earlier runs.
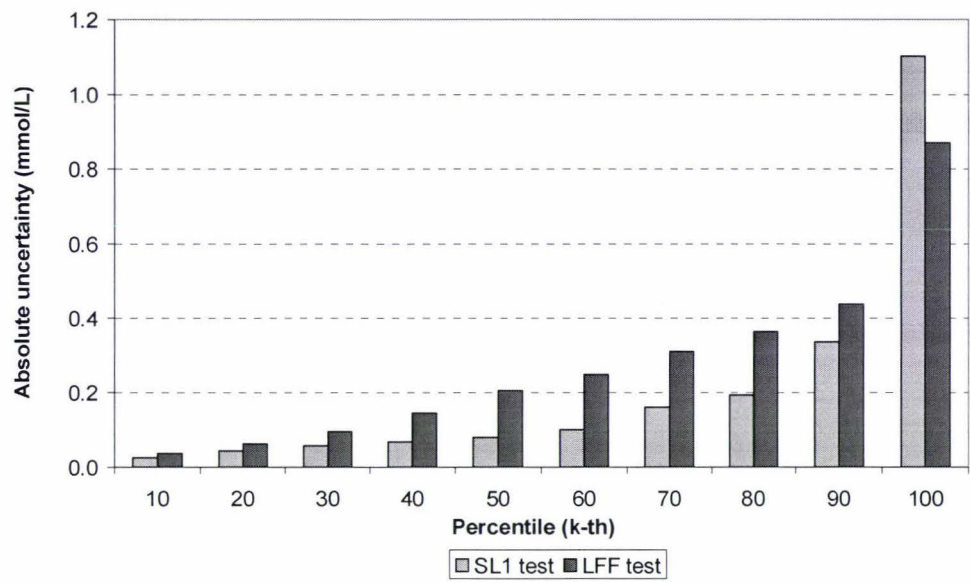
Figure 4.4: Reproducibility of the LFF and SL1 tests. Columns show the k-th percentile absolute uncertainty in the each measurement technique. Values were calculated from sample populations of 92 and 73 averages of duplicate samples, respectively.

The absolute uncertainty of these tests gives an idea of the sensitivity for the minimum level of NMFGM damage that can be detected.

A plot of percentage uncertainties of the tests is shown in Figure 4.5. Whilst the percentage uncertainty of the SL1 test is still larger than the percentage uncertainties of the IFA and OFA tests, the SL1 test compares much more favourably with these tests on this basis than it does on the basis of absolute uncertainty (Figure 4.3). This is because the amount of FFAs measured in the OFA and even the IFA tests are relatively small and require a much more sensitive test for detection. The percentage uncertainty of the LFF test is surprisingly small compared with the other tests but this result is deceptive. It does not indicate greater sensitivity of the measurement but simply reflects the fact that the amount of FFAs produced after 24 hours incubation is much larger than in the other tests. As noted in Section 4.1.6, the growth of microbes during the lengthy incubation of the LFF test appears to result in substantial damage to fat that was previously protected by the NMFGM.
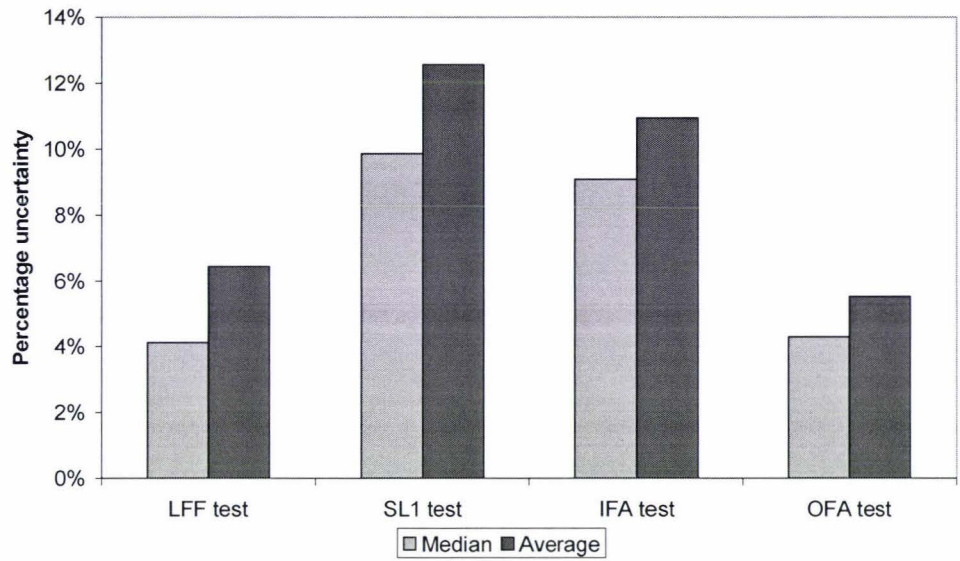
Figure 4.5: Reproducibility of FFA tests used in this work. Columns show the median and average percentage uncertainty in the measurement. Results were calculated from sample populations (from left to right) of 92 (LFF), 73 (SL1), 23 (IFA) and 18 (OFA) averages of duplicate samples.

The SL1 test was analysed in terms of titration replicates in addition to the sample replicate statistics presented above. The average half-range uncertainty from titration replicates in runs J03-06 and J13-20 was subtracted from the average uncertainty between replicate runs to give an assumed value for the uncertainty as a result of sampling and incubation. The comparison between the reproducibility of sampling and incubation, compared with the titration error in replicates is shown in Figure 4.6. The actual increase was 0.20 mmol/L above the titration uncertainty or about 67% of the total uncertainty. Most of this increased error would be due to variations in sampling and incubation rather than any other variations in the experimental operating conditions. Therefore, the sampling and incubation steps did contribute a significant proportion of the total uncertainty.
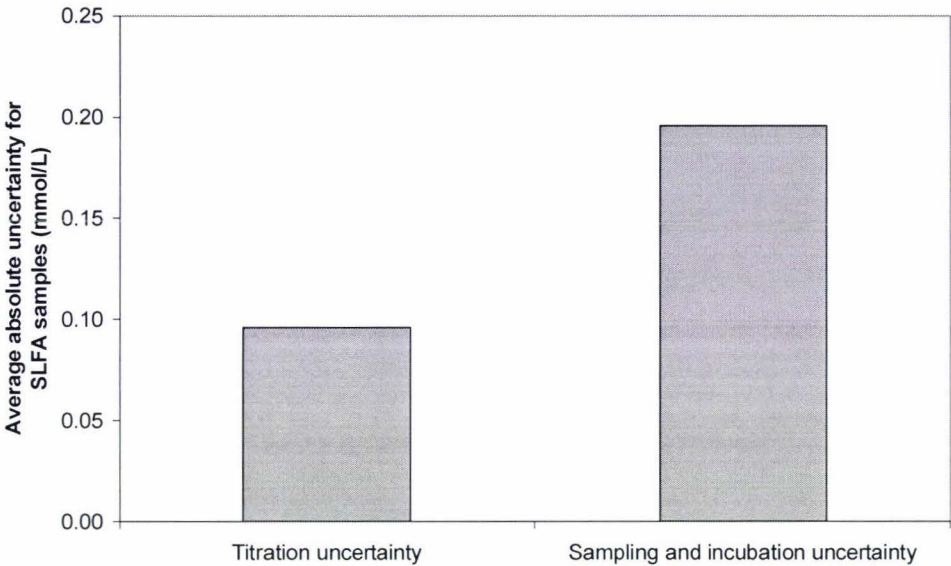
Figure 4.6: Comparison of average absolute uncertainties in measurement of SLFA in terms of sample and titration replicates. The "sampling and incubation uncertainty" was calculated by subtracting the uncertainty from sample replicates with the uncertainty from titration replicates. The number of duplicate samples used to calculate each average was 28, from runs J03-06 and J13-20.

4.1.3   SLFA as a measurement of NMFGM damage

Figure 4.7 gives typical values of IFA and OFA during a pumping experiment (runs T28-30). The variation in OFA during the experiment was 0.01 mmol/L at the 95% level of confidence, which compares well with the overall uncertainty reported in Figure 4.3. This indicates, first of all, that the amount of OFA in the batch of milk did not increase during the experiment (approximately 1 hour at 10°C). Whatever microbial or endogenous enzymes were present in the batch of milk were not sufficient within the range of time and temperature to create more FFAs.

This does not imply that substantial damage to the NMFGM was not created, as shown by the increase in SLFA over the course of the experiment. However, the amount of IFA measured also remained reasonably stable during the experiment. This is a clear indication that:

1.   Lipolysis by endogenous and microbial enzymes present in the sample did not change substantially during the experiment, and

2.   These enzymes could only convert a fixed proportion of the damaged fat into FFAs within a 6 hour incubation at 37°C. The average amount of IFA obtained

in this experiment was 0.37±0.10 mmol/L, substantially lower than the maximum amount of 1.33 mmol/L obtained in the SL1 test.

By taking the difference in SLFA values after 40 minutes re-circulation compared with that before pumping, a measure of the FFAs produced by selective lipolysis of the UPF produced by pumping was obtained, giving an average of 0.60 mmol/L. This is a measure of the damage created by the pumping system. The absolute value of SLFA at any point in time in these experiments represents the sum of:

1.   The IFA produced (which includes OFA), and

2.   Conversion of the UPF to FFAs.

SLFA cannot by itself be a measure of the amount of NMFGM damage in the sample. The difference between SLFA at that point in time (e.g. after 40 minutes re-circulation) and SLFA in the original sample, however, does represent the extra damage that is created by the pumping process. The technique of taking the difference is justified because the values of OFA and IFA remained constant during the experiment and any difference in SLFA must logically have resulted from new NMFGM damage.
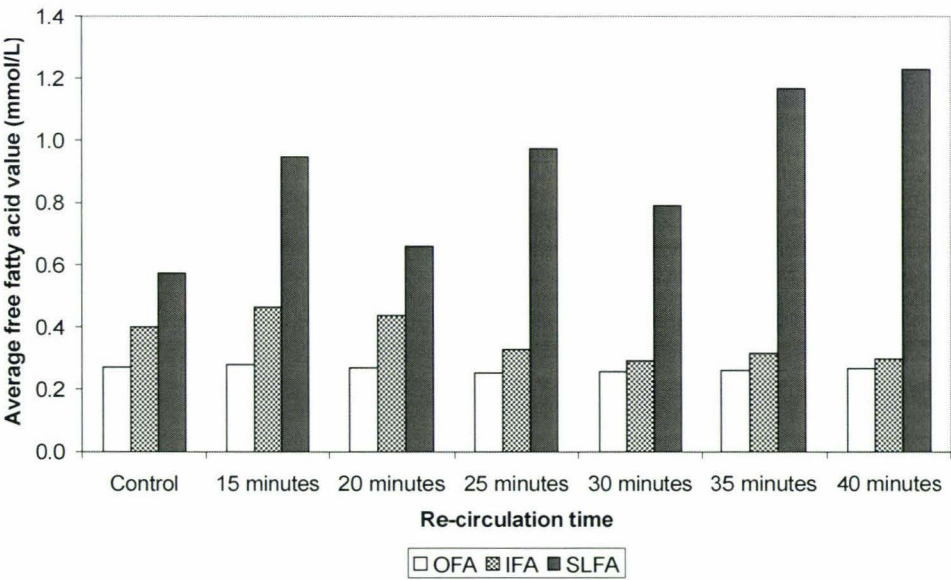
Figure 4.7: Typical variations in OFA, IFA and SLFA averaged over three pumping runs under conditions reported in Figure 4.1. Runs T28-30.

4.1.4   Sensitivity of the selective lipolysis test

The selective lipolysis test can detect clear damage created in a number of common operations in milk powder plants. Figure 4.8 shows a large amount of damage created in a commercial evaporator (run S10) measured by the SL1 test.

A second typical example has been given above in Figure 4.7 for a pumping system. Note that the amount of SLFA produced after 25 minutes pumping was 0.97 mmol/L, which is considerably smaller than the SLFA produced in the most right-hand column of Figure 4.8 (6.7 mmol/L feed) after approximately 15 minutes of circulation through a 5-effect thermal vapour recompression evaporator. Since the same amount of lipolytic enzymes was added in every test, clearly the difference between these sets of data indicates that damage in an evaporator is much more serious than damage in a pumping system.
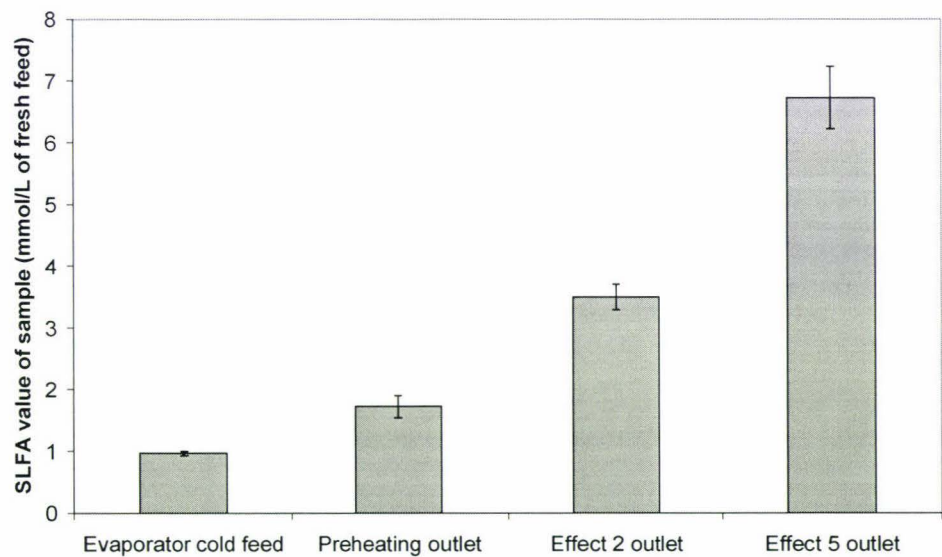
Figure 4.8: SLFA in a commercial 5-effect thermal vapour recompression evaporator (run S10). Error bars indicate the uncertainty in duplicate samples.

Because of the uncertainties in the SL1 test, some levels of damage cannot be detected. Table 4.1 shows the increase in SLFA by passing Farmhouse milk through a pump over a number of passes (run J01). Clearly the difference between three and six passes through the pump is below the sensitivity of the test but a detectable increase in the SLFA occurred over the initial three passes.

Table 4.1: SLFA and IFA results (±95% confidence intervals) for Farmhouse milk passed between two tanks using an Anema-Sneek centrifugal pump running at 1450 rpm. Run J01.

	Control	**3 passes**	**6 passes**
SLFA (mmol/L)	0.77±0.06	1.05±0.07	1.15±0.09
IFA (mmol/L)	0.63±0.01	-	0.66±0.03

4.1.5   Effect of preservatives on the FFA tests

Published studies of lipolysis of milk were conducted under a wide variety of conditions. As suggested in the literature review, there were no standard conditions of incubation. No effort was made to separate the issues of NMFGM damage and production of FFAs; these are related but are not the same. Only two previous studies (Deeth & Fitz-Gerald, 1978; Fang, 1998) have used the principle of adding an exogenous lipolytic enzyme to increase the lipolytic activity of the sample, and only Fang aimed to ensure a sufficient amount of lipolytic enzyme was present to convert all of the UPF into FFAs. The first effort in the present work was to provide a clear protocol of testing that gave some control of all the variables involved in the production of FFAs from UPF.

Two of the variables that could not be easily controlled or even measured were:

1.   The amount of enzymes present in the milk sourced for experiments. It would be expected that these would represent a very complex mixture of native enzymes and microbial enzymes. The latter would depend on how many microbes had been growing in the milk and for how long, and what types they were.

2.   The identification of individual enzymes in a milk sample is a complex and time-consuming exercise that clearly cannot be attempted in routine tests. This exercise is reserved for research undertakings (McKellar, 1989). To ensure better reproducibility of data it is preferable in lipolysis trials to deactivate the microbes in some sense, either by thermal treatment or by the addition of preservatives. Unfortunately, a large number of authors do not report that step and it is not clear whether they took it at all.

In the present work an attempt was made to select preservatives that could control the microbes present in the milk. Of the common preservatives considered, two were tested, bronopol and sodium azide. The effectiveness of these agents in controlling microbial growth is well documented (e.g. Ardo, 1979; 1982; Benda, 1995; Ubben et al., 1997). The tests conducted here centred on any interference by these preservatives on the titration of FFAs and also on the activity of PPL. To test each issue separately, the preservatives were tested in milk samples under the following two conditions and compared with controlled incubation of milk containing neither preservative or PPL at 37°C for 24 hours:

1.   Incubation of milk plus preservative only at 37°C for 24 hours.

2.   Incubation of milk plus preservative and PPL at 37°C for 24 hours.

Figure 4.9 shows the effect of 0.03% sodium azide and 0.02% bronopol (often used levels of preservative in previous studies) on the first test described above. The addition of sodium azide created a dramatic increase in the amount of potassium hydroxide (KOH) required to titrate the extracted FFAs after incubation, whilst the addition of bronopol produced a decrease in the amount of KOH required.

As preservative would be expected to reduce the growth of microbes in the sample and therefore reduce the amount of lipolytic enzymes produced by the microbes during incubation, an increase in the amount of KOH required for titration (1.24 mL more than the control in this case) would not be expected. This result shows clearly that extraneous effects in the titration step were introduced by the addition of sodium azide. Addition of sodium azide to olive oil also resulted in a marked increase in potassium hydroxide required for titration but in that case there were no microbes or lipases present in the oil sample. Thus clearly sodium azide simply increases the acidity of the sample.

The bronopol result was lower than the controlled incubation with milk only but this could be interpreted either as a reduction in acidity or an interference of bronopol with microbial lipolytic activity in the sample. Imhof & Bosset (1995) reported that bronopol interfered with lipase activity.
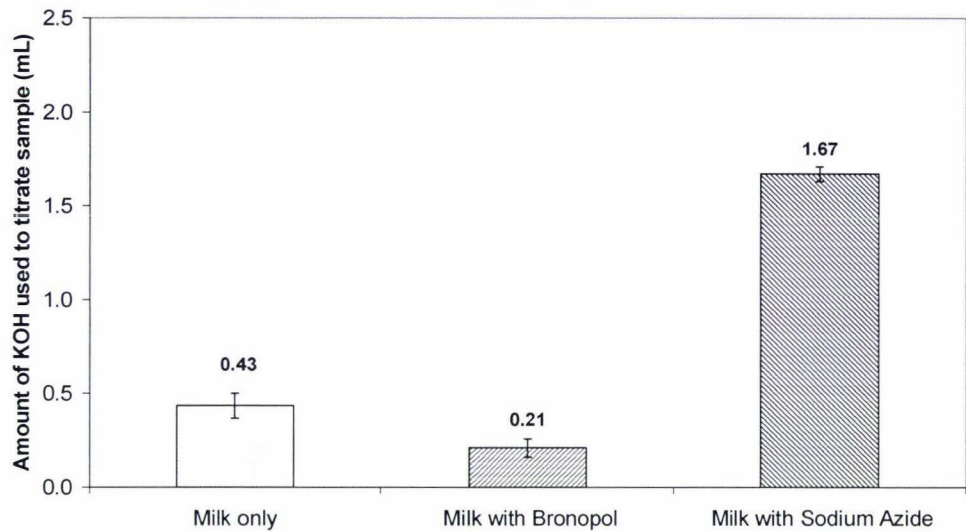
Figure 4.9: Effect of two preservatives (bronopol and sodium azide) on the amount of alkali (potassium hydroxide – KOH) titrated in Farmhouse milk. Run T32.

Figure 4.10 shows the effect of the two preservatives in the second test, incubation with PPL. The difference between the sample incubated with bronopol and the control sample is again -0.21 mL of KOH compared with -0.22 with first test in Figure 4.9 but the absolute value of KOH required for both the control sample and that with bronopol in the presence of PPL increased. Thus it appears that bronopol does not interfere with the action of PPL but has decreased the acidity of the sample.

The difference between the amount of KOH used for samples with and without sodium azide in the presence of PPL was 1.50 mL (Figure 4.10) compared to 1.24 in the absence of PPL (Figure 4.9). Thus, the presence of sodium azide may not only have changed the acidity of the sample but promoted greater activity of PPL during incubation, however the difference in results between test 1 and test 2 is not large enough to make this point conclusively.
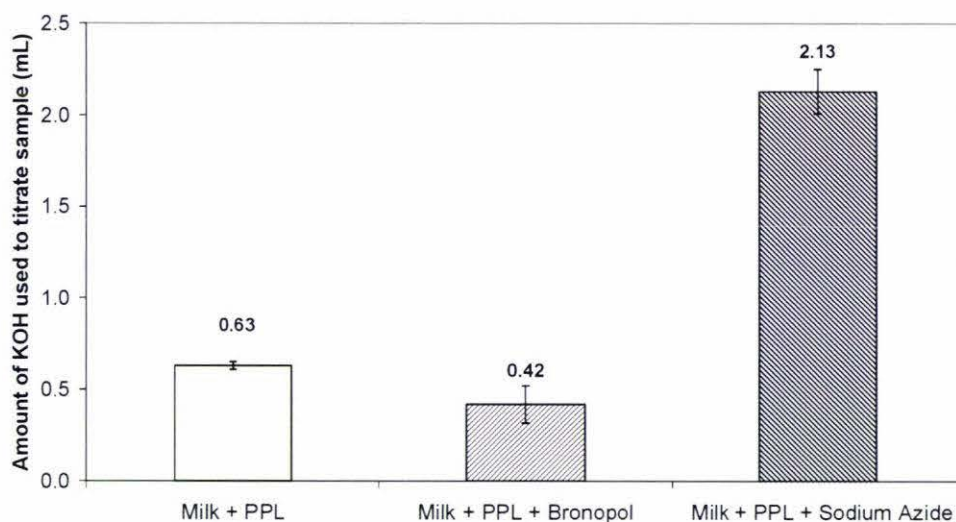
Figure 4.10: Effect of two preservatives (bronopol and sodium azide) with PPL on the amount of alkali (potassium hydroxide – KOH) titrated in Farmhouse milk. Run T32.

Both sodium azide and bronopol appeared to have an influence on microbial growth and subsequent enzymatic activity in the sample during incubation. The control sample contained white aggregates after incubation (Figure 4.11). This indicated the presence of proteases causing the coagulation of casein. These aggregates were absent in the samples treated with either bronopol or sodium azide. It is interesting to note that in the selective lipolysis test these flakes were also not present.
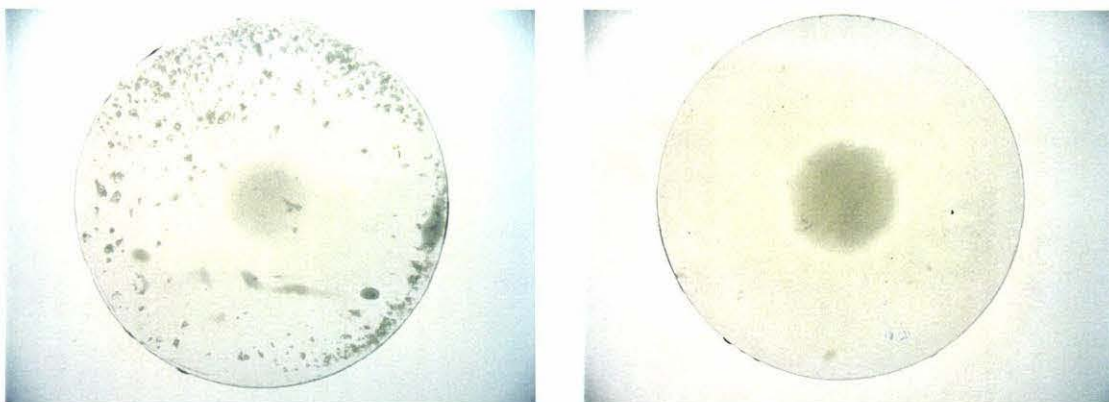
Figure 4.11: Photo of white aggregates found in Farmhouse milk samples incubated at 37°C for 24 hours (left photo). These were not present in the presence of either bronopol (right photo) or sodium azide.

While the effect of the different added preservatives on the activity of enzymes in the incubated samples would undoubtedly be of interest to enzymologists, it is not a major

issue in this work since the results have shown clearly that both sodium azide and bronopol interfered with the incubation test. Their incorporation in the test was deemed unwise and avoided.

Imhof & Bosset (1995) have looked at three methods of conserving raw milk samples for the determination of FFAs:

1.   Immediate extraction of FFAs (OFA).

2.   Addition of different preservatives, such as bronopol.

3.   Heat treatment (2 minutes in a boiling water bath) to inactivate the lipase activity.

They recommended that the heat treatment offered many advantages, but found no improvement "concerning the stability of the samples used to determine the total lipolysable fat after 24 or 48 hours at 37°C."

4.1.6   Effect of microbial contamination on the measurement of FFAs

With the loss of the use of preservatives as an option to control the microbial contamination of milk samples, a new method was devised. Heat treatment as proposed by Imhof & Bosset (1995) was not an option because it can be shown to inhibit the activity of some lipolytic enzymes (Section 4.3). One option was to use only milk samples with an extremely low microbial count, such as that attained by Miller & Puhan (1986a) by milking the cow themselves. While it is possible for research purposes to carefully milk cows to obtain the milk samples required, that procedure was not acceptable in this case where the analysis targeted industrial milk supplies that had been stored and transported over significant periods of time under less than perfect conditions of cooling, sanitation and hygiene.

The solution proposed here sought to minimise the effect of microbial contamination on the results of the SL1 test. For that purpose, a number of milk samples from the same batch of milk were incubated at 37°C for different periods (run T31). At the same time the microbial population was monitored by a plate count using skim milk agar and incubating plated samples for 48 hours. Farmhouse milk bought from a supermarket

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was used. The results in Table 4.2 and Figure 4.12 show that the microbial count of 3×10^2 cfu/mL at the beginning of the experiment had increased to 7×10^2 cfu/mL after 6 hours incubation at 37°C, but grew explosively to at least 1×10^9 cfu/mL after 24 hours incubation. Similarly, the level of FFAs measured after 6 hours incubation (0.29 mmol/L) was, within experimental uncertainties, no different from the measurement at 0 hours, but the FFAs measured after incubation for 24 hours was 1.6 mmol/L, which was significantly higher.
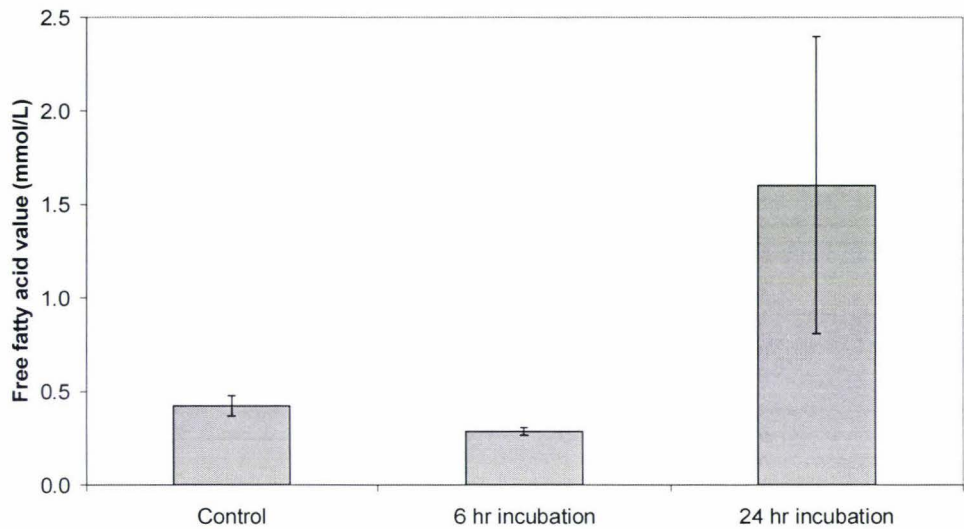
Figure 4.12: FFA value of Farmhouse milk incubated at 37°C for set periods. Error bars indicate the uncertainty in triplicate samples. Run T31.

Table 4.2: Effect of incubation time on microbial plate counts of samples shown in Figure 4.12.

Incubation period	Microbial value (cfu/mL)
0 hours	3×10^2
6 hours	7×10^2
24 hours	$>1\times10^9$

Suhren et al. (1975) showed that microbial levels above 1×10^6 per mL caused an increase in FFA levels after incubation at 9°C for 48 hours using pure cultures of *Pseudomonas*. In addition, most authors agree that flavour problems from microbial growth (therefore the result of either lipolysis or proteolysis) in milk are only a problem once the microbial count exceeds 1×10^6-1×10^7 cfu/mL (Downey, 1975).

Thus shortening the incubation period by a factor of four from 24 to 6 hours (and in the case of the selective lipolysis test, adjusting the amount of PPL added from 5 µg/L to 20 µg/L) successfully dealt with the microbial problem in commercial milk samples. No protein aggregation or gelation of samples was observed after incubation for 6 hours (or in any SL1 test), but both were often observed in samples incubated as part of the LFF test. This observation would agree with the reduced amount of FFAs produced after incubation for 6 hours compared with 24 hours (Figure 4.13). The author believes that after 6 hours incubation the microbes were still in the lag phase of their growth curve. Therefore at that relatively low level of microbial contamination the amount of enzymes secreted by the microbes was still minimal.

4.1.7   Limitations of the selective lipolysis test

The selective lipolysis test was designed for use with milk commonly encountered within the dairy industry. This milk usually contains some small amounts of microbial enzymes in addition to the endogenous milk lipase. It is possible that some of these enzymes are non-selective in the sense that they may attack both the NMFGM and fat in the globules. Thus, unusually high contents of microbial enzymes in exceptional samples may cause so much damage to the original protected fat (PF) upon incubation that the selective lipolysis test results obtained in these cases would no longer be dominated by the conversion of UPF to FFAs. The selective lipolysis test works best when the IFA values are only a small proportion of the SLFA values.

The use of difference in SLFA values before and after a passage through process equipment also does not work when there is simultaneous mechanical damage and heating to temperatures above 75°C. This is because the heating step may alter the amount of endogenous or microbial enzymes in the sample after the process equipment, and therefore change the SLFA value. Therefore a comparison before and after the equipment is not accurate.

In runs T24 and T25 described in Section 1 of Chapter 3, Farmhouse milk from the chilled vat (4°C) of the Massey University milk processing pilot plant was transferred to the large-volume pumping rig and re-circulated around the large-volume pumping rig tank for 12 minutes. Finally, it was pumped through a plate heat exchanger where its

temperature was raised to 80°C. Samples were taken from the vat, after the pumping rig, and after the plate heat exchanger. In run T24 the plant had been through a full clean (as described in Section 4 of Chapter 3), 2 days before the run. In run T25 a further nitric acid rinse was conducted immediately before the run to kill microbes that might have grown since the previous cleaning process. Table 4.3 shows that the nitric acid rinse prevented further microbial contamination during the run. The difference in microbial counts between the vat value of $2x10^4$ cfu/mL and the pump value of $5x10^4$ cfu/mL in run T25 was well within the uncertainties of plate counts. In contrast, run T24 showed a significant increase in microbes between the vat and pump, which indicates that significant microbial contamination had occurred in the plant since the last cleaning process. As expected in both runs, heating above 80°C killed a significant proportion of the microbes (down to less than or equal to $3x10^3$ cfu/mL). Thus, the issue of contamination in the milk samples heated to 80°C milk was not a serious one.

Table 4.3: Effect of acid pre-rinse of experimental equipment on microbial plate counts from Farmhouse milk. Values are the average number of colony forming units per mL of milk sample. Runs T24 (no acid rinse) and T25 (with acid rinse).

Run #	Vat	Pumping rig exit	Heat exchanger exit
T24	$7x10^3$ cfu/mL	$3x10^6$ cfu/mL	$2x10^2$ cfu/mL
T25	$2x10^4$ cfu/mL	$5x10^4$ cfu/mL	$3x10^3$ cfu/mL

Both IFA and SL1 tests were conducted on the samples collected. Figure 4.13 shows the levels of FFAs obtained in the OFA, IFA and SL1 tests for duplicate samples.
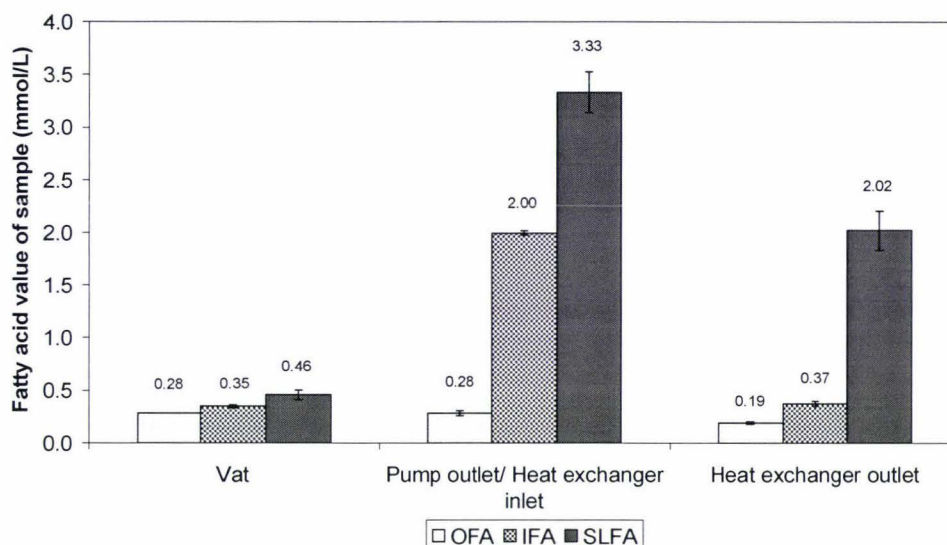
Figure 4.13: FFA values in runs T24 reported in Table 4.3.

As expected, both the levels of IFA and SLFA in run T24 increased significantly between samples from the vat and the pumping rig exit because of microbial contamination, whereas the OFA results remained constant. The difference between the SLFA and IFA results for, say, the pumping rig sample represents in principle the extra conversion of UPF to FFAs generated by the addition of PPL.

In contrast, the SLFA and IFA results for the sample after the plate heat exchanger were considerably lower than for the pump sample. The difference between the IFA values was 1.63 mmol/L, which is very large. Interestingly enough, the difference between the SLFA and IFA values for samples taken after the plate heat exchanger (1.65 mmol/L) was roughly the same as the difference in those values for the pump sample (1.33 mmol/L), suggesting the extra conversion of UPF to FFAs had not changed significantly. Therefore these results indicate that:

1. Some bacteria able to produce lipases were killed by the 80°C treatment. This reduced the overall lipase activity, and therefore the IFA value.

2. When the IFA values change between samples taken before and after the heat exchanger, taking the difference in the SLFA values before and after the equipment to assess NMFGM damage is not acceptable.

3. Because the difference between IFA and SLFA before and after the heat exchanger did not change significantly, the heat exchanger is not likely to have

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created any further damage to the NMFGM. This is confirmed in Section 2 of Chapter 5.

4.   The large decrease in IFA before and after the heat exchanger indicated a drop in microbial lipase activity, and coupled with the unchanged difference between the IFA and SLFA values seems to support the view of Downey (1980) that the incubation of microbial lipase in milk samples, (run T24), can increase the amount of lipolysis (of PF and UPF) over and above the mechanical act of pumping.

4.2   Particle size zoning test

Physical changes to fat globules when the NMFGM is damaged cannot be measured through any lipolysis test and are best captured through analysis of particle size distributions (PSDs) of fat globules in the milk. As previous workers, notably Walstra et al. (1984) and Michalski et al. (2002a) have observed, the particle size of fat globules can be either increased (flocculation or coalescence of globules) or decreased (disruption) when the NMFGM is damaged. Figure 4.14 gives examples of the PSD of globules in Farmhouse milk sourced from a local company (Mainland Milk Products factory at Longburn, New Zealand) after pumping through a re-circulation system incorporating a storage tank (run T29).

Clearly there is a difference in the PSD after damage, notably the occurrence of three peaks in the damaged sample. The new peak, situated on the right-hand side of the graph is linked with emergence of coalesced or flocculated globules. As noted in Chapter 3, comparisons of raw particle size distributions can be qualitative but do not allow easy estimation of the extent of damage. When both disruption and coalescence occur then the comparison between damaged and undamaged samples becomes even more difficult. The PSZ test described in Section 2 of Chapter 3 allows the quantification of the levels of size reduction and size enlargement of the original fat globules.
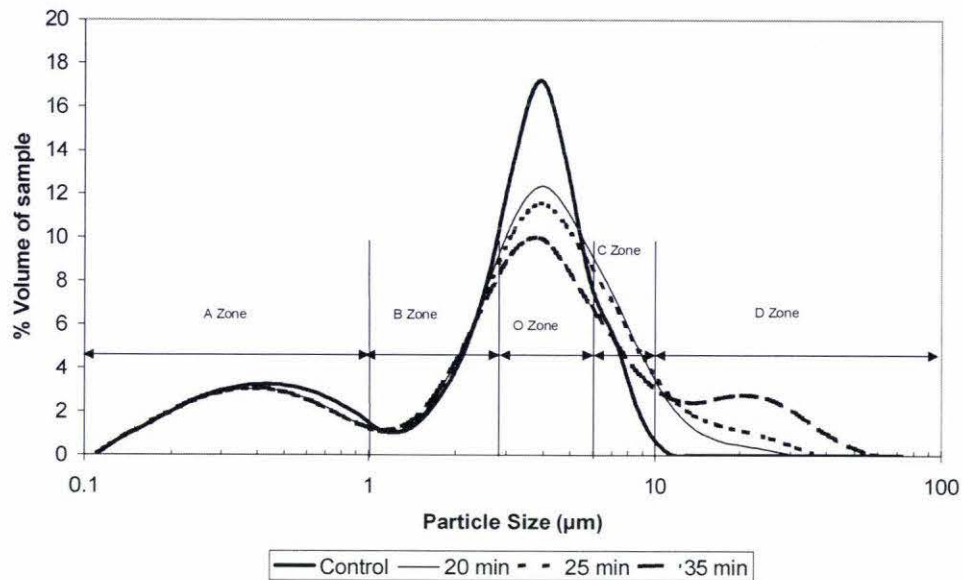
Figure 4.14: Example of typical PSDs obtained using the Malvern Mastersizer E. Run T29 with Farmhouse milk re-circulated via a tank.

When the PSZ test is applied to the data in Figure 4.14 the results can be plotted as in Figure 4.15. In this example, the number of particles (reflected as a volume percentage) in the A+B Zone remains relatively stable and the number of particles in the C+D Zone increases. The number of particles in the O Zone decreases. The results in Figure 4.14 indicate that:

1.   A number of size-dominant fat globules (at least 14 volume % in the O Zone) have been altered but approximately 24 volume % remain untouched.

2.   A number of small fat globules in A and B Zones (2 volume %) have flocculated to form larger fat globules in the size range of the O Zone or above.

3.   A number of new fat particles of sizes above 5.0 μm in diameter have been created by flocculation or coalescence (16 volume %).

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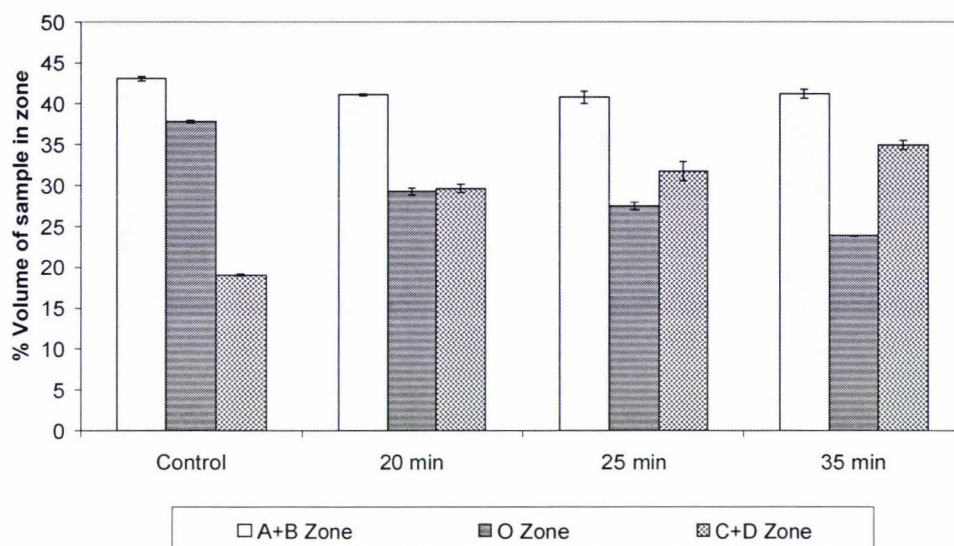
Figure 4.15: Example of typical particle size zoning test results obtained using the Malvern Mastersizer E. Calculated from PSD (run T29) shown in Figure 4.14. Error bars indicate the uncertainty in duplicate samples.

4.2.1   Uncertainties of measurement

PSDs can be measured quickly and accurately. The absolute uncertainty of the PSZ test, calculated from 37 half-range uncertainties obtained in 37 duplicate samples taken in a number of experiments in this work, is shown in Figure 4.16. The percentage uncertainty of PSZ test measurements is shown in Figure 4.17. The charts show that the results of the PSZ test are much more precise than the SL1 test. For example the average absolute uncertainty of the C+D Zone was ±0.55 volume % and is not large compared with the total size of the C+D Zone, such as the typical one shown in Figure 4.15. The very high percentage uncertainty in the D Zone is due to the number of very small values for the D Zone. In undamaged Farmhouse milk the D Zone is almost zero.

The measurement uncertainty of the PSZ test was further tested using 10 replicate measures of a Farmhouse milk sample taken from a supermarket. The results shown in Table 4.4 clearly show that the measurement uncertainty of the test (i.e. from replicate determinations of the same sample) is similar to its sampling uncertainty (i.e. from single determinations of replicate samples), shown in Figure 4.16.
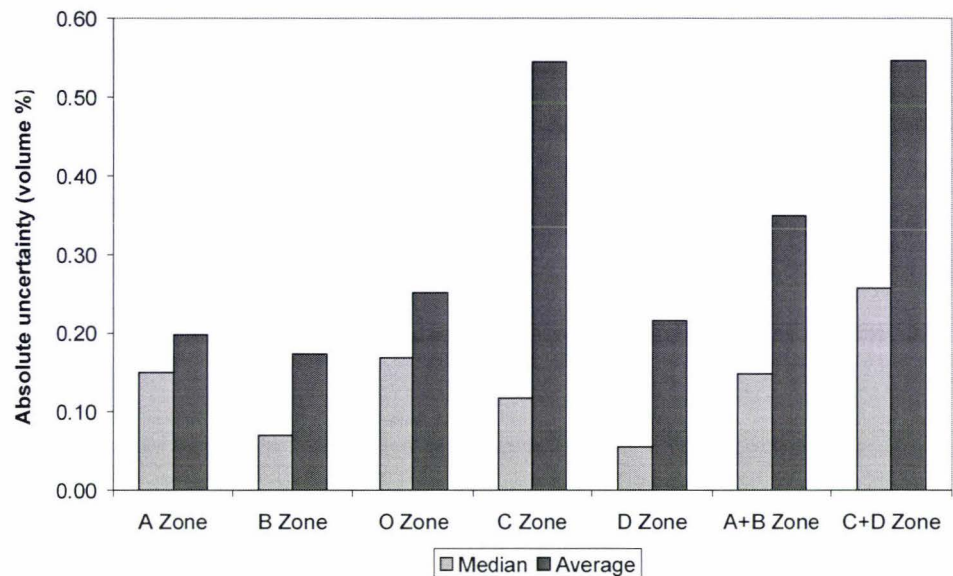
Figure 4.16: Uncertainties for each zone in the PSZ test. Columns show the median and average absolute uncertainties in the measurement. Results were calculated from 37 duplicate samples.
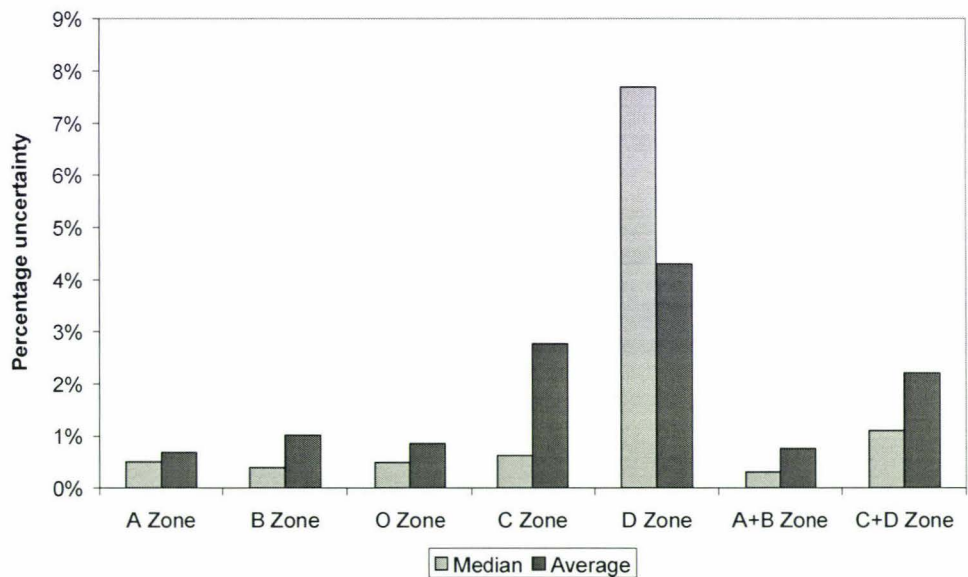
Figure 4.17: Percentage uncertainties for each zone in the PSZ test. Columns show the median and average percentage uncertainty in the measurement. Results were calculated from 37 duplicate samples.

Table 4.4: Measurement uncertainty in the PSZ test using 10 replicate measurements of an undamaged Farmhouse milk sample.

Zone	Average Value (volume %)	95% Confidence Interval (volume %)
A	27.21	0.34
B	18.44	0.12
O	36.11	0.27
C	18.14	0.16
D	0.08	0.00
A+B	45.65	0.42
C+D	18.23	0.16

In addition, changes in the A, B, A+B, O, C and C+D Zones when milk is damaged are quite significant; typical examples of the variations in the A+B, O and C+D Zones are given in Figure 4.18 for an evaporator, which shows mainly disruption, and above in Figure 4.15 for a pumping rig which shows flocculation/coalescence.
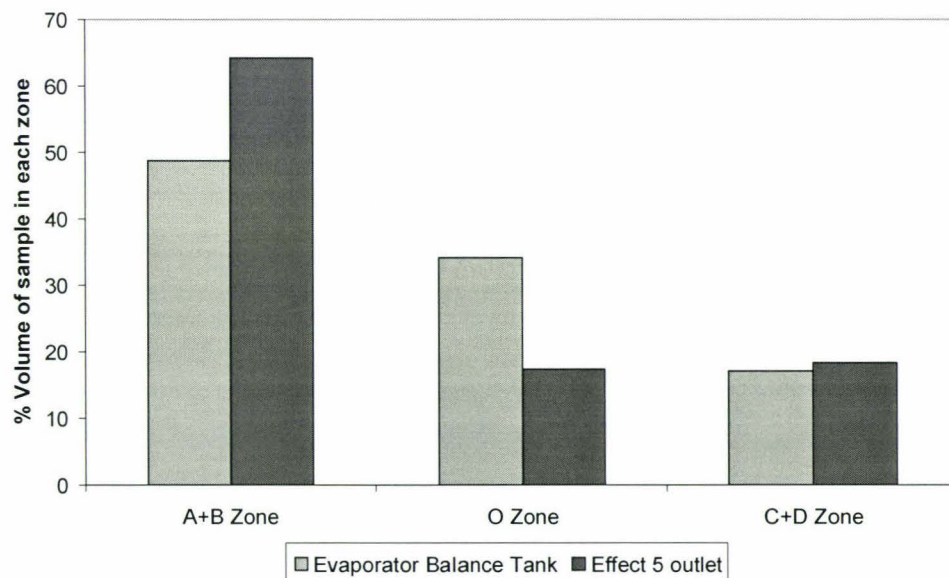
Figure 4.18: Results of PSZ test using samples from a commercial 5-effect thermal vapour recompression evaporator. Cold feed sample was 3.7% fat and finisher sample was 13% fat. Run S10.

4.3   Sample Handling

The reliability of measurement techniques in the selective lipolysis and PSZ tests cannot be determined accurately if the sample is not representative of the milk in the equipment. Several issues of sample and sample handling are worth considering:

1.   Damage during sampling.

2.   Creaming of the sample in storage.

3.   Unrepresentative sampling.

4.3.1   Damage during sampling

The NMFGM is very easily damaged by mechanical handling. This is well documented as discussed in Chapter 2. Sampling of milk in the New Zealand dairy industry is usually done with the Vacutainer needle and tube apparatus (Section 1 of Chapter 3). In this work, raw milk samples obtained using the Vacutainer often tended to gel during incubation. When a Vacutainer is pressed into the sample port of a process line milk could be seen to rush rapidly into the test tube of the Vacutainer device because of the large pressure difference between the line and the Vacutainer. The jet of milk entering the 10 mL test tube filled the tube in less than 1 second, giving an impact velocity estimated as greater than 10 m/s. Since splashing can be shown to damage the NMFGM significantly (shown in Chapter 5) the effect of the sampling linear velocity was tested in experimental runs T06-09, as shown in Table 4.5. The amounts of solvent-extraction free fat (FF) measured in these early experiments - when the SL1 test protocol had not been developed and no PSD analysis on site was available - were obtained for average velocities of 3.9 and 0.33 m/s. The data show that the average FF in samples taken using the higher velocity was 0.029% and for the lower sampling velocity was 0.024%. The standard errors were 0.002% and 0.001% FF. The difference between the two FF values was significant at the 95% level of confidence using a one-tailed t-test. All sampling subsequent to this experiment was made with a sterile plastic 30 mL syringe and a 0.9 mm needle. The milk filled the syringe more slowly, because unlike the Vacutainer the syringe was not under vacuum, and in addition there was friction between the cylinder and the piston of the syringe.

Table 4.5: Effect of sampling velocity on free fat (data collected at the Waitoa dairy factory reception). Runs T06-09.

Average sampling velocity (m/s)	Number of samples	Average value (% FF in sample)	Standard error (% FF in sample)
3.9±0.9	8	0.029	0.002
0.33±0.09	16	0.024	0.001

4.3.2   Creaming of the sample during storage

It was visually observed that samples tended to cream during storage before measurement since they were not homogenised. Because of flocculation and coalescence the creaming effect was heightened in the damaged sample. This is shown in Figure 4.19 where different levels of damage were created in the agitation rig at agitation speeds of 2000 rpm for 0, 1, 2 and 4 minutes. Milk samples were stored at two temperatures, 5 and 20°C for up to 48 hours in runs T19-22. PSDs were measured and the size of the C+D Zone was calculated using the PSZ method. The results indicate that there was flocculation of fat globules, leading to faster creaming in the more severely damaged milk.

Because creaming and flocculation are the result of physical changes in the milk system, a selective lipolysis test that measures chemical consequences of NMFGM damage would not provide an adequate picture of these changes and therefore was not performed in these runs. Figure 4.19 shows that the percentage volume of the C+D Zone in the sample increased gradually for all four samples over time up to 48 hours of storage. The extent of flocculation/coalescence after 24 hours storage was on average 40 volume %, up from 26% at the beginning of the run. However, after 48 hours storage, the extent of flocculation/coalescence increased dramatically. For example, the C+D Zone in the undamaged milk increased from16.7 volume % to 58.1 volume %.
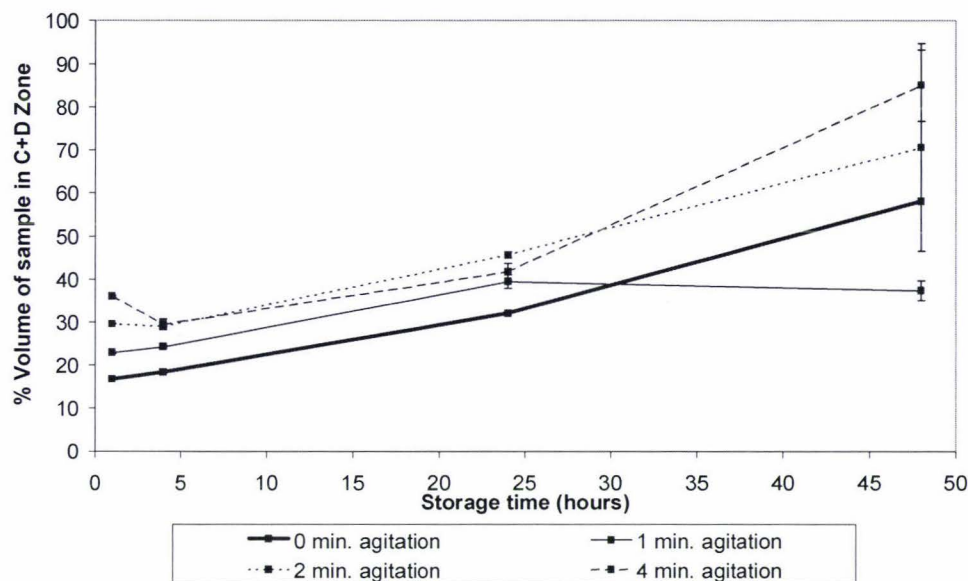
Figure 4.19: Effect of 20°C storage of bulk samples on C+D Zone of Farmhouse milk. Error bars indicate the uncertainty in duplicate samples (runs T19-22).

Storage at 5°C resulted in a much smaller change in the PSD, for example the C+D Zone of the undamaged milk only increased from 16.7 volume % to 17.7 %, as shown in Figure 4.20. The extent of flocculation/coalescence after 24 hours storage was on average 27.7 volume %, virtually identical to the 27.1% at the beginning of the run. Storage of milk at 5°C for up to 24 hours does not significantly alter the PSD of a milk sample unlike storage at 20°C. Therefore a milk sample stored at 5°C for up to 24 hours can confidently be used as a true reflection of the PSD of the sample at time zero.
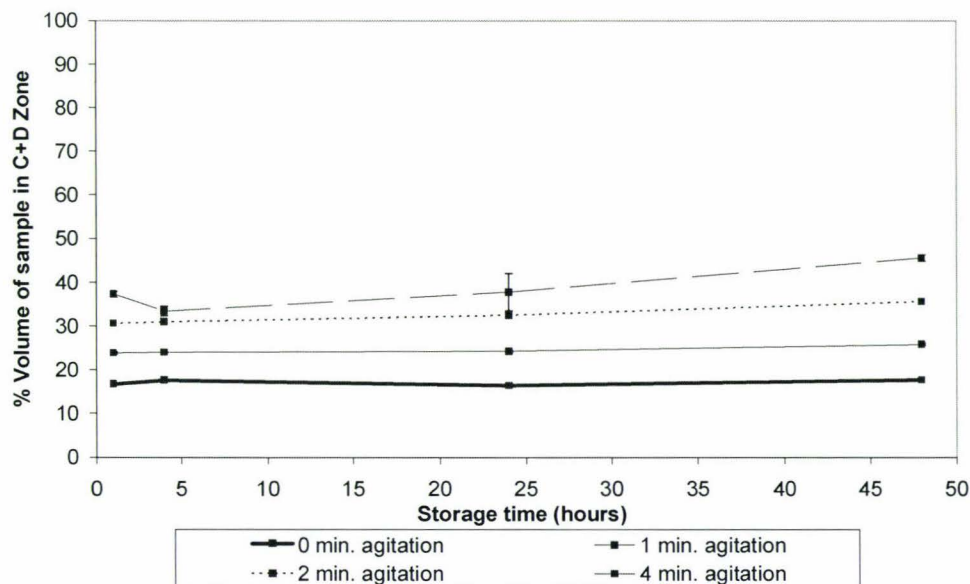
Figure 4.20: Effect of 5°C storage on C+D Zone, runs T19-22 reported in Figure 4.19.

Plots of uncertainties of the measurements of the C+D Zone are shown in Figures 4.21 and 4.22. The uncertainties were calculated from duplicate samples that were performed for all samples in these tests. If these uncertainties are larger than the measurement uncertainty of 0.3 volume % for the A+B Zone, or 0.4 volume % for the C+D Zone (shown in Table 4.4) then the increase is likely due to inhomogeneity in the sample, created by the increased rate of creaming.
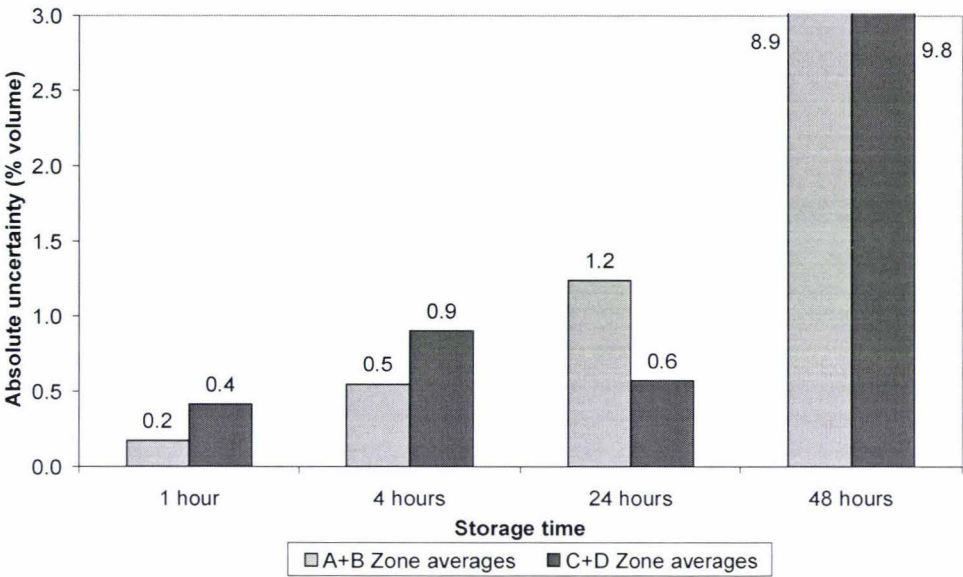
Figure 4.21: Effect of 20°C storage on average absolute uncertainties in the A+B and C+D Zones, runs T19-22 reported in Figure 4.19.
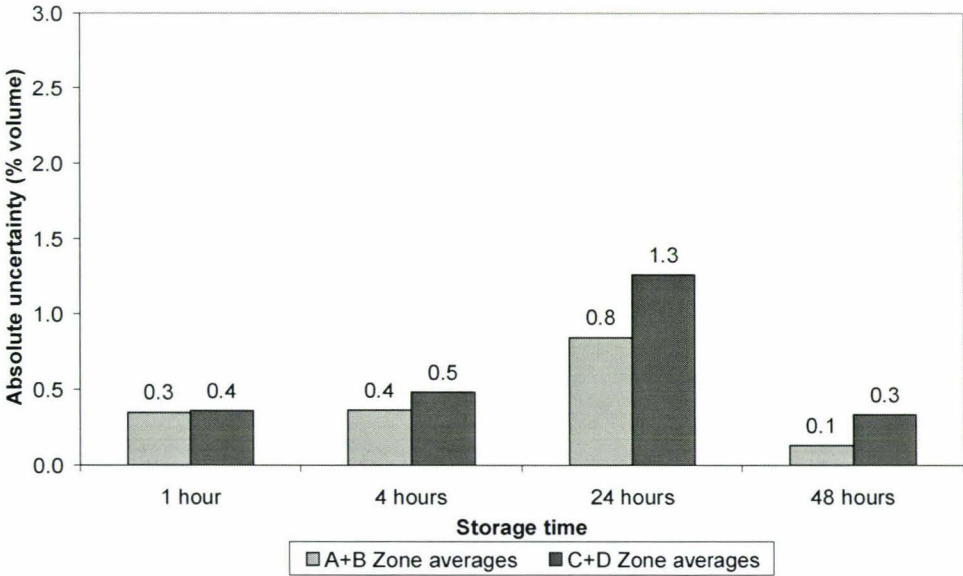
Figure 4.22: Effect of 5°C storage on average absolute uncertainties in the A+B and C+D Zones (runs T19-22) reported in Figure 4.20.

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The milk container used for sampling in the PSZ test was always gently inverted five times before a 5-10 mL portion was sampled. This sample was inverted 5 times immediately before 1 mL was added to the Mastersizer for measurement. The fact that the uncertainty in that measurement increased for all storage times indicates that it was more and more difficult to take a representative sample the longer the milk was stored. This effect is most clear at 20°C storage. It is interesting to note that the higher uncertainties found in the sample agitated for 4 minutes and stored at 20°C for 48 hours (Figure 4.19) corresponds to the largest C+D Zone of 85 volume % (and therefore the greatest flocculation, and therefore creaming), compared with the low uncertainties for undamaged milk stored for 48 hours at 5°C (17.7 volume %).

Clearly the protocol for sample inversion before PSD measurement helps but does not completely negate the effect of creaming during storage. The data indicated that when samples had to be stored between the time they were taken and the time they were measured for logistical reasons (unavailability of PSD measurement instrument on site) or because of large numbers of samples, this had to be done at 5°C for no longer than 24 hours. This is the recommended procedure in the PSZ test protocol described in Section 2 of Chapter 3.

4.3.3   Representative sampling

Forms of damage in milk

It is well known that damage to the NMFGM can result in either a decrease in the size of the fat globules in the liquid milk (disruption) or an increase through flocculation and/or coalescence (Walstra et al., 1984). Examples of flocculation and/or coalescence that occur under relatively low conditions of shear, shown previously in Figure 4.19, are reflected by an increase in the C+D Zone of the PSZ test. Homogenisation is a typical example of disruption of the fat globules that results in a massive increase in the A+B Zone and occurs during extreme turbulence or shear as shown in Figure 4.23. Kaw (1998) also showed this effect.
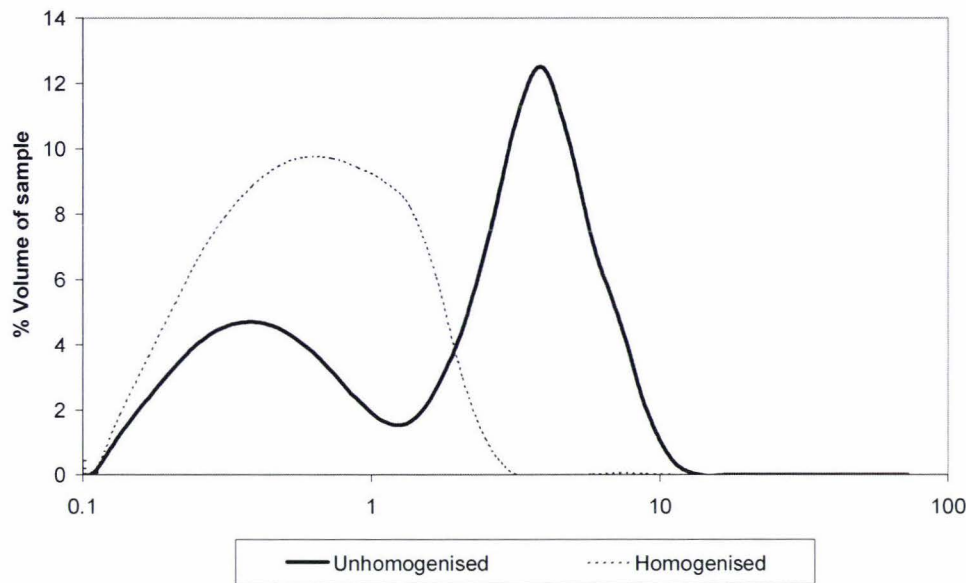
Figure 4.23: Effect of homogenisation on the PSD of Farmhouse milk homogenised at 200 bar and then 50 bar at 50°C.

Damage to the NMFGM does not always reside in the liquid phase of milk systems alone. Many workers have observed foaming of milk systems, especially during agitation, splashing and pumping with air inclusion. Figures 4.24 to 4.26 show photographs of these phenomena observed in this work. Many researchers have reported high levels of NMFGM damage (in terms of FFAs) in the foam (e.g. Mulder & Walstra, 1974; Miller & Puhan, 1986b). Walstra et al. (1999) argued that foaming phenomena tend to occur at lower temperatures because there is not enough liquid fat to form "granules". He further described the formation of "whipped cream" at temperatures where "there is very little liquid fat during beating in of air".

Figure 4.24: Photograph of foam produced from agitating Farmhouse milk at less than 10°C.

In this work, foaming tended to occur when the milk was cold (<10°C) and was increased by splashing (Figure 4.25) and air inclusion (Figure 4.26).
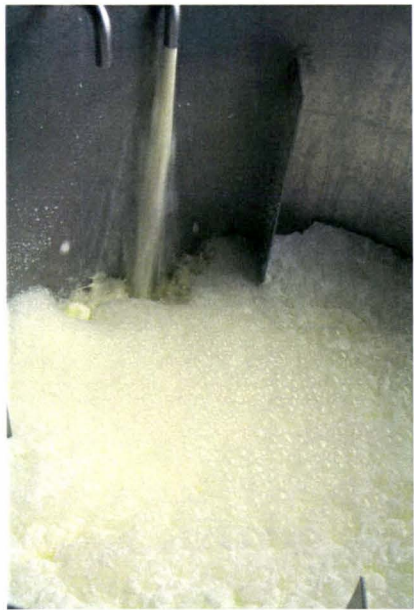
Figure 4.25: Photograph of foam produced
from splashing Farmhouse milk at less than
10°C into a tank of milk.

Figure 4.26: Photograph of foam produced by adding air into the pipeline carrying Farmhouse milk at less than 10°C re-circulated via a tank. The hose in the photograph was designed to allow no splashing of the milk.

In addition, when damage was created in this work in the temperature range of 20-25°C phase separation was observed in the sense that butter-like particles separated from the liquid milk and often attached to the wall of the vessel or rose to the surface of the milk (Figures 4.27 and 4.28). Mulder & Walstra (1974) described the visual occurrence of solid fat "granules" and the strong dependency of their formation on the turning (or agitation) rate, fat content, fat globule size and temperature (i.e. proportion of solid fat). It is noted that the optimum churning temperature of butter, which requires the total disruption of the NMFGM, is in the region of 10-20°C but is very dependent on the above variables.
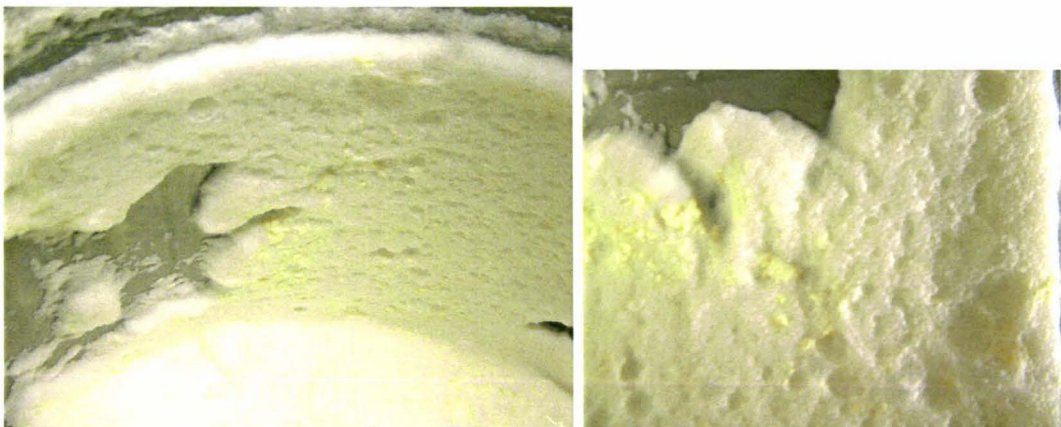
Figure 4.27: Photographs of "butter-like" particles attached to the wall of a tank after 17°C Farmhouse milk was re-circulated via a tank using a cavitating centrifugal pump.

Figure 4.28: Photograph of "butter-like" particles attached to the wall of a tank after 25°C Farmhouse milk was agitated at 2000 rpm.

In early experiments in this work, when the temperature of the milk was not controlled in a single-pass pumping rig, clear evidence of phase separation was observed (Figure 4.29). During pumping, heat was generated by viscous dissipation and the outlet temperature would reach between 15°C and 26°C. Most of the observations of phase separation were observed during those runs, before later runs carried out in the last year of this work. The largest particles could be observed on the side of the tank wall after pumping, as in Figure 4.27 or floating on the top of the milk in agitated tanks, as in Figure 4.28. In both cases the deposits could be collected in the tank as yellowish fat clumps (Figure 4.29). The formation of these clumps, or churned fat can be explained based on flotation churning theory which has been described by Mulder & Walstra (1974). This theory requires the presence of liquid and solid fat and also the presence of air. In the temperature range 10-20°C a fraction of the milkfat is liquid but a significant proportion is still solid. "Fat globules touch these [air] bubbles, spread some liquid fat over the air-water interface and become attached to the bubbles". As the air bubbles coalesce the "fat globules attached are driven towards one another, so that they form small clumps".

Figure 4.29: Solid phase extracted from 50 litres of Farmhouse milk passed backwards and forwards between two tanks using a cavitating Fristam centrifugal pump (run C02).

Representative measurement of NMFGM damage

Since there can be three different phases, liquid milk, foam, and churned fat, sampling of the liquid milk alone will clearly give an underestimate of the amount of UPF.

Figures 4.30 and 4.31 give the PSD and the FFAs measured by the LFF test in milk samples during an early experimental run (C02) where churned fat was observed (Figure 4.29 above). The churned fat was not sampled. The particle size measurements in Figure 4.30 show that the peak in the O Zone (3.2 to 5.9 µm) diminished dramatically after the first and second pass through a cavitating pump rig that included a globe valve and a centrifugal pump located between two milk tanks. This is clear evidence that the original size-dominant fat globules were disrupted. As a consequence of this, both the C+D and A+B Zones were altered, with the creation of large particles shown by the increase in the C+D Zone after the first pass.
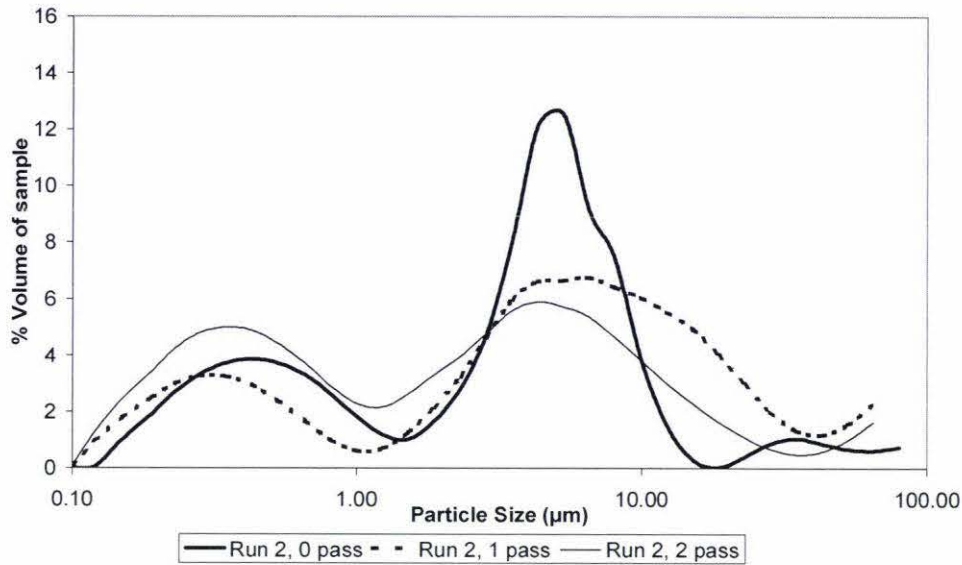
Figure 4.30: PSDs of damaged Farmhouse milk producing churned fat. Run C02.

However, the FFAs measured in the LFF test used at that time actually showed a decrease with an increasing number of passes through the pumping system (Figure 4.31). Simultaneously, the importance of the C+D Zone in the PSD decreased from the first- to the second-pass sample. If all the analysis of the system performance had relied on the liquid phase sample alone, one would be forced to conclude that the amount of NMFGM damage decreased as the number of passes increased from 1 to 2. However, churned fat, which was not seen during the run, could be photographed at the end of the run. Clearly, if the churned fat had formed a representative part of the sample subjected to the LFF test, then the sample FFA values measured might have been higher than those illustrated in Figure 4.31 for one and two passes. Further, the FFA value for two passes might have been higher than for one pass.

Churned fat also occurred in an early agitation experiment (runs C39-44), as previously illustrated in Figure 4.28. When only the liquid phase was sampled, the FFAs from the LFF test again did not reflect the increasing NMFGM damage with agitation time. Because of the difficulties of sampling the churned fat during pumping or agitation runs, later experiments (except when studying temperature effects) were always conducted at temperatures below 10°C to ensure churned fat was not produced.

Churned fat can be considered to have consisted partly of UPF because it had been separated from the milk emulsion. This required that the milk emulsion be stabilised,

which in turn required damage to the NMFGM whose primary function was to stabilise the emulsion and protect the fat globules.
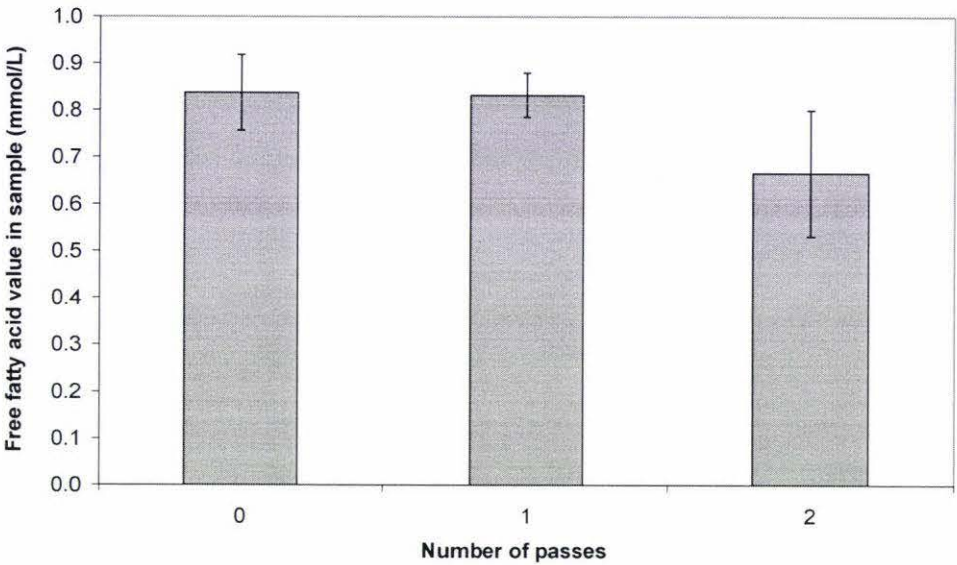
Figure 4.31: FFA values using the LFF test from run C02 reported in Figure 4.30.

The same problem of unrepresentative sampling was encountered when significant foaming occurred in the system. This issue became significant when pumping and agitation runs were carried out at temperatures below 10°C. (Generally, when churned fat was observed there was no foam, and when foam was observed no churned fat was present). In the cavitation runs, described in Section 1 of Chapter 5, liquid milk samples showed random variations in SLFA. This indicated that the fat content in the foam was very unevenly distributed, and this lack of uniformity in the foam, which could be seen as differently coloured areas (e.g. Figure 4.26), made representative sampling of the foam very difficult.

In run T30 there was a significant amount of foaming, and both the liquid and foam portions were sampled. The PSD analysis can be summarised for 15 and 40 minutes of pumping in a re-circulation system. The results from run T30 are shown in Figure 4.32. This chart shows the larger C+D Zone of the foam portion of the milk. The mass of foam measured after 40 minutes accounted for 4% of the total mass of the original batch of milk. For samples re-circulated for 15 minute and 40 minutes, 90% of the PSD of the foam samples was located in the C+D Zone. This compared to only 26% and 42% respectively for the liquid samples.
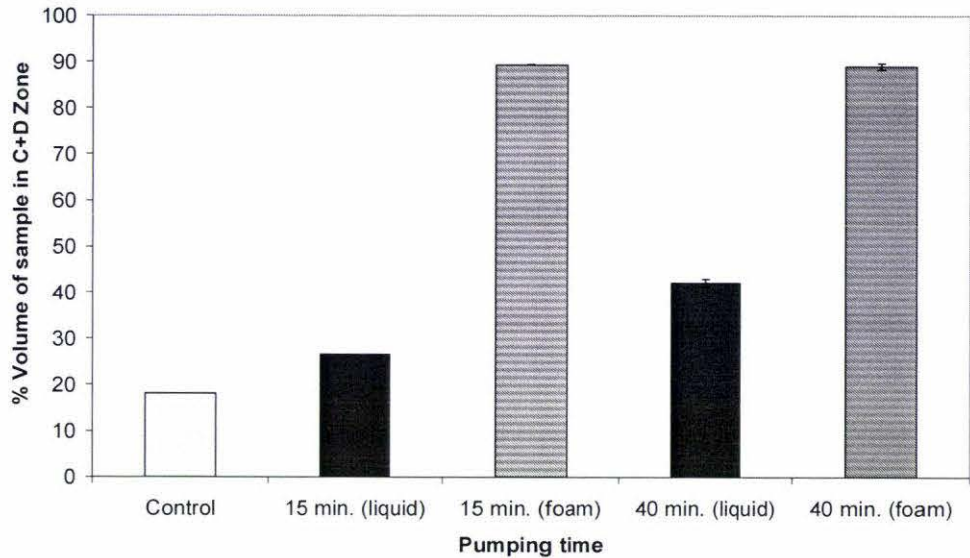
Figure 4.32: Illustration of C+D Zone of milk and foam produced from 10°C Farmhouse milk. Run T30.

It appears impossible to take a representative sample of the system when all three phases – liquid milk, foam and churned fat – are produced. Indeed, it was quite disturbing that most of the publications involving FFA and FF testing do not include methods of sampling in sufficient detail for the reader to know whether samples were truly representative. The author found that early attempts to take representative samples in systems with just liquid milk and foam were extremely difficult and gave very variable results. In the end it was decided that the only reliable approach to deal with the problem was to sample the liquid milk, foam and churned fat separately. In addition, the three phases had to be weighed separately. This required laborious work, especially when recovering foam, and could only be done at the ends of runs. This tripled the amount of work required. Thus the method was only applied in selected experiments to provide a complete description of damage done to the NMFGM.

In order to obtain a mass balance of fat in a milk sample with NMFGM damage the fat composition of all phases had to be determined. A typical set of results of relative masses of the different phases obtained during an agitation experiment (C107) carried out specifically for the analysis of fat in the different phases is shown in Table 4.6. The fat content of these samples and the distribution of the fat between the different phases is shown in Table 4.7. In all cases there was a liquid phase but there could also either be churned fat or foam depending on the temperature of agitation. If the churned fat was

not sampled in the agitation experiment then 70% of the fat would not have been accounted for (Table 4.7). However, the error at 10°C would have been only 5% because the mass of foam was relatively small. This clearly shows that the churned fat phase constituted a very significant proportion of the total fat whereas the foam contribution at both 10°C and 45°C was much less. These results highlight the need to take representative samples from all phases of a milk sample, especially when churned fat is present.

Table 4.6: Effect of the form of the non-liquid phase on the distribution of mass between the liquid, foam and churned fat phases of Farmhouse milk agitated at 2000 rpm for 4 minutes at various temperatures. Run C107.

Agitation temperature (°C)	Form of non-liquid phase	Mass of phase (g)		Total Mass (g)
		Liquid	Non-liquid	
10	foam	385	3.44	388
20	churned fat	379	12.3	391
45	foam	394	12.0	406

Table 4.7: Fat content of samples reported in Table 4.6.

Agitation temperature (°C)	Fat content of phase (%)		Proportion of fat outside liquid phase
	Liquid	Non-liquid	
Control	3.8	NA	0%
10	3.0	18	5%
20	1.0	72	70%
45	3.2	13	11%

* NA = not applicable

An example of the contribution the churned fat makes to the total FF measured by the solvent-extraction test in run C98 is shown in Table 4.8. Milk was agitated at 2000 rpm for 5 minutes at 20°C. Only liquid milk and churned fat were present. This shows clearly that the churned fat phase contributed a significant proportion of the FF of the sample. Interestingly, fat accounted for only 72% of the mass of the churned fat sample

(Table 4.7). This is similar to the composition of butter (80% fat or higher). A detailed analysis of the other components present was not made but water content would likely have been significant, and NMFGM material would be likely to have been present.

Only 58% of the fat present in the churned fat was extracted as FF (Tables 4.7 and 4.8). This observation lends support to the theory of Mulder & Walstra (1974) that a number of undamaged fat globules are present in the structure of butter – shown diagrammatically in Figure 4.33.

Table 4.8: FF content of a milk sample with churned fat (run C98).

Mass of phase (g)		FF content of phase (g)		FF content of phase (%)		Contribution of phase to total FF value (mg FF/g of sample)	
Liquid	Churned fat	Liquid	Churned fat	Liquid	Churned fat	Liquid	Churned fat
395	16.6	0.15	6.9	0.039	41	0.36	16.8

fat globules   membrane   fat crystals   aqueous droplet   air cell
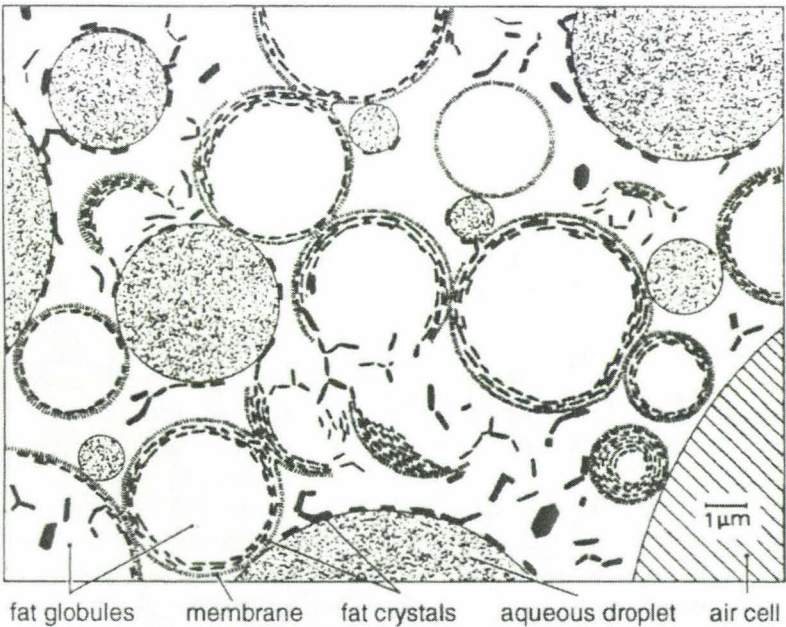
Figure 4.33: Diagram of the structure of butter at room temperature. From Mulder & Walstra (1974). Note – membrane thickness has been exaggerated.

4.4   Development of the SL2 test

Section 4.1 showed that the SL1 test is a significant improvement on the LFF test in terms of reproducibility of measurements. This is likely to be due to the shorter incubation time (6 hours compared to 24) that yields samples that were observed not to have protein coagulation (white aggregates) on top of the milk or to gel after incubation, as was the case with the LFF test.

The results in Section 4.3 highlighted the need to representatively measure milk samples with NMFGM damage in all phases whether liquid milk, foam or churned fat. In the SL1 test, large fat globules were often observed on the surface of samples of liquid milk incubated with addition of PPL for 6 hours at 37°C. This separation of a fat phase was not present in the original milk sample before incubation. Figure 4.34 shows a clear yellow layer of liquid fat on top of the sample container after incubation at 37°C for 6 hours. In most applications of the SL1 test attempts were made to obtain a representative sample of both these phases by inverting the container holding the incubated sample five times, as described in Section 1 of Chapter 3. The original reasoning for the inversion procedure was that it would allow uniform dispersion of two phases yet was gentle enough to avoid any further damage to the NMFGM. It was noticed that the inversion protocol did not succeed in mixing the fat and aqueous phases very well, and those samples that created enough NMFGM damage to result in separation of phases after incubation gave very scattered results. The SL2 test was therefore developed to give a more representative sub-sample of the incubated milk for titration purposes. It was realised belatedly, at the end of this work, that very vigorous agitation of already incubated samples would not change the results of the titration for FFAs even if more NMFGM damage was created because the titration procedure was conducted immediately after the sub-samples were taken and FFAs need time to develop.
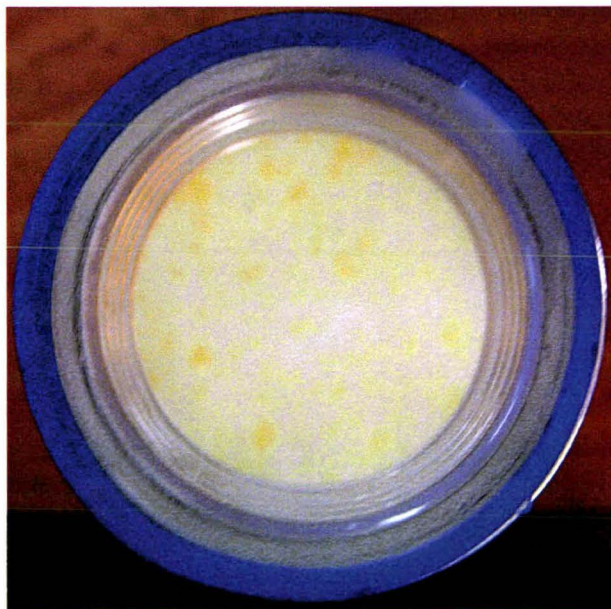
Figure 4.34: Liquid milk sample incubated at 37°C for 6 hours. Note, yellow layer is liquid fat.

In the SL2 test the incubated sample was vigorously agitated for 15 seconds using a Heidolph DIAX 600 homogenising unit running at 10,000 rpm. All other conditions are identical to the SL1 test. The effect of homogenisation of the incubated sample in Figure 4.34 is shown in Figure 4.35. An agitation experiment was conducted using the SL1 test (runs C85-90) and then repeated using the SL2 test (runs C97-102). The results for the liquid samples at different temperatures are shown with both the SL1 and SL2 tests in Figure 4.36. Clearly the trends observed for the two tests were similar but the absolute value of FFAs measured was much larger in the SL2 test. This is probably due to the fact that when the fat phase is finely dispersed in the liquid emulsion more fat is present in the sub-sample used for titration. The similarity of trends in this case arises from the fact that incubated samples of the liquid milk showed relatively small amounts of fat separation from the emulsion and the SL1 test still captured a reasonably representative sample by the inversion protocol.

Figure 4.35: Liquid milk sample shown in Figure 4.34 after homogenisation for 15 seconds.
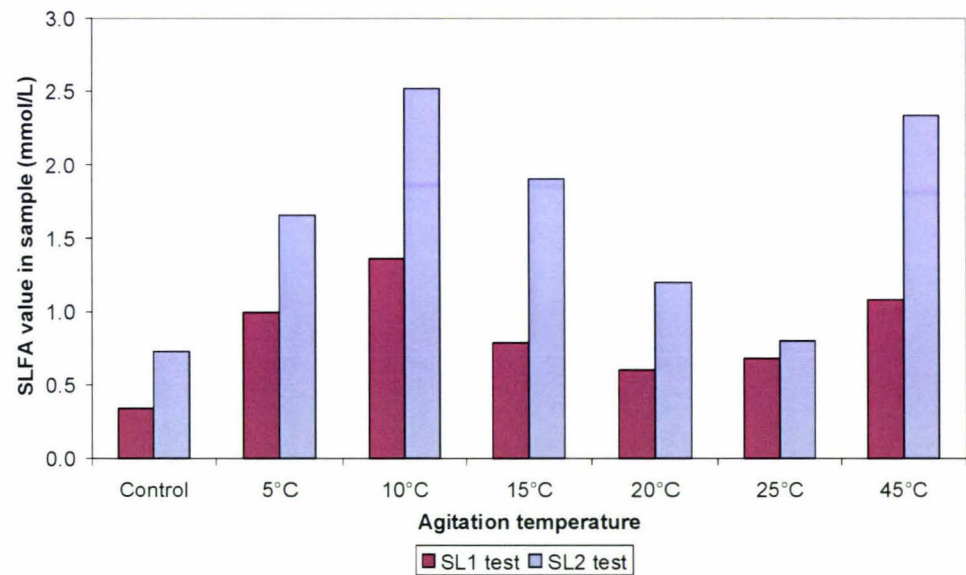
Figure 4.36: Comparison between the SL1 and SL2 test when measuring liquid milk samples from Farmhouse milk agitated at various temperatures for 4 minutes at 2000 rpm. SL1 data from runs C85-90 and SL2 data from runs C97-102.

4.5 Comparison between different test measurements of NMFGM damage

At the beginning of this work, a number of different tests, particularly the solvent-extraction FF and FFA tests were often conducted on milk samples to compare the information given by these tests with the information given by the two new tests

proposed (the selective lipolysis and PSZ tests) to assess some of the consequences of NMFGM damage.

Traditional FFA tests, called OFA tests in the present nomenclature, are usually unable to measure NMFGM damage. For example, Evers et al. (2000b) concluded that no significant increase in damage was found across the evaporators they monitored in the New Zealand dairy industry using the BDI method to determine the OFA content of the milk. Their work was the only one that attempted to analyse the effect of evaporators on NMFGM damage. Results of the SL1 test, presented in Figure 4.8, show on the contrary a significant change in SLFA across the evaporator monitored. Since the OFA of the milk is not likely to change substantially over the 15 minute residence time of the evaporator, the lack of change in the BDI measurement was not surprising but cannot be taken as evidence of no damage. The SL1 test reflects the damage much more successfully. Another example was shown for a pumping system in Figure 4.7. The OFA and IFA did not change throughout the duration of the run but the SLFA increased dramatically.

Thus the simple measurement of FFAs without incubation (i.e. at above 30°C) does not reflect the amount of NMFGM damage. In a significant number of publications the reported storing of the milk samples at low temperatures, typically 5°C for 24 hours, and measuring the increase from the initial FFA value (the OFA in this nomenclature), was called spontaneous or induced lipolysis. Because the activity of lipolytic enzymes at 5°C is low, these results must be differentiated from those following incubation at 30°C or above, as pioneered by Miller & Puhan (1986a). To differentiate the FFAs produced using incubation above 30°C, only these experiments will be referred to as IFA. As shown previously in Figure 4.7, the IFA do not change as the milk flows through processing equipment because, in most cases, the residence time is too short to produce more microbes or enzymes. An exception occurs when the equipment is very dirty and the milk becomes significantly contaminated as it passes through the equipment as shown in runs T24 and T25. Thus the IFA themselves are no better a measure of NMFGM damage done by processing operations than the OFA measurement. A major reason for this failure is the amount of lipolytic enzymes normally present in industrial milk samples. This is often insufficient to transform all of the UPF into FFAs within practical incubation times. Deeth & Fitz-Gerald (1978) were

the first to realise this problem and proposed the addition of an exogenous enzyme to buttress the lipolytic activity of enzymes already in the milk sample. Deeth and Fitz-Gerald (1975, 1978) and Fang (1998) proposed that the added enzymes needed to be substrate-selective in the sense that they should attack only the UPF and not fat globules with an intact NMFGM.

In this sense the present selective lipolysis test attempts to extract the same material as the solvent-extraction FF test, except that in the selective lipolysis test the UPF is first converted to FFAs. Previous authors have questioned the reliability of the FF test (Walstra et al., 1984; Evers et al., 2001). In particular, Evers et al. have shown the test is highly sensitive to the type and amount of solvents used for extraction as well as to changes in procedures of extraction. The reproducibility of FF tests is considered poor. But the procedure for the measurement of FFAs has been adopted by the International Dairy Federation and is considered much more reliable. The averaged results of the SL1 and FF tests for a number of pumping runs (T28-30) in Figure 4.37 show, as an example, that both SLFA and FF increase with duration of pumping but the trends for the FF results are somewhat steeper. Since there are differences in solvents and extractive methods for the FF and SL1 tests the difference might simply reflect differences in extraction efficiency.
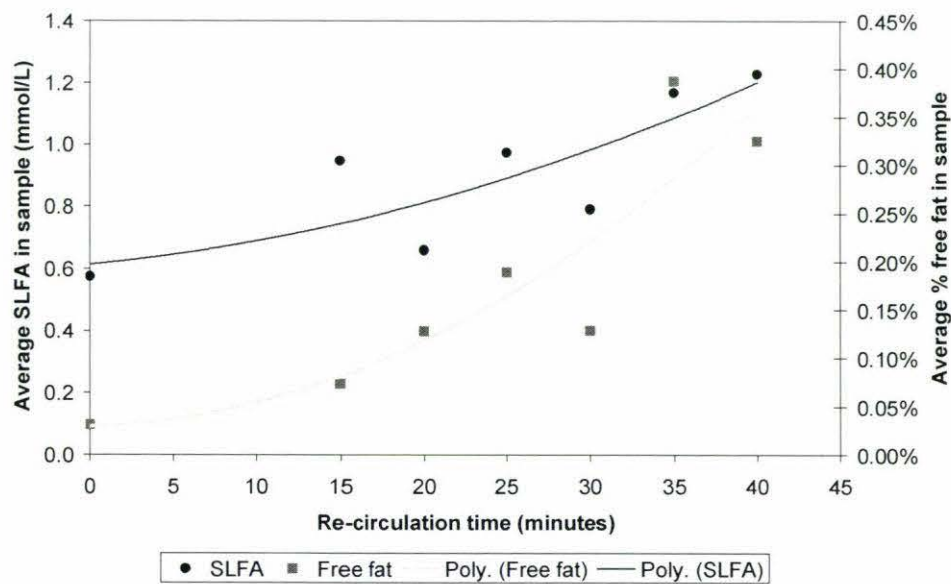
Figure 4.37: Comparison of SLFA and FF results for Farmhouse milk subjected to different durations of re-circulation with a 2800 rpm Fristam centrifugal pump (runs T28-30). Trend lines show second-order polynomial fits.

The PSZ test is a measure of the changing PSD of the fat globules. In some situations, such as the pumping runs of T28-30, the increase in the C+D Zone (Figure 4.38) over the duration of the run has quite a similar trend to the SLFA and FF tests. However, the PSZ test measures a physical consequence, whereas the selective lipolysis test measures a chemical consequence of NMFGM damage. These two pieces of information should be treated as complementary, not necessarily similar. For example, considering runs T28-30, the SL1 test shows that as NMFGM damage increases during the run, more fat can be converted to FFAs in the SL1 test. At the same time, under the conditions of shearing of this run the damaged fat globules tended to coalesce or flocculate, as shown by the increase in the C+D Zone (Figure 4.38).

In other situations the trends in the PSZ and SLFA results may be considerably different. For example the results for the evaporator samples, shown in Figure 4.8 indicate an increase in SLFA across the evaporator due to NMFGM damage. But there was no corresponding increase in the C+D Zone (Figure 4.18) even though there was a decrease in the O Zone. The physical change resulted in disruption, as evidenced by an increase in the A+B Zone.
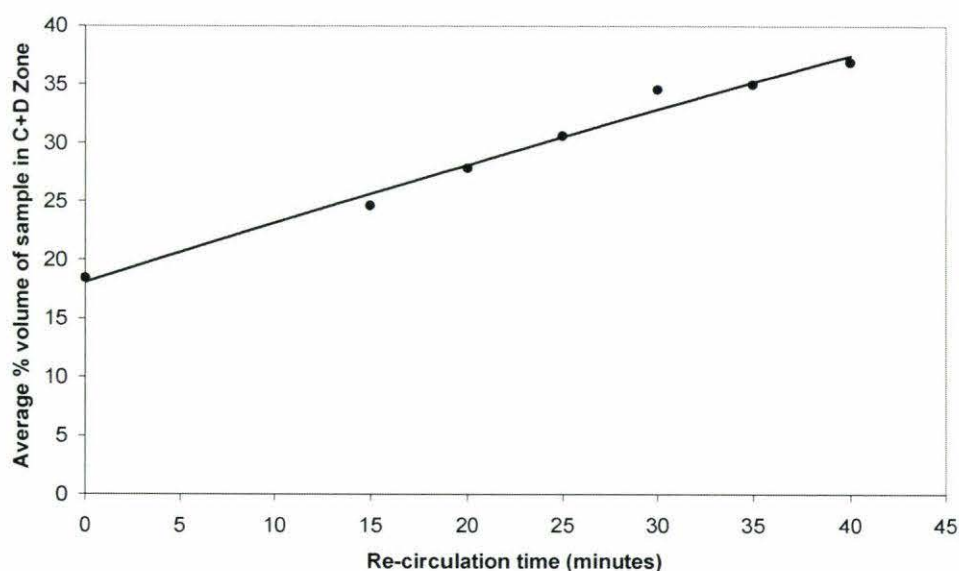
Figure 4.38: Particle size zoning test, C+D Zone results from runs T28-30 reported in Figure 4.37. Trend line shows a second-order polynomial fit.

In experiments on pumping Farmhouse milk through a valve (Section 1 of Chapter 5), NMFGM damage was found only under cavitating conditions. There was no foam when

the system did not cavitate but foam was present under cavitating conditions. The SLFA results for the liquid milk samples showed a small amount of damage and the PSD did not change, indicating that the sizes of the globules were almost completely unaffected. However, the C+D Zone for the foam samples was substantial and increased continuously during the run. This suggests that all of the damaged fat globules had left the milk and moved into the foam. The SLFA results for the foam increased on average compared with the fresh (before-pumping) samples of milk, but showed considerable random variations. Thus, the information from the PSZ and SL1 tests are complementary.

4.6   Conclusion

This chapter reported the work carried out to develop two measures of NMFGM damage: the selective lipolysis (SL1 and SL2) and PSZ tests.

It was shown that the addition of PPL was crucial in making it possible to measure NMFGM damage in samples. Simply measuring the initial FFAs (OFA) or those produced by incubating the sample at 37°C for 6 hours (IFA) after a damage treatment did not show a trend. A consistent trend was only seen in SLFA values.

Pooled data from over 200 duplicate samples showed that the SL1 test was more reproducible than the LFF test of Fang (1998) with a median uncertainty of ± 0.08 mmol/L. This vindicates the decision to change the PPL concentration from 5 to 20 μg/mL sample, and the incubation conditions from 30°C for 24 hours to 37°C for 6 hours. The inconsistencies with the LFF test were attributed to microbial contamination and increased production of lipolytic enzymes during incubation (Section 4.1.6). The SL1 test was shown to be sensitive enough to detect NMFGM damage produced in a pumping rig or in an industrial evaporator. Work is still required to improve the test with regards to operations involving heating.

Pooled data from 37 duplicate samples showed a low median uncertainty in all 5 zones of the PSZ test, ranging from 0.07 to 0.26 volume %. In percentage terms this equated

to less than 3% uncertainty, except for the D Zone, which was due to the typically small size of the D Zone in undamaged milk.

The PSZ test could also differentiate NMFGM damage created in a pumping rig or an industrial evaporator.

Three sample handling issues were identified that can reduce the accuracy and precision of these two tests:

1.   Damage during sampling.

2.   Creaming of the sample in storage.

3.   Unrepresentative sampling due the production of foaming or churned fat.

Results showed that sampling at high velocity could induce an increase in the FF level in the milk. It was also shown that storage of samples for 24 hours at 5°C did not result in significant creaming in samples awaiting analysis of the PSD.

It was clearly shown that measurements of the results of foaming and churning need to be included in measurements of NMFGM damage as they contribute a significant portion of the damage. The SL2 test protocol has improved the efficiency of sampling the incubated milk, but more work is required to refine this protocol.

Comparisons between the SL1, PSZ, and solvent-extraction FF test of Fang (1998) showed that all three tests gave different profiles of NMFGM damage. The conclusion was that the tests are complementary and no one test gives the full picture of NMFGM damage in a sample. The present work yields a more comprehensive and complex picture of the damaged system than could be obtained with any single test in previous attempts to measure consequences of NMFGM damage.

Chapter 5
Mechanical Factors affecting NMFGM Damage

The physical factors affecting NMFGM damage can be separated into two types:

1.   Process operations and

2.   Operating variables, which are a function of the operating conditions and equipment design.

Several common operations within milk powder processes were investigated in this work:

Section 5.1 presents the results of pumping experiments that investigated variables such as pump speed, air inclusion, temperature, shear rate through a pipeline and valve cavitation.

Section 5.2 presents the results of heating experiments using both direct and indirect heating operations. The variables investigated were temperature, temperature difference and pressure difference.

Section 5.3 presents the results of agitation experiments that investigated variables such as temperature, agitation time and agitation speed.

Finally, Section 5.4 presents the results of evaporation experiments that investigated the process of removing of water by a falling film evaporator.

The detailed experimental protocols have already been presented in Section 4 of Chapter 3.

5.1   Pumping experiments

Four different pumping rigs were used in these experiments, each designed to study rigorously a particular variable. In the first experiments (runs C63-70), the large-volume pumping rig was used to identify the effect of air inclusion and splashing created in a recirculation system. Samples were analysed using the PSZ test. More work was then conducted with this rig, using both the PSZ and SL1 tests, in runs C103-106. In order to isolate the effect of pumping speed with respect to NMFGM damage, a second single-pass pumping rig was built where the number of passes of the milk through the pump could be counted (runs J01-06). A third pumping rig, named the BT1000, with interchangeable piping capillaries of different sizes, was used to investigate the effect of high shear rate in pipelines, in runs J08-20. The small-volume pumping rig was used to study the effect of valve cavitation on NMFGM damage, in runs P01-04.

5.1.1   Re-circulation of Farmhouse milk with and without air inclusion and splashing

50 litres of Farmhouse milk was re-circulated under various conditions of operation in the large-volume pumping rig and sampled regularly over time as described in detail in Section 4 of Chapter 3.

In the first experiments (C63-70) samples were taken during the runs and analysed with the PSZ test. The results are summarised in Figures 5.1 to 5.3. When the milk was re-circulated with the pump operating at 2800 rpm there was a significant change in the particle size distribution (PSD) of the milk. The C+D Zone increased with time, even under the action of pumping alone, as shown in Figure 5.1. However, the A+B Zone showed very little change and in general tended to decrease slightly with time when a change was observed (Figure 5.2). Thus damage during pumping was reflected by flocculation and/or coalescence. The effect of splashing of the milk from a height of 1 metre, or including air in the system at 0.5 L/min (3% air by volume at a flow rate of 3 m^3/hr), was to accentuate the rate and level of damage. The worst case scenario was recirculatory pumping with both air inclusion and splashing (Figure 5.1).
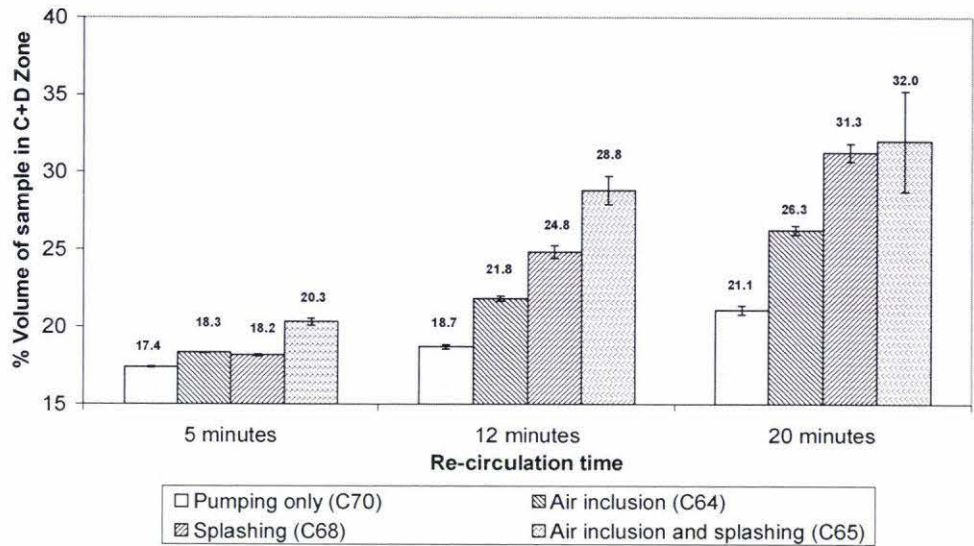
Figure 5.1: Effect of re-circulation of Farmhouse milk using a Fristam centrifugal pump running at 2800 rpm on the C+D zone. Runs C64, C65, C68 and C70. Error bars reflect the uncertainty in duplicate samples. 0 time value averaged for all runs (3 measurements) was 17.1 volume %.
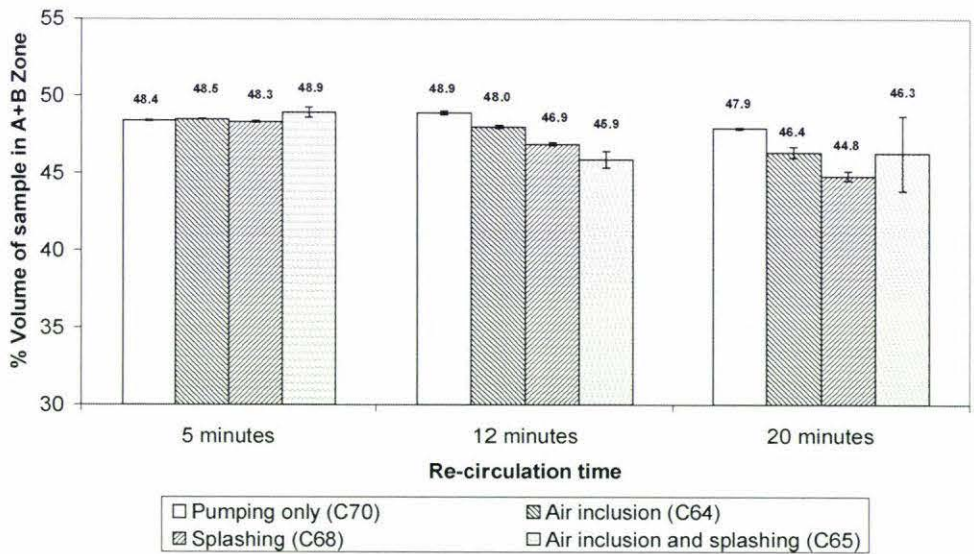
Figure 5.2: Effect of 2800 rpm pumping on the A+B Zone from runs C64, C65, C68 and C70, reported in Figure 5.1. Error bars reflect the uncertainty in duplicate samples. 0 time value averaged for all runs (3 measurements) was 48.7 volume %.

When the pump was operated under the same conditions but with a speed of 1960 rpm the increase of damage with re-circulation time was much smaller (Figure 5.3).
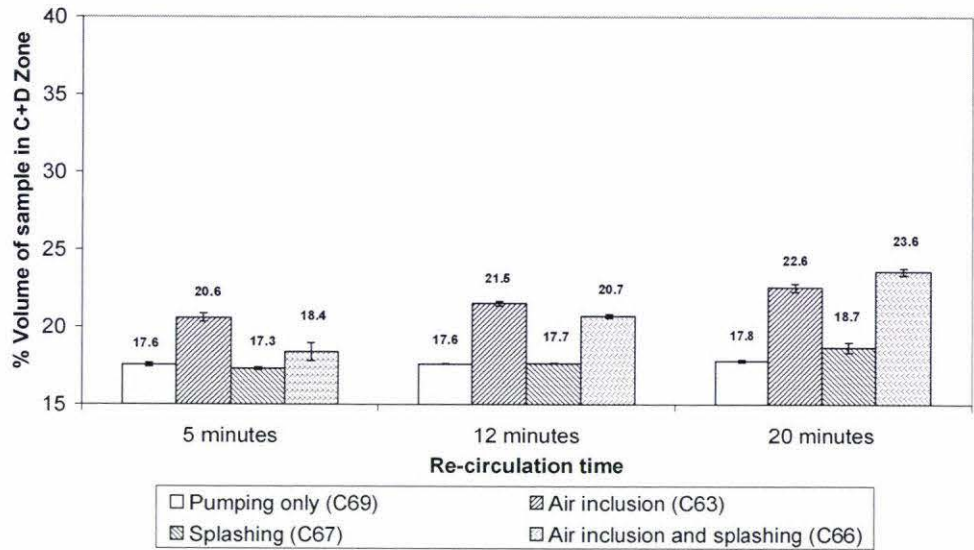
Figure 5.3: Effect of 1960 rpm pumping on the C+D zone from runs C63, C66, C67 and C69 in the experiment reported in Figure 5.1. Error bars reflect the uncertainty in duplicate samples. 0 time value averaged for all runs (3 measurements) was 17.1 volume %.

To confirm that the operation of the system was stable and the protocol of operation reliable, replicate runs were conducted on the same batch of milk on the same day (T26-27) to minimise any variation owing to the raw material used. The replicate runs showed that the level of damage obtained under a given set of operating conditions was highly reproducible (Figure 5.4). The reliability of the PSZ test measurement has already been discussed in Section 2 of Chapter 4.
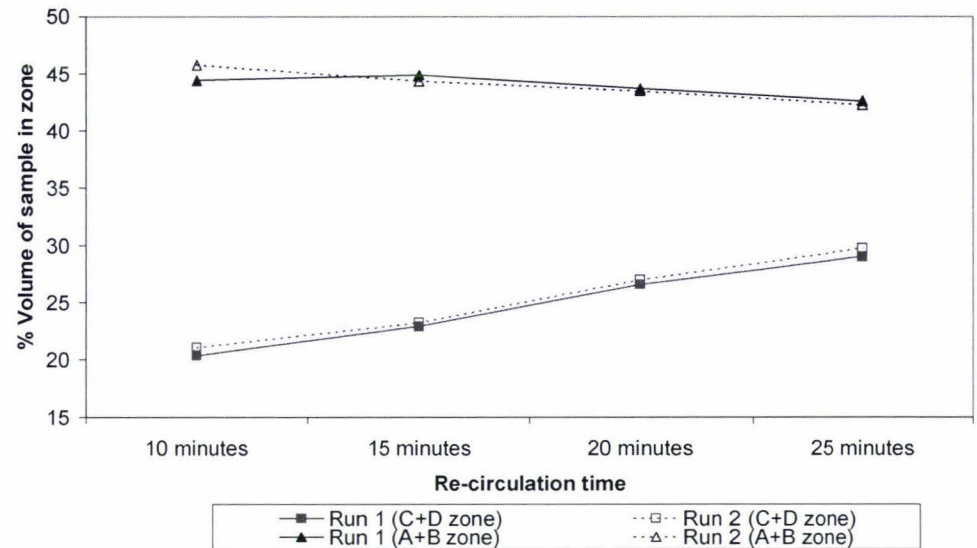
Figure 5.4: Repeatability of two identical runs, using the same batch of Farmhouse milk, re-circulated around a tank with a Fristam centrifugal pump. Runs T26-27.

Foaming was observed in all pumping runs with splashing (C65-68) and/or with air inclusion (C63-66) but foaming was not observed with pumping alone (C69-70). Since the fat contained in the foam consisted mainly of damaged fat, as reported in Section 3 of Chapter 4, it was not surprising that the incidence of foaming was observed more with operations that damaged the milk fat globules to a greater extent. The mass of fat in the foam was only a fraction of the total fat in the milk; therefore the weighted contribution of damaged fat in the foam to the rest of the milk was still relatively small. The nature of the foam differed between the runs with splashing and those with air inclusion. Visual observation of the foam indicated that splashing tended to create foam that was less dense.

The distribution of damaged fat among three phases, liquid, foam and solid, presented a particular challenge in representative sampling, as discussed in Section 3 of Chapter 4. The selective lipolysis test, SL1, used throughout most of this work, relied on gentle inversion of the sample bottle to mix the foam or churned fat with the liquid milk. During the drafting of the thesis a number of new runs were made (C103-106) where damage in the liquid and in other phases was measured separately and the weighted contributions added. This gave a much better estimate of total damage done to the original milk fat globules. These runs used the improved selective lipolysis test, version 2 (SL2). The pump was again operated at 2800 rpm and air was fed at 0.5 L/min to

match conditions in runs C64 and C70 but the plate heat exchanger was used to control the temperature of the re-circulating milk to two values, 10°C and 20°C. Samples of liquid milk, the foam and the churned fat where it could be separated from the foam and the liquid were taken and analysed with the PSZ, free fat (FF) and SL2 tests.

The PSD changes with pumping time at 10°C in runs C103 and C106 (Figures 5.5 and 5.6) are quite similar to those obtained in runs C64 and C70, also run at approximately 10°C (Figures 5.1 and 5.2). When air was included the C+D Zone volumes were in general greater than when milk was pumped without air, both at 10°C and 20°C (Figures 5.5 and 5.7). This confirms the observations made in runs C64 and C70 (Figure 5.1).
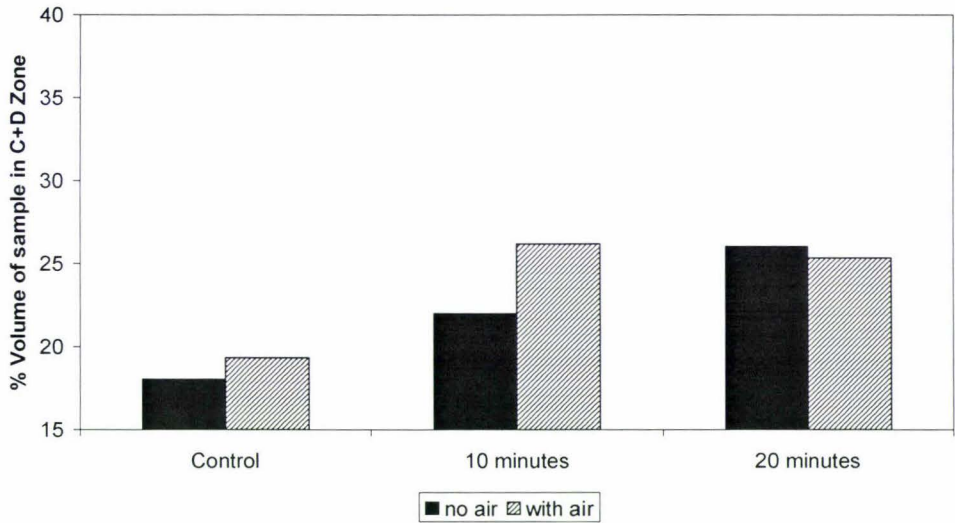
Figure 5.5: Effect of re-circulation of Farmhouse milk at 10°C using a Fristam centrifugal pump running at 2800 rpm on the C+D zone of liquid milk samples. Runs C103 and C106.
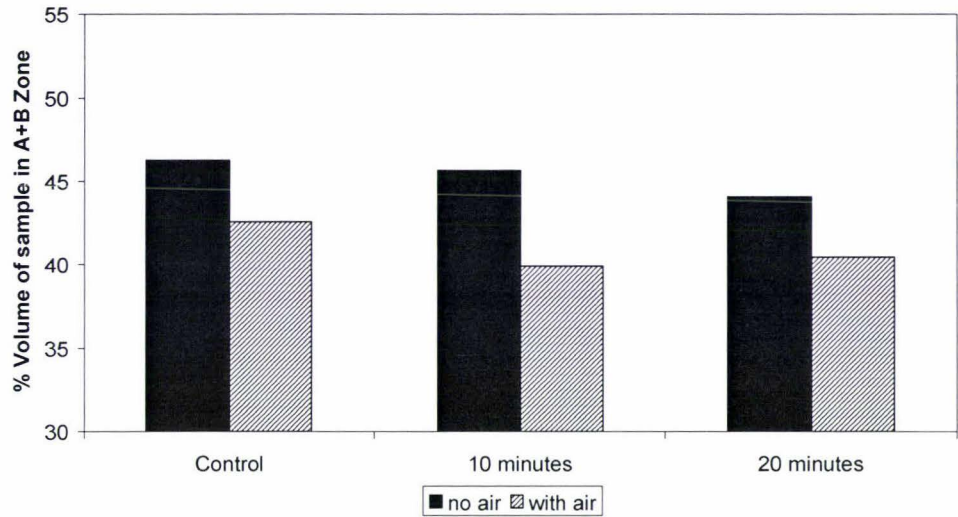
Figure 5.6: Effect of pumping at 10°C on the A+B Zone from runs C103 and C106, reported in Figure 5.5.
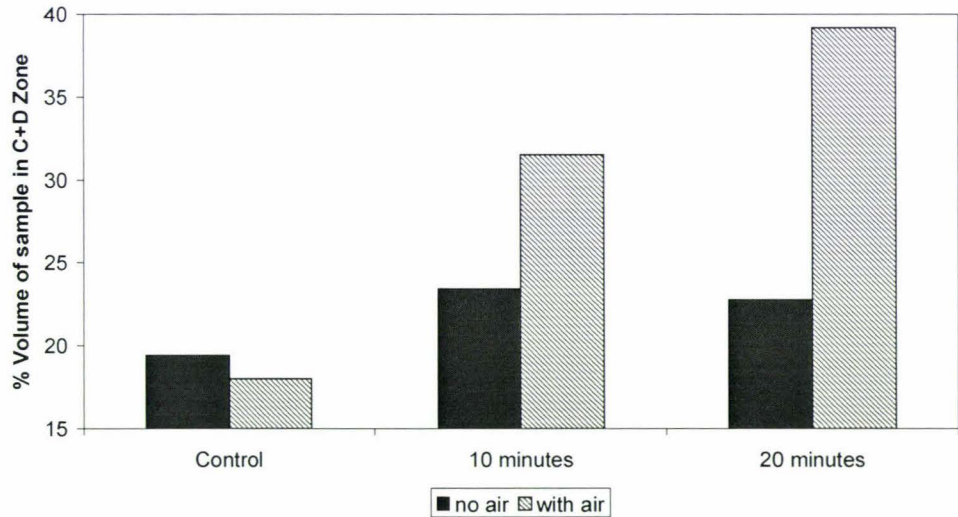
Figure 5.7: Effect of re-circulation of Farmhouse milk at 20°C using a Fristam centrifugal pump running at 2800 rpm on the C+D zone of liquid milk samples. Runs C104 and C105.

The FF results also support the conclusion that air inclusion creates much more NMFGM damage, both at 10°C and 20°C (Figures 5.8 and 5.9). These observations are in agreement with the work of Aule & Worstorff (1975) and Miller & Puhan (1986b) who also investigated the role of air in NMFGM damage caused by pumping. It is interesting to note that the FF in the liquid alone was much smaller than the FF

detectable in the whole system after the contribution of churned fat and foam had been added (Figures 5.8 and 5.9). It is unfortunate that some authors in this field of research do not describe their sampling procedure in sufficient detail (e.g. Miller & Puhan, 1986b; Kessler & Fink, 1992) to determine whether their sampling was confined to the liquid alone, which sounds possible, or whether they devised a technique of proportional sampling of both the liquid, and either foam or churned fat. Aule & Worstorff (1975) reported that they did try to "take out a representative sample from milk with churned-out fat" but found that the "conformity between parallel samples was poor" but also mentioned that other methods (Diller-Zulauf & Wirsekara, 1943-1944; Tamsma et al., 1959; Lagoni & Peters, 1959) "gave still poorer results". Clearly the method of sampling has a significant effect on the amount of FF detected.
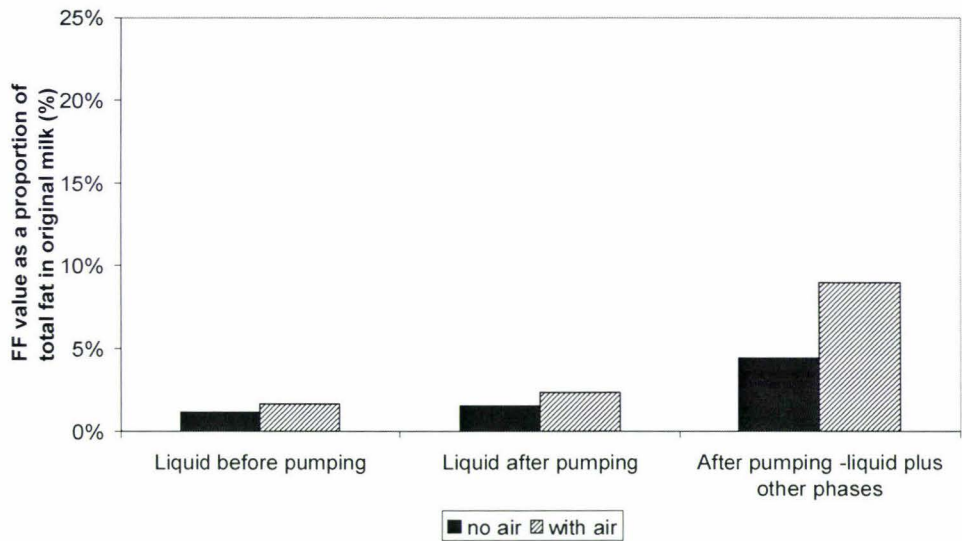
Figure 5.8: Effect of pumping at 10°C on solvent-extractable FF in runs C103 and C106, reported in Figure 5.5.
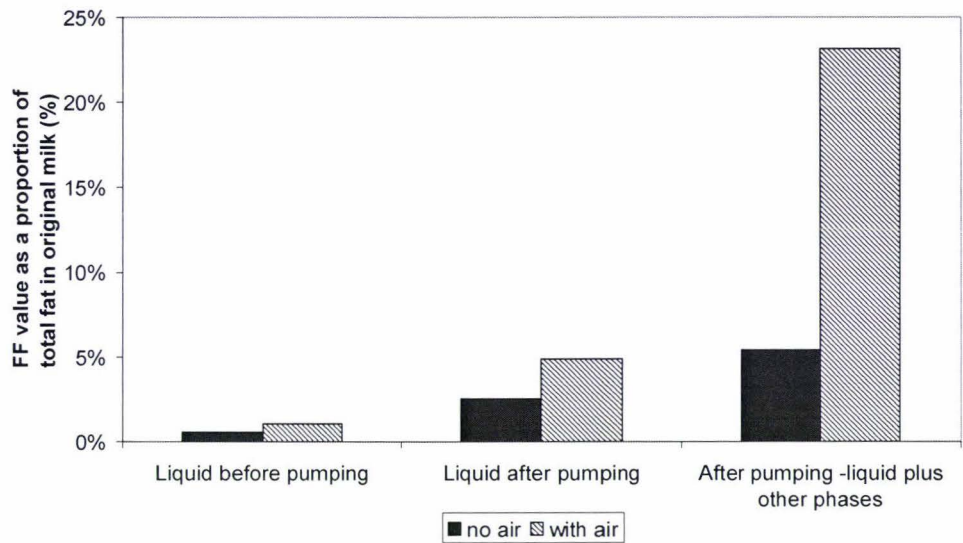
Figure 5.9: Effect of pumping at 20°C on solvent-extractable FF in runs C104 and C105, reported in Figure 5.7.

5.1.2 Effect of pumping temperature

The FF results in Figures 5.8 and 5.9 show that pumping at 20°C created much more damage than at 10°C. It should be noted that the increase in the FF of the liquid phase caused by 10°C and 20°C pumping was small, but became much larger when the contributions of the foam and churned fat were added. At 10°C, pumping resulted only in foaming, but at 20°C churned fat could be seen incorporated in the foam, as evidenced by the yellowish tinge in Figures 5.10 and 5.11. The foam was recovered and weighed. The relative proportion by mass of foam and liquid milk in the four runs are shown in Table 5.1.

Figure 5.10: Photograph of foam layer after Farmhouse milk was pumped at 10°C for 10 minutes (Run C103).

Figure 5.11: Photograph of foam layer after Farmhouse milk was pumped at 20°C for 10 minutes (Run C104). Note yellow patches of churned fat.

Table 5.1: Effect of pumping temperature and inclusion of air on amounts of foam and/or churned fat produced from a 50 litre batch of Farmhouse milk (runs C103-106).

Run #	Milk Temperature	Air Inclusion ?	Mass of foam plus churned fat	% mass of foam plus churned fat	Visual observation
C103	10°C	no	274g	0.5%	foam only
C104	20°C	yes	744g	1.4%	foam and churned fat
C105	20°C	no	128g	0.2%	foam only
C106	10°C	yes	312g	0.6%	foam only

When air was included, the weighted contribution of the foam with churned fat to overall FF values at 20°C was significantly higher than the corresponding effect of air inclusion at 10°C, which did not result in churned fat (Figures 5.8 and 5.9). A sample calculation is given in Appendix C4.

This is in general agreement with the results of Kessler & Fink (1992) who pumped cream at temperatures in the range 5°C-55°C and found a maximum FF value at roughly 25°C. However their data were very scattered, presumably because the method of sampling did not succeed in capturing representative proportions of churned fat. Herbst et al. (1984) noted that the FF and FFAs in samples treated by stirring and pumping decreased as the temperature increased from 5°C to 10°C then increased to a point where a marked "oiling off [occurred] at 20-30°C".

The SL2 test results for pumping at 10°C and 20°C are more complex (Figures 5.12 and 5.13). At 10°C the SLFA value in the liquid sample almost doubled after 20 minutes of recirculatory pumping. The weighted contribution of SLFA from the foam phase was added to the SLFA obtained for the liquid phase to give a better estimate of total SLFA produced from the original batch of milk (see sample calculation in Appendix C3). For pumping both with and without inclusion of air the overall SLFA was always greater than that for the liquid phase alone.
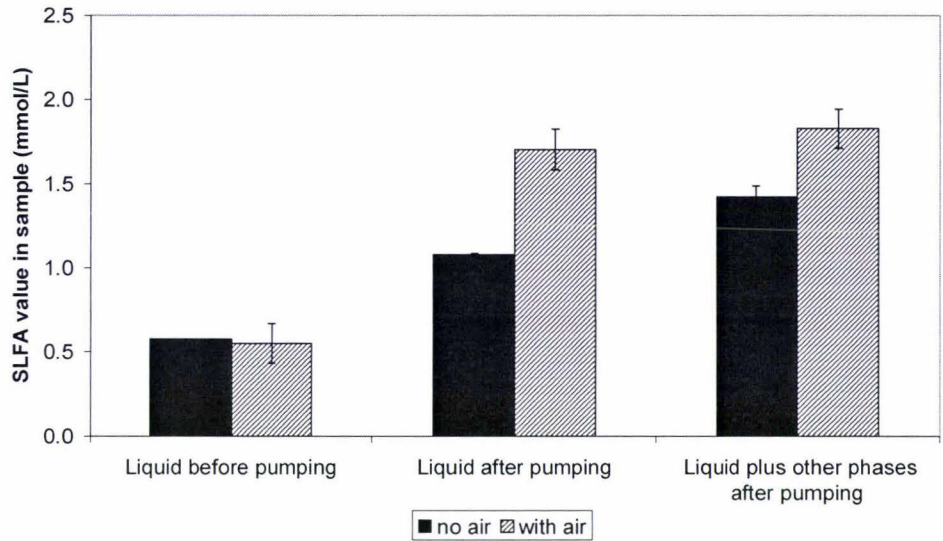
Figure 5.12: Effect of pumping at 10°C on SLFA from runs C103 and C106, reported in Figure 5.5.

The SLFA produced in the liquid milk sample pumped at 20°C also increased from the initial value, and this proportional increase was slightly smaller than the proportional increase at 10°C, with air inclusion, and slightly higher without air inclusion (Table 5.2). The relative increases in SLFA in the total sample caused by pumping in runs C103-C106 is summarised in Table 5.2. It can be seen clearly that at both temperatures the inclusion of air does produce a greater increase in SLFA than pumping alone. Thus, both the FF (Figures 5.8 and 5.9) and SLFA data agree on the influence of air on pumping damage done to the NMFGM.

However, the actual value of SLFA after pumping at 20°C with air inclusion was actually lower than the SLFA obtained without air inclusion. This may have been partly due to the fact that the SLFA in the sample before pumping was different in runs C104 and C105. Another factor was the presence of vortices in the runs. These vortices varied in intensity between each run. In runs C103-104 these vortices reduced the flow rate through the rig for a few seconds, but in runs C105-106 they did not. The likely effect of this variable was an increase in air inclusion in runs C103-104 and the production of more foam. This appears to have been the case as shown in Table 5.3. Though absolute values cannot be certain, the trends were not significantly affected, since the two runs with air inclusion were both much higher than those without air inclusion.
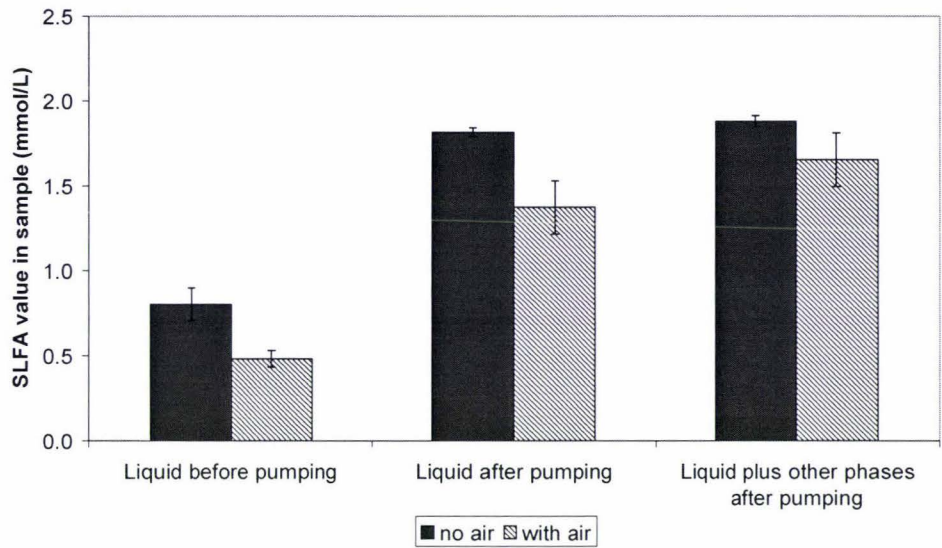
Figure 5.13: Effect of pumping at 20°C on SLFA from runs C104 and C105, reported in Figure 5.7.

Table 5.2: Effect of pumping temperature and addition of air on SLFA in Farmhouse milk (runs C103-106).

Run #	Temperature (°C)	Air Inclusion?	Initial SLFA (mmol/L)	Liquid SLFA (mmol/L)	Relative increase from initial	Final SLFA (mmol/L)	Relative increase from initial
C103	10	no	0.58	1.08	88%	1.42	147%
C104	20	yes	0.48	1.37	185%	1.65	243%
C105	20	no	0.80	1.82	126%	1.88	135%
C106	10	yes	0.55	1.70	210%	1.83	233%

Herbst et al. (1984) noted that "at 20-30°C, marked oiling-off occurred with FF content attaining [the] highest and FFA content attaining [the] lowest values". Oiling-off is evidence of a large amount of FF. This observation agrees with the data in Figures 5.8-9 and 5.12-13, which indicate that the SLFA measurement and FF measurement in the liquid phase give two different pictures of the effect of pumping temperature on NMFGM damage.

The weighted contribution of SLFA in the foam, or foam plus churned fat, was determined and incorporated in the results as discussed in Section 3 of Chapter 4. The result is shown as the third set of bar graphs in Figure 5.12 and in Figure 5.13. The contribution of the foam and churned fat to the total SLFA produced from the original

milk was relatively small whereas their contribution to the FF results (plotted in Figures 5.8 and 5.9) was much more substantial. The percentage contribution of the foam/churned fat to the FF and SLFA values is shown in Table 5.3.

Table 5.3: Proportional contribution of foam/churned fat phase to the total FF and SLFA value in Figures 5.8-5.9 and 5.12-5.13, from samples in runs C103-106.

Run #	Contribution of foam/churned fat to total SLFA value	Contribution of foam/churned fat to total FF value
C103	24%	66%
C104	17%	95%
C105	3%	53%
C106	7%	74%

The author believes that the technique of application of the SL2 test to churned fat still needs to be improved, and therefore the results in Table 5.3 still do not reflect the true nature of the phenomena investigated. Since the churned fat is essentially fat stripped completely of its NMFGM, as occurs in the churning process in the manufacture of butter (Walstra et al., 1999), it should be possible to convert all of this fat to FFAs with a selective enzyme given enough incubation time. When all of the fat content in the foam or the churned fat is hypothetically converted into SLFA (sample calculation in Appendix C5) and this weighted contribution added to the SLFA of the liquid sample the trends shown in Figures 5.14 and 5.15 become very similar to the trends observed by the FF test, as shown in Figures 5.8 and 5.9.
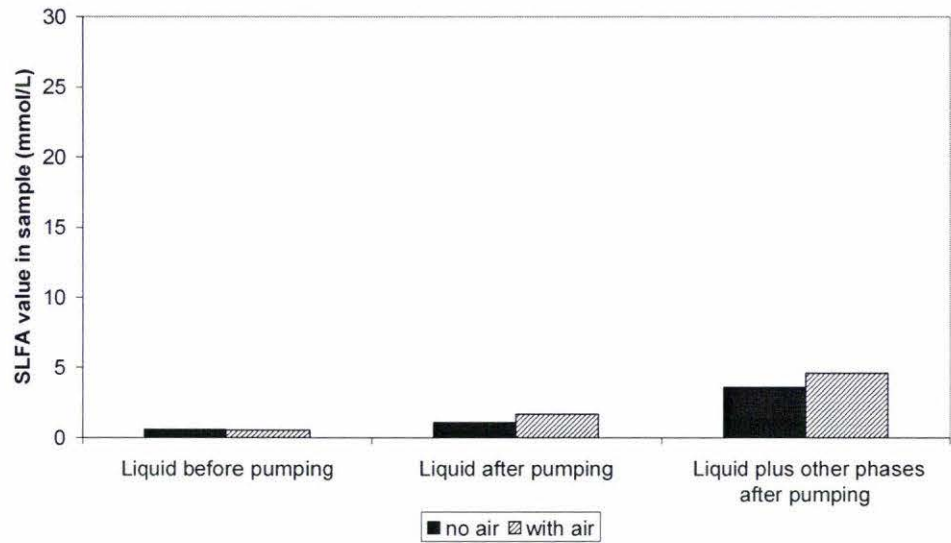
Figure 5.14: Modified SLFA values from runs C103 and C106 (10°C) reported in Figure 5.12. SLFA contributions from the other phases were calculated by assuming the total fat content of that phase could be converted to FFAs. Note that the maximum value on the Y-axis in this graph is 12 times larger than that on the Y-axis of Figure 5.12.
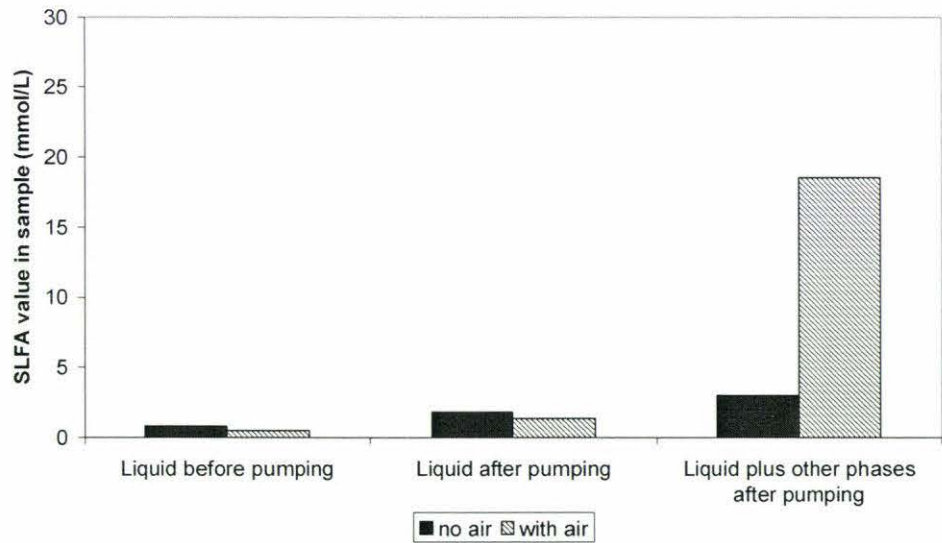
Figure 5.15: Modified SLFA values from pumping runs C104 and C105 (20°C) reported in Figure 5.13. SLFA contributions from the other phases were calculated by assuming the total fat content of that phase could be converted to FFAs. Note that the maximum value on the Y-axis in this graph is 12 times larger than that on the Y-axis of Figure 5.13.

5.1.3 Effect of pump speed

The results of runs C63-70 suggested that pump speed was an important variable in the control of NMFGM damage. Escobar & Bradley (1990) found a higher FFA level after

pumping raw milk with a 3500 rpm pump compared with a 1750 rpm pump, and Rudzik (1987) found FFA levels and FF levels increased when pumping was performed for 3 minutes or more at 1000, 2000 and 3000 rpm. Kammerlehner & Kessler (1980) found an increase in cream from 50% to 90% in centrifugal FF when centrifugal, eccentric-screw, piston, "cog-wheel", peristaltic and diaphragm pumps were operated at performance levels between 10%-100%. The performance levels were set by continuously adjusting "speed gears" for rotary pumps, adjusting the stroke of the piston pump or throttling a valve downstream of the centrifugal pump.

Upon more careful analysis of the pumping systems used and previous literature data (Kammerlehner & Kessler, 1980; Miller & Puhan, 1986b; Rudzik, 1987; Escobar & Bradley, 1990; Fang, 1998) it appeared that most experimental set-ups could not show clearly what parts of pumping systems contributed most to NMFGM damage. For example, Fang (1998) claimed that NMFGM damage could be created in a controlled manner by passing milk from one tank to another through a cavitating pump. However upon closer examination the piping system was found to incorporate two globe valves located at the pump inlet, and it is not quite clear whether the pump or the valves were cavitating. Because the work of Fang was focussed on the effect of NMFGM damage on fouling by whole milk in heat exchangers a detailed analysis of the contribution to damage by the different components of the pumping rig was not available.

A number of runs in the present work were conducted specifically to look at the effects of the pump and pumping speed on NMFGM damage (J02-06). The single-pass rig described in detail in Section 4 of Chapter 3 was operated at 5 speeds: 725, 870, 1015, 1160 and 1305 rpm in run J02 at a temperature of approximately 10°C, and in runs J03-06 at 2 speeds (870, 1305 rpm) that were replicated four times. No significant foaming was observed in all runs.

The SLFA results for runs J03-06 are shown in Table 5.4. Since the uncertainty in the SL1 test is much greater than in the PSZ test, as discussed in Chapter 4, the acceptable levels of uncertainty found in these runs give confidence that reproducible amounts of damage could also be achieved in this rig without the need to also measure particle size.

Table 5.4: Reproducibility of results obtained using the single-pass pumping rig with Farmhouse milk passed between two tanks using an Anema-Sneek centrifugal pump (runs J03-06). Samples were analysed using the SL1 test.

Pump Speed	Control	870 rpm	1305 rpm
Average value (mmol/L)	0.81	1.05	1.33
95% confidence interval (mmol/L)	±0.11	±0.12	±0.18
Percentage uncertainty	±13%	±11%	±14%

The PSD results of run J02 (Figure 5.16) show that in relative terms the importance of the C+D Zone increased as the pumping speed increased. On the other hand the relative importance of the A+B Zone decreased. This indicates that the system was flocculating more and more as pumping speed increased. At the same time the difference in SLFA values in the liquid before and after the test (Figure 5.17) also increased with pumping speed. Thus, the trends obtained in the single-pass and large-volume pumping rigs were compatible. NMFGM damage was increased with increased duration of pumping or the number of passes. Damage was also increased with pump speed.
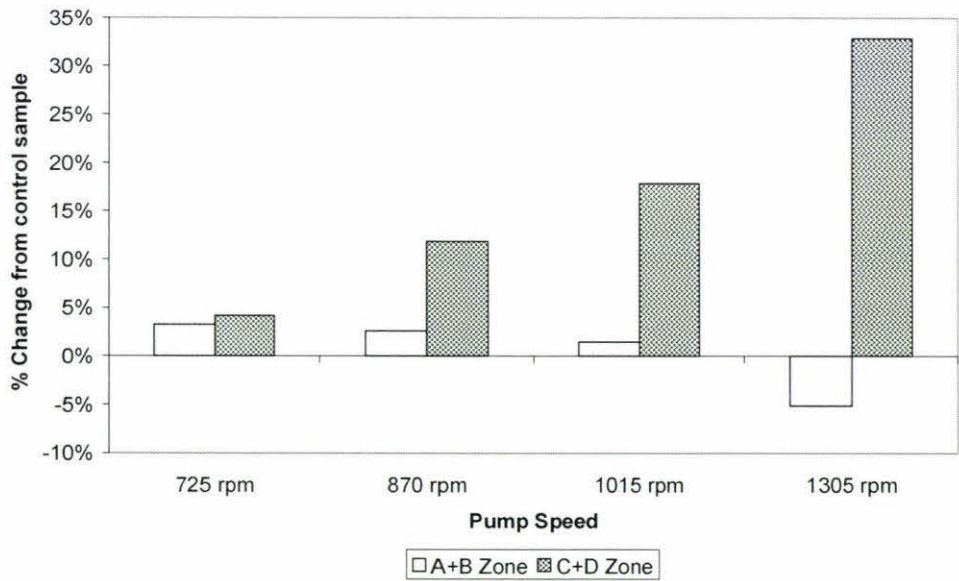
Figure 5.16: Change in PSD due to pump speed of an Anema-Sneek centrifugal pump (run J02). Control values of A+B and C+D Zone were 49 and 18 volume %, respectively.
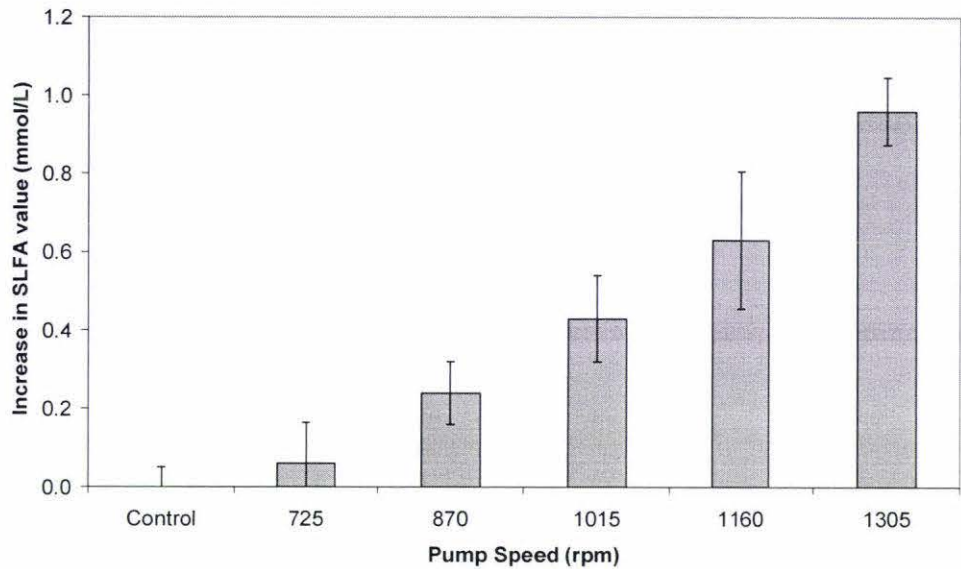
Figure 5.17: Increase in SLFA in run J02, reported in Figure 5.16. Error bars show the uncertainty in triplicate measurements. Control value was an average of 0.86 mmol/L.

It should be noted that increasing the pump speed automatically increases the flow rate in the rigs. Table 5.5, for example, shows the relationship between pump speed and flow rate in run J02. As they stand, the experiments conducted do not provide enough information to separate the effect of pump speed and flow rate. In other words, one cannot say whether damage is created by the higher friction between the pump impeller and the milk at higher pump speeds or whether it is due to the higher friction in the piping system that is caused by higher flow velocities.

Table 5.5: The flow rates measured in run J02 (Anema-Sneek centrifugal pump).

Pump speed (rpm)	725	870	1015	1160	1305
Flow rate (L/hr)	350	1070	1870	2540	3460

5.1.4   Effect of shear rate

Hinrichs (1994; 1998) determined a critical shear rate at which NMFGM damage occurred in a Couette-flow viscometer. This critical shear rate was found to be a function of both fat content and temperature. The results were transferred to the flow of cream in pipes (under the influence of compressed air, which avoided damage by pumping). Hisserich & Reuter (1984) used a Couette-Spalt apparatus to determine the critical energy flux at which FFAs in the milk, measured after incubation at 6°C for 20

hours, started to increase. The effect of changing the shear rate in the piping system with milk was analysed separately in the BT1000 rig (runs J08-20) described in Section 4 of Chapter 3.

Four replicate runs were made at two shear levels (J13-20) to test the reproducibility of damage in this rig. Again, NMFGM damage was measured by the amount of SLFA produced. In this case, the 95% confidence limits for each flow velocity are shown in Table 5.6. Clearly these were much higher than for the previous two pumping rigs. It was later determined that the poor reproducibility was probably due to sampling errors arising because the monopump could not be drained between runs (since running a monopump dry damages it). Thus the relatively large uncertainties probably reflect cross-contamination between runs.

Table 5.6: Replication uncertainty in the BT1000 pumping rig with Farmhouse milk re-circulated with a Dressler monopump. Two pairs of experiments were carried out. Samples were taken from the same batch of milk (runs J13-20).

Velocity in capillary	Control	0.78 m/s	Control	9.1 m/s
Average SLFA value (mmol/L)	1.3	1.4	0.88	1.5
95% confidence interval (mmol/L)	±0.5	±1.0	±0.20	±0.3
Percentage uncertainty	±38%	±70%	±23%	±19%

As the capillaries decreased in diameter from 7.42 mm to 1.76 mm the wall shear rate increased from 550 s^{-1} to 41,400 s^{-1}. There was a general increase in the difference between the SLFA of liquid samples taken before and after a re-circulation treatment, as shown in Figure 5.18. At the same time there was an increase in flocculation/ coalescence as evidenced by the increase in the C+D Zone and decrease in the A+B Zone with shear rate, as shown in Figure 5.19.

The increase in SLFA owing to shear ranged from 12% to 113%. This increase seems to have been much more substantial than the increase of 3% FF obtained by Hinrichs (1998) when cream was pushed through a 10 mm diameter, 164 metre-long pipe by compressed air. However, direct numerical comparison is not possible because this

work presents relative increases in SLFA while Hinrich's paper reports absolute differences in % FF. Nonetheless it is evident that the amount of damage produced in the present experiments is much larger than the damage produced in the experiments of Hinrichs. This is probably related to the high shear rate in the present experiments, 550 s^{-1} to 41400 s^{-1}, compared with the shear rate that can be calculated from the flow velocity and pipe diameter data to Hinrichs of 112 to 208 s^{-1}.
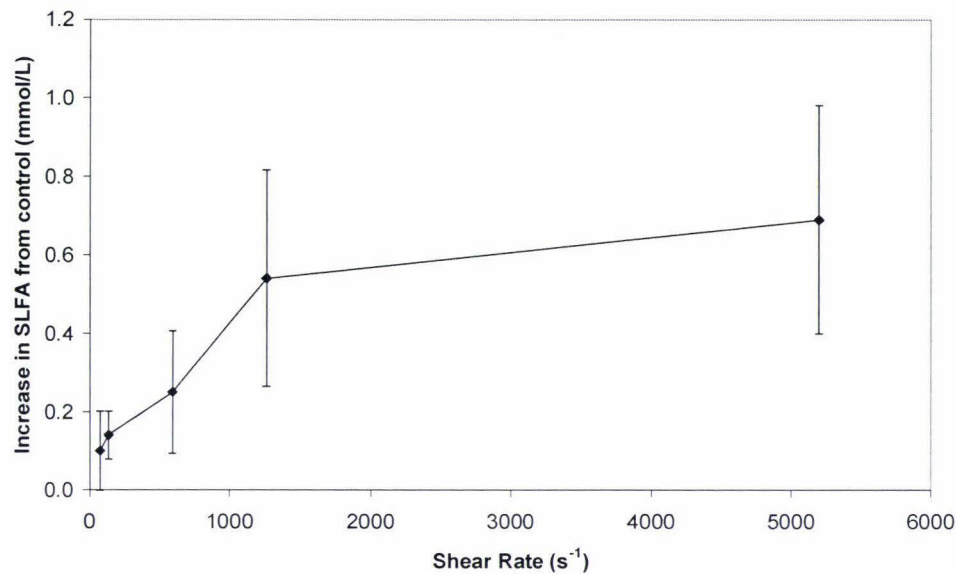
Figure 5.18: Increase in SLFA value as a function of shear rate in the BT1000 pumping rig (runs J08-12). Error bars show the uncertainty in triplicate measurements. Control SLFA values (mmol/L) of milk not passed through the rig for each run were 0.86 (550 s^{-1}), 1.24 (1040 s^{-1}), 1.15 (4700 s^{-1}), 1.13 (10100 s^{-1}) and 0.61 (41400 s^{-1}).
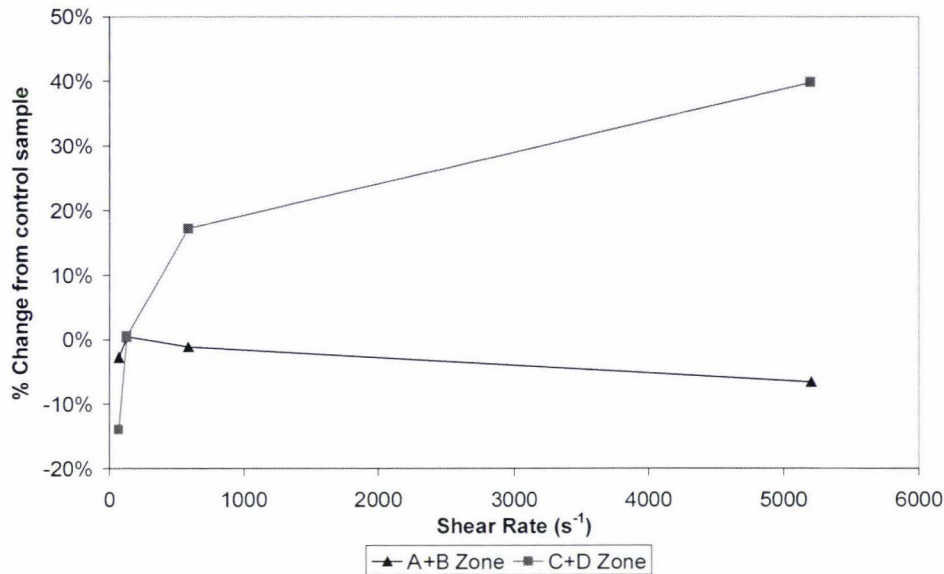
Figure 5.19: PSD changes owing to shear rate in runs J08-12, reported in Figure 5.18. Control values for the A+B Zone were (from left to right for 550, 1040, 4700 and 41400 s^{-1}) 48.6, 47.5, 47.8 and 47.9 volume %, respectively. Control values for C+D Zone were (from left to right) 17.5, 17.8, 17.6 and 17.5 volume %, respectively.

5.1.5   Effect of valve cavitation

Valves are pipeline accessories that tend to generate a fair amount of turbulence because the path of the fluid changes direction several times within the valve, especially in globe and needle valves. Intuitively, this situation could lead to a contribution to NMFGM damage by valves. As noted before, it is difficult to conclude from the data of Fang (1998) whether the damage was created by cavitation in the valve or in the pump of his system. The single-pass pumping rig used in experiments J02-06 was modified by incorporating a valve upstream of the pump. This was done because it made valve cavitation more likely than if it was placed downstream of the pump. Two valves were tested: a needle valve and a globe valve. Pressure measurements were made before and after the valves using internationally recognised standard configurations for pressure taps to monitor the level of cavitation (Instrument Society of America, 1995). A number of runs were made with water pumped at various flow rates by varying the pump speed and valve setting independently. The pressure drop over each valve was used to calculate the cavitation index, using Equation 5.1 (Ross Valve Manufacturing Company Incorporated, 2004).

$$\sigma = \frac{(P_2 - P_V)}{(P_1 - P_2)} \qquad\qquad (5.1)$$

where:

σ = cavitation index

P_1 = valve inlet static pressure

P_2 = valve outlet static pressure

P_V = absolute fluid vapour pressure of liquid at inlet temperature

The process of measurement is set out in detail in a booklet by the Instrument Society of America (1995). Comparing the cavitation index obtained under different conditions (summarised in Table 5.7) with published norms of assessment, shown in Figure 5.20, allowed identification of the conditions of operation of the pumping to achieve preset levels of cavitation rig with different valves. The lower the cavitation number, the more severe the level of cavitation was likely to be.

Table 5.7: Illustration of different conditions of valve cavitation achieved using small-volume pumping rig. Vapour pressure of water was taken as 0.338 psia at 20°C (Perry et al., 1997).

Valve type	Valve Position	Pump Speed (Hz)	Inlet pressure (psia)	Outlet pressure (psia)	Cavitation index*	Flow rate (L/hr)
Needle	10% open	35 Hz	17.25	1.59	0.08	180
	50% open	20 Hz	17.25	10.00	1.33	53
Globe	5% open	35 Hz	17.25	1.59	0.08	1070
	5% open	20 Hz	17.25	10.00	1.33	560
Gate	4% open	35 Hz	16.82	1.59	0.08	1020
	5% open	20 Hz	17.20	9.57	1.21	480

*Cavitation index calculated using Equation 5.1.

Cavitation Guide
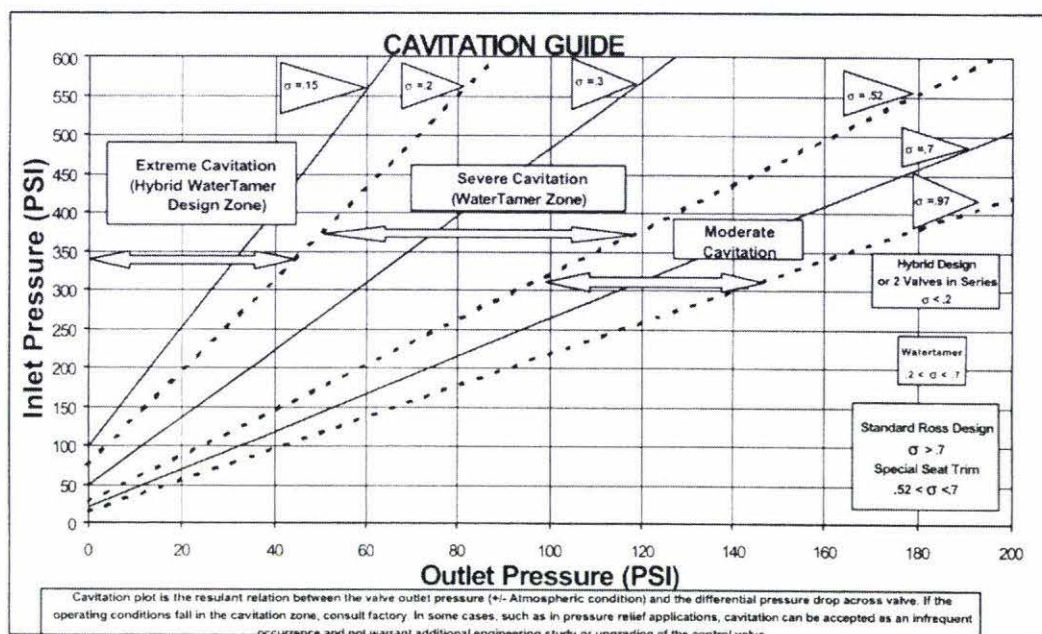
Figure 5.20: Cavitation conditions as a function of inlet and outlet pressures for a valve. Source: (Ross Valve Manufacturing Company Incorporated, 2004).

After a number of preliminary experiments, during which analysis and measurement techniques were refined, there was only time to choose runs with distinctly high and low cavitation indices. Two valves (needle and globe) were tested in the final runs (P01-04) with Farmhouse milk and samples were taken at 5, 10, 15 and 20 minutes for measurement by the PSZ and SL1 tests. The cavitation index was calculated from pressure measurements across the valve using Equation 5.1. Two runs were made with each valve.

It was important to ensure that the pump did not cavitate in this experiment. Cavitation in a pump occurs only when the pressure at the eye of the impeller is lower than the vapour pressure of the fluid. The pressure at the valve exit was quite close to the inlet pressure of the pump because the pressure drop between that point and the pump inlet was quite small since the connecting pipe was large (1 inch internal diameter). Thus, it is unlikely that the pump itself would have cavitated since the pressure of the fluid, water or milk, was above the vapour pressure of water. (The saturated vapour pressure of water is 2.3 kPa absolute (0.34 psia) at 20°C (Perry et al., 1997)).

It was observed that the milk did not foam when the cavitation number of the valve was high (above 1.0). When the cavitation number of the valve was low (below 0.27), and therefore representing what is considered extreme cavitation according to Figure 5.20, foaming was observed. A reasonable explanation for this observation is that the pressure at some point in the system was low enough for bubbles to form in the milk. These could have been water vapour bubbles produced from the pressure drop within the valve or air bubbles coming out of solution due to the pressure being almost zero (according to Henry's Law, where the mole fraction of solute (air) in the liquid phase is proportional to the partial pressure of the solute in the gas phase). In cavitation the bubbles would collapse again after the pressure had increased above the vapour pressure (i.e. after the valve, in this case), but there is no information on how a foam may stabilise this effect. It is known that air or nitrogen mixed with milk causes foaming and an increase in NMFGM damage (Fitz-Gerald, 1974). In either case, whether air or water vapour, the presence of foam indicated that NMFGM damage was present.

When the cavitation number of the valve was high the SLFA values of the milk samples did not change with time, within the uncertainty limits of the measurements. This was true for both the needle and the globe valve tested (Figure 5.21). Similarly, the C+D Zone remained constant with re-circulation time (Figure 5.22).
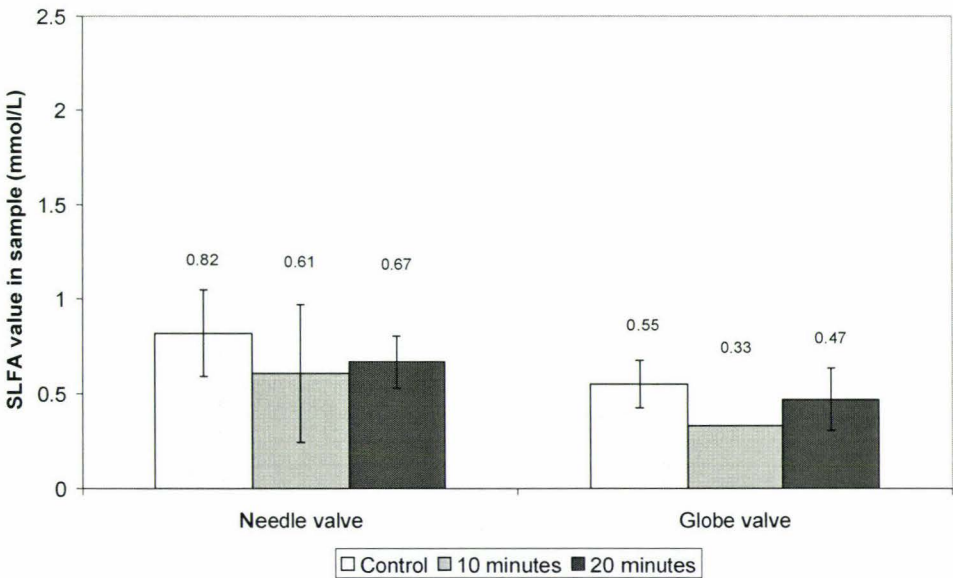
Figure 5.21: SLFA values for liquid milk samples of Farmhouse milk re-circulated around a pumping rig under non-cavitating conditions. Runs P01-02. Cavitation index was 1.9 and 3.9 for the needle and globe valve, respectively. Error bars indicate the uncertainty in triplicate measurements.
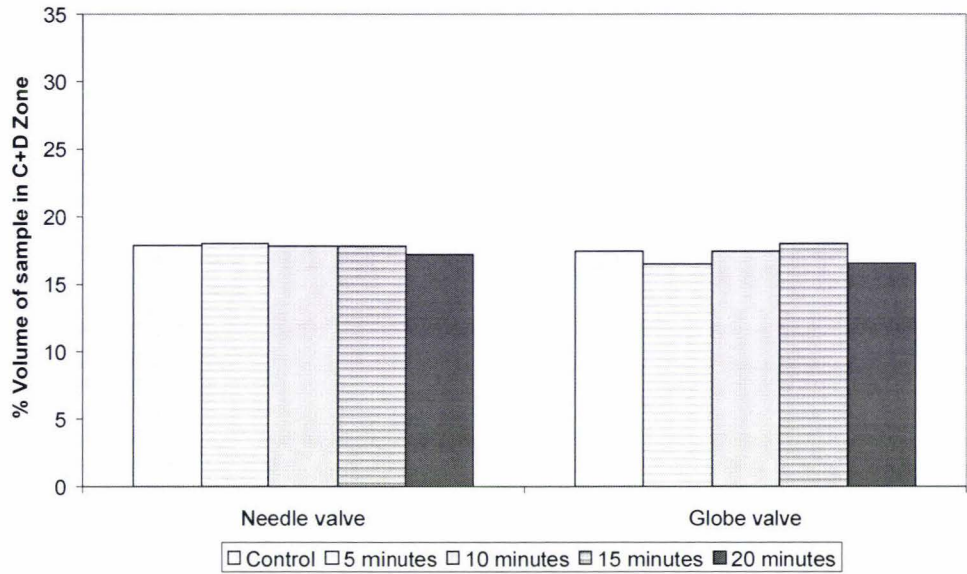
Figure 5.22: PSZ test values from runs P01-02, reported in Figure 5.21.

Surprisingly, the C+D Zone for the milk samples showed no consistent trend in the runs (P03-04) at low cavitation number (Figure 5.23). However, there was a clear increase in the SLFA values of the liquid sample with time (Figure 5.24).
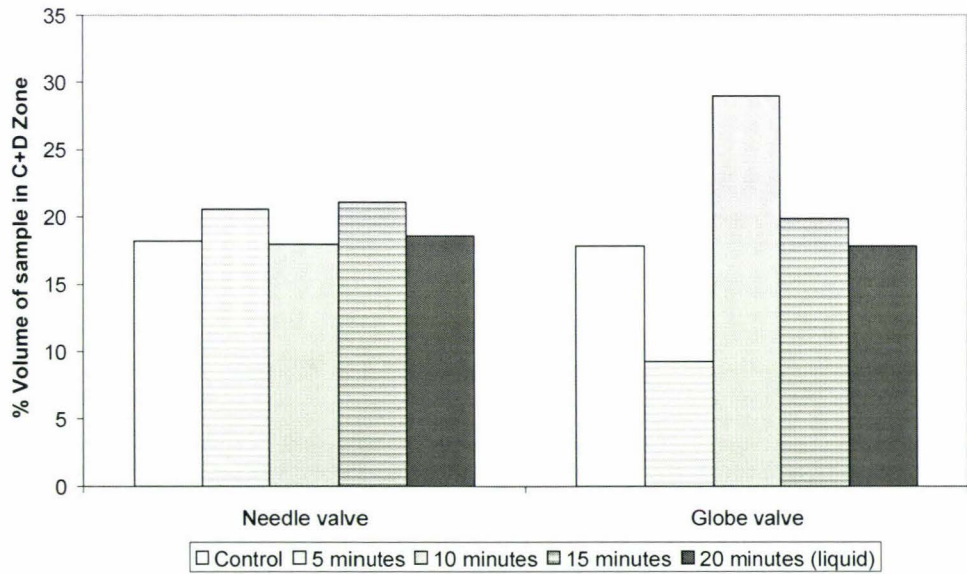
Figure 5.23: PSZ test values from liquid milk samples of Farmhouse milk re-circulated around a pumping rig under cavitating conditions. Runs P03-04. Cavitation index was 0.03 and 0.26 for the needle and globe valve, respectively.
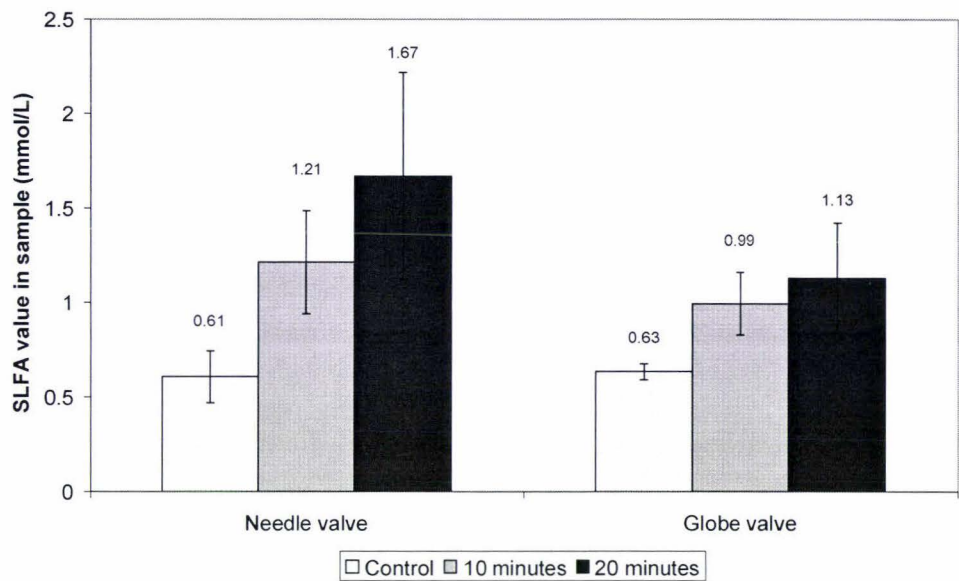
Figure 5.24: SLFA values from runs P03-04, reported in Figure 5.23. Error bars indicate the uncertainty in triplicate measurements.

The foam in these runs was also sampled and its PSD measured. The C+D Zone was much larger in the foam sample (Figure 5.25) and this was consistent with a much higher SLFA concentration in the foam than in the liquid sample. Nonetheless, because the mass of foam was small, the proportion of damage in the foam was not significant compared with the total amount of NMFGM damage when the liquid and foam fractions were combined, as shown in Figure 5.26.
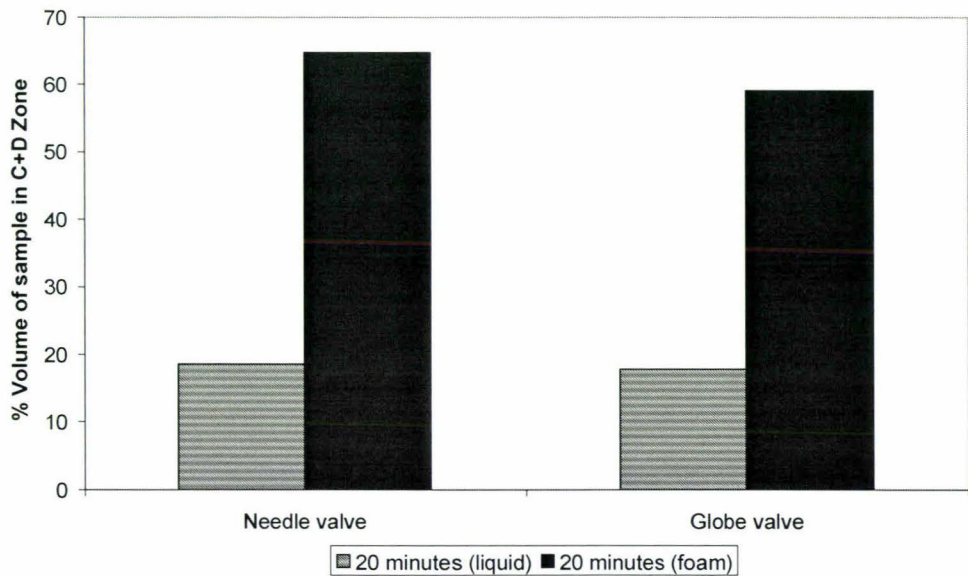
Figure 5.25: PSD comparison between samples of liquid milk and foam from runs P03-04, reported in Figure 5.23.
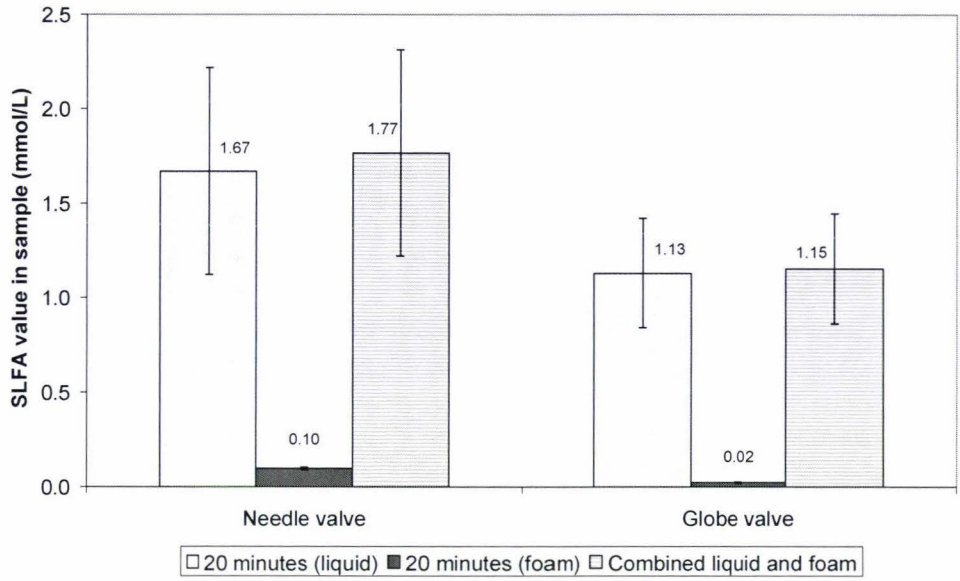
Figure 5.26: SLFA contribution from liquid and foam samples from runs P03-04, reported in Figure 5.24. Error bars indicate the uncertainty in triplicate titrations.

All this information indicates that damage to the original milk system occurred only at low valve cavitation numbers. Because the foam was not very homogeneous sampling replication was quite poor, although in general NMFGM damage can be seen to increase with re-circulation time. At high valve cavitation numbers, no foam was detected at all. Results for the globe valve show the same trends as those for the needle valve.

5.2 Heating experiments

Thermal processing is a core feature of milk powder manufacture. Examples of heating operations are:

1. Pasteurisation, to control the level of microbes in the milk,
2. Preheating, to condition the functionality of the proteins,
3. Evaporation, to remove most of the water, and
4. Concentrate heating, to manipulate the rheological properties of the concentrated milk.

These operations can be conducted in heat exchangers, especially plate heat exchangers and tubular heat exchangers, by allowing the heat to flow indirectly from a hot source through a wall into the milk, or by mixing hot steam directly with the milk, for example

through a direct steam injection (DSI) unit. Two series of experiments were performed, one with indirect heating in a plate heat exchanger, and another with direct heating by a DSI unit.

5.2.1   Changes in FFAs upon heating

Significant variations in the SLFA values of milk samples were observed when the temperature of the milk was varied between the range 4°C and 95°C. Earlier experiments with DSI units performed before the SL1 test was developed show that the LFF values (runs C08-11, reported in Figure 5.27) were quite constant at 3.25 mmol/L at temperatures below 60°C, dropped sharply within a narrow range of temperatures between 65°C and 80°C and started to level off again at 0.75 mmol/L for higher temperatures. This experiment was repeated using the SL1 test and the results are shown in Figure 5.28 (runs C80-84). Whilst it is difficult to define the exact shape of the change, it is clear that the SLFA value dropped before levelling out to a lower plateau of approximately 1.0 mmol/L.
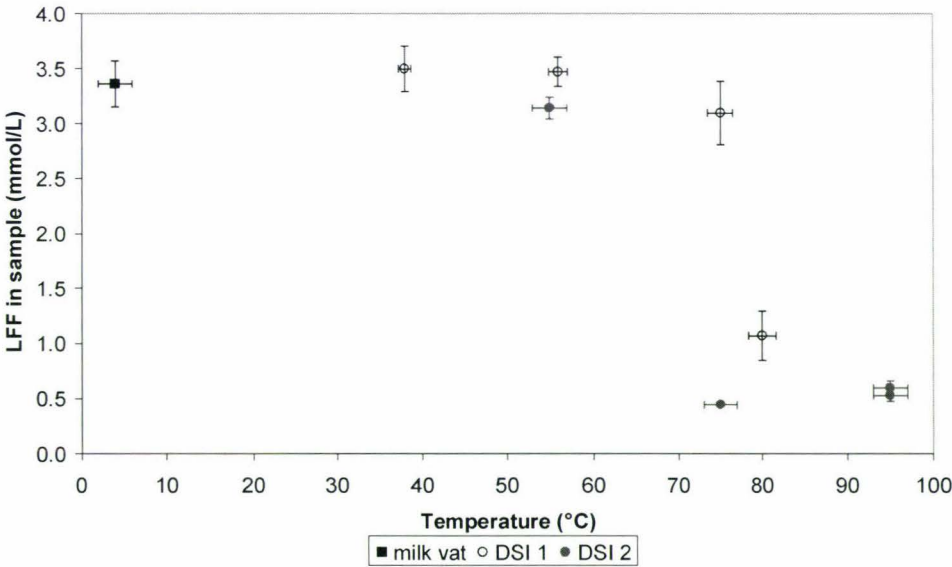
Figure 5.27: Effect of direct heating with a DSI unit on lipolysable FF (LFF) content of Farmhouse milk. Runs C08-11. Vertical error bars indicate the uncertainty in triplicate titrations. Horizontal error bars indicate random fluctuations in the controlled temperatures.
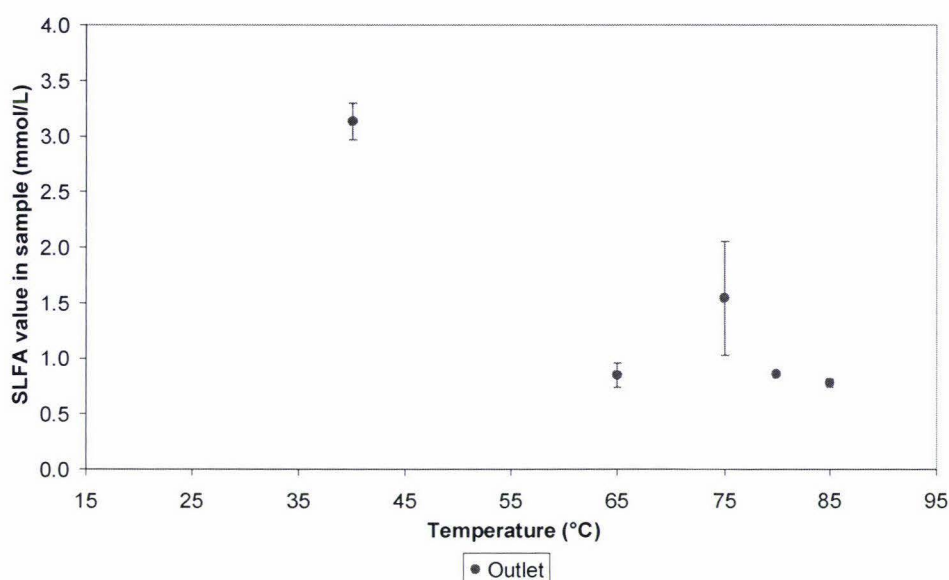
Figure 5.28: Evolution of SLFA with temperature of Farmhouse milk in direct steam injection heating experiments. Runs C80-84. Error bars show the uncertainty in duplicate samples.

This result was perplexing as some damage to the NMFGM as the milk flowed through the heaters would be expected, especially the DSI units which involved the implosion of a significant number of steam bubbles. According to the principle of the selective lipolysis test, presented in Chapter 3, one would have expected this damage to be reflected by an increase in the SLFA value of the milk as it passed through the heater. It was suspected therefore that heating reduced the activity of some of the lipolytic enzymes already present in the original Farmhouse milk. An analysis of the variations in IFA with temperature would have revealed clearly how the original enzymes present in the milk had been affected by heating. Unfortunately these IFA measurements were not carried out in the three series of experiments because the practice of making routine measurements of SLFA, IFA and OFA on the same sample was applied only later in the course of this work.

In order to better understand the LFF and SLFA trends in Figures 5.27 and 5.28, two runs (T24, T25) were performed with heating in a plate heat exchanger. In one run (T25) the milk contained a very low microbial count, because the plant was rinsed with nitric acid immediately before the run, the other run (T24) did not have this rinsing step. It should be noted that even in run T24 the plant had been cleaned several days before by the last user, but significant microbial growth still occurred in the intervening days before the experiment. This phenomenon was proved by microbial counts of the water

used to flush plants that remained idle for several days after CIP (Trinh et al., 2002). Standard plate counts for runs T24 and T25 showed that the original milks contained respectively $2x10^4$ and $7x10^3$ cfu/mL. OFA, IFA and SLFA measurements were made on milk samples from the milk vat (at 4°C), milk entering the plate heat exchanger (at 10°C) and milk leaving the plate heat exchanger (at 80°C). All measurements were made in duplicate (Figure 5.29).

The IFA values in Figure 5.29 show an increase from the vat to the pump outlet. This was likely due to the milk picking up a large number of microbes between these sample points as shown in the plate counts reported previously. But there was a significant decrease in IFA values between samples from the plate heat exchanger inlet and exit. The pattern of SLFA increase and decrease between these three samples is very similar to the pattern of IFA increase and decrease. The difference between the SLFA and IFA values for the sample entering the heat exchanger in experiment T24 was 1.33±0.19 mmol/L and the difference for the sample exiting the heat exchanger was 1.65±0.19 mmol/L. These two values are not significantly different within the uncertainty of measurement. One may conclude, therefore, that the change in SLFA values across the heat exchanger in run T24 was simply due to change in the IFA values.
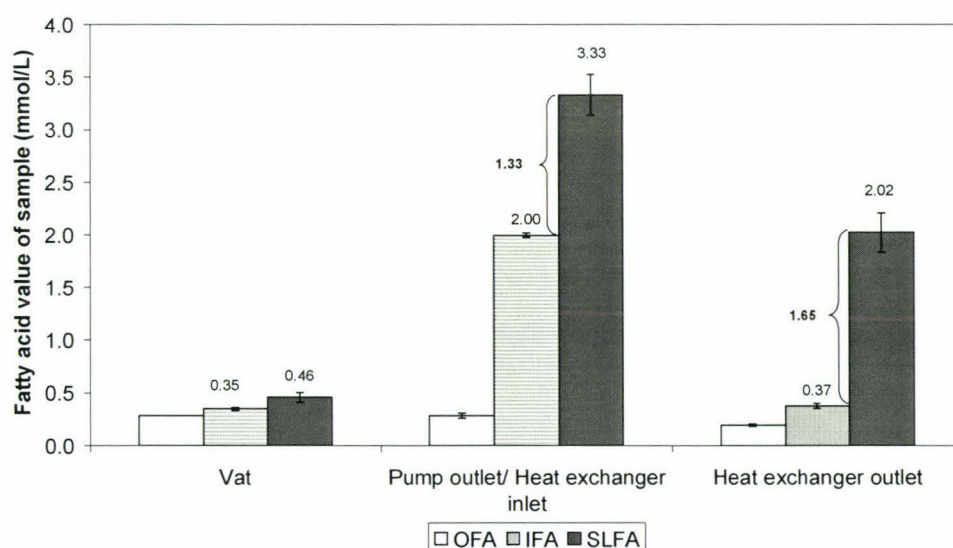
Figure 5.29: Effect of heating with a plate heat exchanger on SLFA, IFA and OFA values (run T24) in Farmhouse milk. Values in bold show the difference between the SLFA and IFA value. Error bars indicate the uncertainty in duplicate samples.

Since pig pancreatic lipase (PPL) was added to milk samples after they were removed from the heat exchanger and cooled, the drop in SLFA clearly cannot be attributed to the inactivation effect of heat on the added PPL. Hence, the effect of heat seen on the SLFA is due to the inactivation of the enzymes or the bacteria that produce these enzymes in the milk. Milk at the vat had a microbial count of 7×10^3 cfu/mL but picked up microbial contamination on its way to the plate heat exchanger to give an increased level of 3×10^6 cfu/mL before entering the plate heat exchanger. The level decreased again to 2×10^2 cfu/mL at the plate heat exchanger exit. The values of OFA of the three samples did not differ greatly because within the short residence time between the vat and the plate heat exchanger exit any lipolytic enzymes in the milk could not have had enough time to convert fat to FFAs.

The IFA results clearly reflect the microbial count in the sample. It can be concluded, therefore, that the IFA results in run T24 reflect the lipolytic activity of microbial enzymes present in the milk. The difference between the SLFA and IFA values remained constant between samples before and after the plate heat exchanger (Figure 5.29) basically because there was no mechanical damage by heating. Low mechanical damage from indirect heating is shown by the PSZ test results reported in Figure 5.30 (Section 5.2.2). One may conclude that the difference between SLFA and IFA values of these samples represents the amount of FFAs produced by the action of the PPL on the unprotected fat (UPF) – that is the difference is the amount of FAUPF (fatty acids from UPF). By inference, the IFA results would reflect the attack of microbial lipases mainly on protected fat (PF) – producing FAPF (fatty acids from PF). FAPF have the ability to cause undesirable flavours, even in milk not mechanically damaged.

It is widely reported in the literature that some microbial lipases are extremely resistant to heat. The present results indicate that only a small fraction of such enzymes were heat stable and that a substantial proportion of them could be deactivated if the temperature of the milk reached a critical value (65°C to 80°C) – where the lower level of SLFA values were observed (Figures 5.27 and 5.28).

Present pasteurisation practice, where the efficacy of pasteurisation is based on assays of alkaline phosphatase, targets pathogenic microbes, and uses only 72.5°C for 15 seconds or the equivalent. In most industrial pasteurisation systems there is a holding

section after the milk has reached the desired temperature. In the present work milk was sampled directly after the plate heat exchanger or the DSI units. Thus the effect seen seems to be much more related to threshold temperature than holding time. The data in Figures 5.27 and 5.28 indicate that this temperature could be between the upper and lower SLFA values of the SLFA-temperature curve. A slight increase in temperature would allow the process to operate clearly within the lower SLFA range and therefore provide the added advantage of reduced rancid flavour due to FFAs. New Zealand regulations already allow for these higher pasteurisation temperatures, so the effect on current practice would be minimal.

Since the microbial populations in different plants may differ slightly it is probable that the mixture of lipolytic enzymes produced would also vary. The exact temperature required for good flavour stability would require a determination of the SLFA-temperature curve on site in the plant.

5.2.2   Changes in particle size distribution upon heating

There was very little change in the PSD curve of Farmhouse milk as it passed through a plate heat exchanger. Figure 5.30 (runs C84-88) illustrates the large amount of data that confirm this observation. Note that the set of A+B and C+D data for milk entering the heat exchanger (10°C) coincides with the line exiting the plate heat exchanger. This observation simply reflects the fact that the shear rate exerted on the milk in the plate heat exchanger is not very large at the types of flow rates encountered in the pilot plant used in this work.
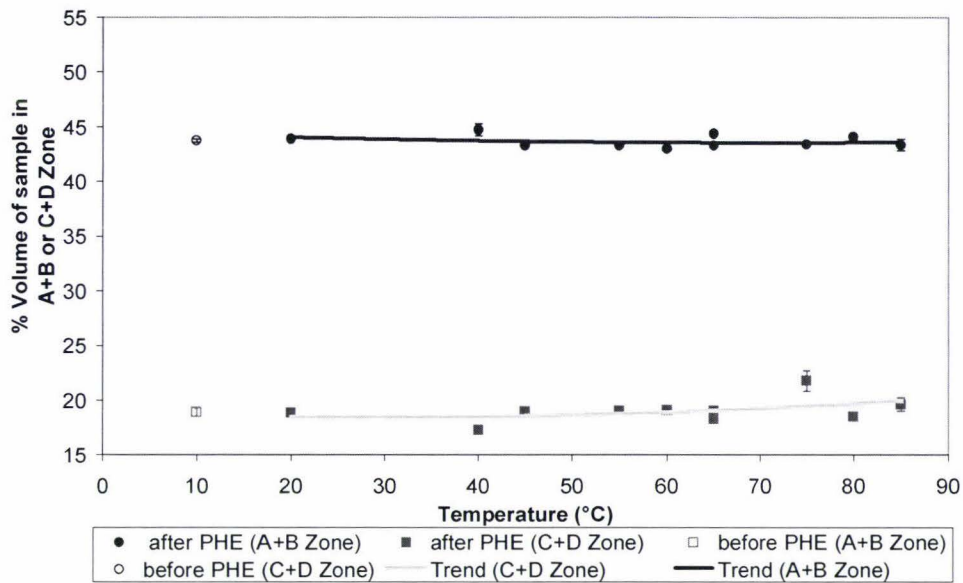
Figure 5.30: Effect of indirect heating using a plate heat exchanger on A+B and C+D Zones of PSZ test. Runs C75-84. All inlet milk samples were at 10°C (shown as an average). Error bars show the uncertainty in duplicate samples.

By contrast, the PSD of milk passing through the DSI unit changed substantially as shown in Figure 5.31. Comparisons between the milk entering (thick line) and the milk exiting the DSI unit (thin line) indicate that the fat globules were both substantially disrupted, and coalesced to the detriment of the O Zone.
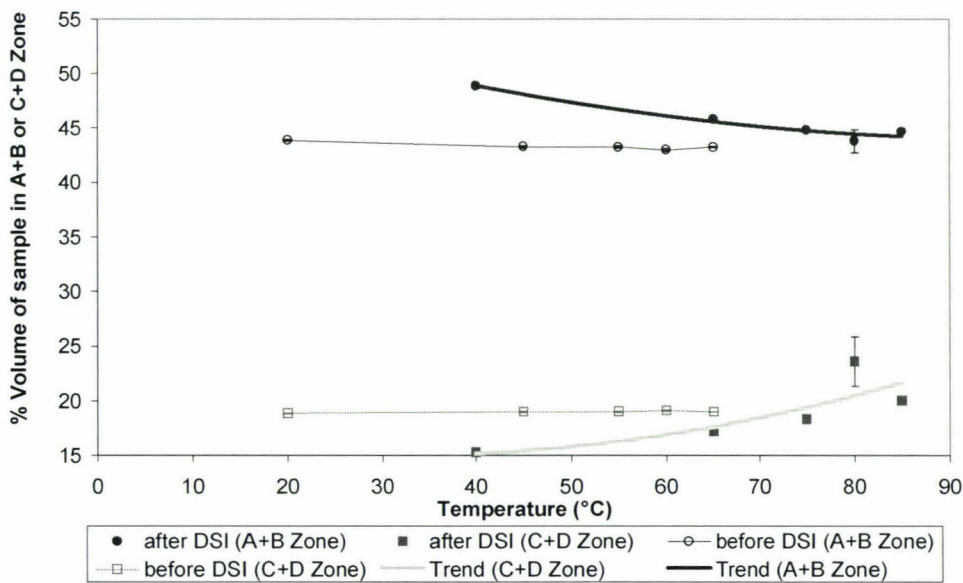
Figure 5.31: Effect of direct heating using a direct steam injection unit on A+B and C+D Zones of PSZ test. Temperatures were measured close to the sample points. Runs C75-84 reported in Figure 5.27. Error bars show the uncertainty in duplicate samples.

Only two operations in milk powder manufacturing processes have been observed to result in disruption of fat globules: DSI preheating, and homogenisation. All others have resulted only in flocculation and coalescence, or no change in the PSD. Walstra et al. (1984) argues that flocculation occurs in low shear rate environments and disruption occurs at very high shear rates. The author believes that disruption in the DSI unit results from two mechanisms:

1. The steam jets entering the flow of milk at an angle along the stream lines can have an impacting effect similar to splashing, especially if the steam velocity is high.

2. The steam jet immediately distributes itself as bubbles, which collapse almost instantaneously as the steam mixes with the milk. The collapse of bubbles would result in an effect similar to cavitation because tiny jets of fluid would rush into the vacuum created by the collapsing bubbles. Cavitation is reported to result in extremely high pressure forces that can damage hardened steel, let alone fat globules.

The Massey University pilot plant used in this work, described in Chapter 3, involved two DSI units (named here DSI 1 and DSI 2) fed from the same steam line but with two different back pressures of milk: DSI 1 at 30 kPa gauge and DSI 2 at atmospheric pressure. The pressure ranges of the steam entering the two DSI units were also different: for DSI 1, a range between 100 and 185 kPa and for DSI 2, a range between 60 and 165 kPa. Figure 5.32 gives a correlation between the A+B Zone and the pressure difference between the steam and the milk sides (runs C21-38). The faster the steam enters the milk the larger the resulting disruption of fat globules, as shown by the increase in the A+B Zone.
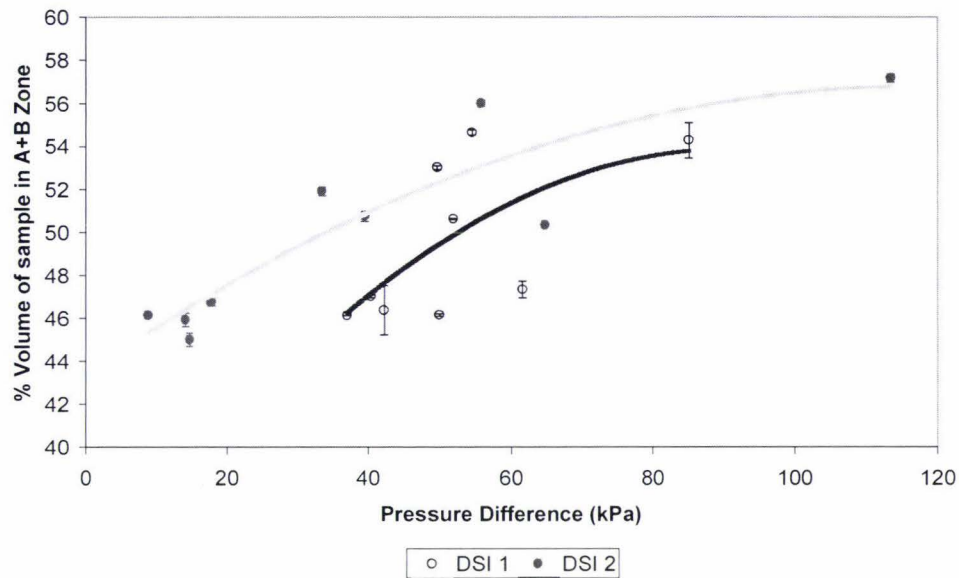
Figure 5.32: Effect of pressure difference between the steam line and milk line in two direct steam injection units on the A+B Zone of Farmhouse milk. Runs C21-38. Control samples for DSI 1 and 2 were 46.2±0.5 and 45.2±0.3 volume % respectively. Error bars indicate the uncertainty in duplicate samples.

The data in runs C21-38 can be re-plotted to show the change in A+B Zone with temperature increase across the DSI unit (Figure 5.33). This temperature increase is a direct measure of the amount of steam mixed with the milk, which is a better correlating variable for disruption as seen by the collapse in the data for DSI 1 and DSI 2 into the same region of the plot shown in Figure 5.33.
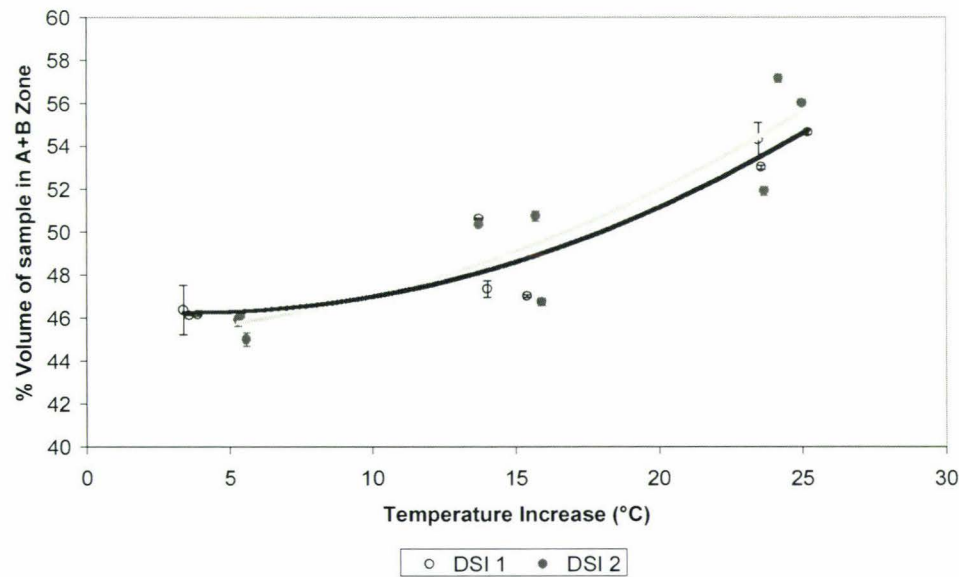
Figure 5.33: Effect of milk temperature increase across DSI units on A+B Zone from runs C21-38, reported in Figure 5.32. Error bars indicate the uncertainty in duplicate samples.

5.3   Agitation experiments

An earlier series of preliminary experiments were carried out with the agitation rig, described in Section 4 of Chapter 3, to refine techniques of sampling and for measuring NMFGM damage. Once the PSZ test was developed a set of three agitation experiments was conducted fairly early in the course of this work to measure damage using this test, because it was the most reliable test available.

Firstly, two sets of experiments were carried out to study the effect of agitation time on NMFGM damage at 5°C (T19-22) and 45°C (C54-56).

A second experiment was conducted to monitor the effect of agitation speed at 25°C (C39-44).

A third set of experiments (runs C50-52) was carried out to study the effect of agitation at three different temperatures. These temperatures were chosen to study the effect on the NMFGM when the fat was in three different states: solid at 5°C, liquid at 45°C, and partly liquefied at 20°C. The agitation speed was 2000 rpm for a duration of 4 minutes. All runs were performed in duplicate.

In all cases only the liquid milk was sampled. The PSZ test was used in all three sets of experiments to measure NMFGM damage.

Once the SL1 test was developed and the importance of sampling of all three layers: liquid, foam and churned fat established, a final set of agitation experiments were performed (runs C85-102). During the performance of these experiments the SL2 test was also investigated. The PSZ test was again applied in this final set of experiments which was conducted in conditions as close as possible to the last three sets of agitation experiments to ensure that replication of the PSZ results was still accurate.

Effect of agitation time

The effect of agitation time at 2000 rpm on the PSD of milk samples for runs at 5°C and 45°C in runs C54-56, T19-22 are shown in Figures 5.34 (A+B Zone) and Figure 5.35 (C+D Zone). At 5°C, the dominant effect was an increase in the C+D Zone with time

representing flocculation or coalescence of fat globules. This trend is re-enforced by a small drop in the A+B Zone, which indicates that even the small fat globules were flocculating. Interestingly enough, the increase in the C+D Zone with time was much slower in the 45°C experiment and there is no hint that the A+B Zone is reduced by agitation at 45°C. Thus the longer the duration of shear whether by pump re-circulation or agitation the greater the changes to the fat globules.
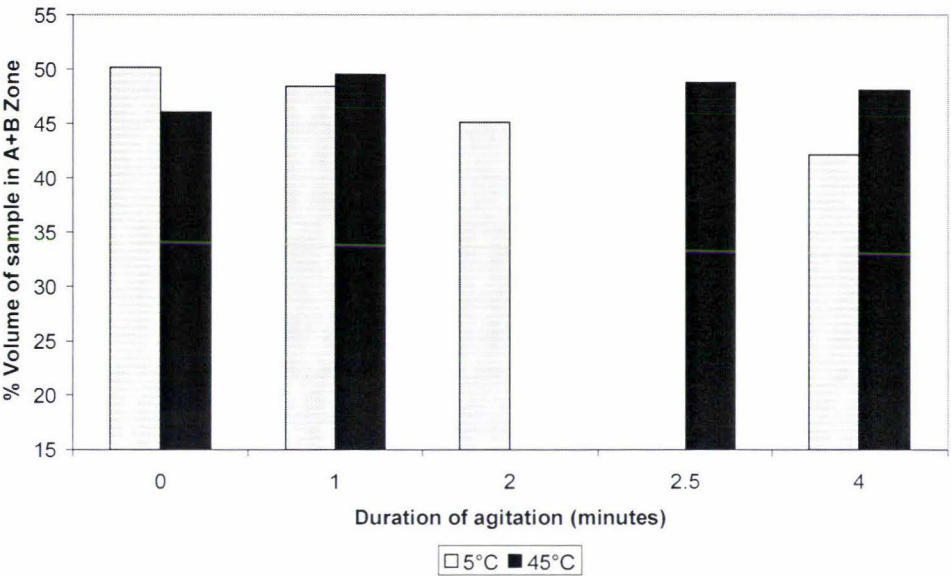
Figure 5.34: Effect of agitation time at two temperatures on the A+B Zone of Farmhouse milk. Runs T19-22 and C54-56.
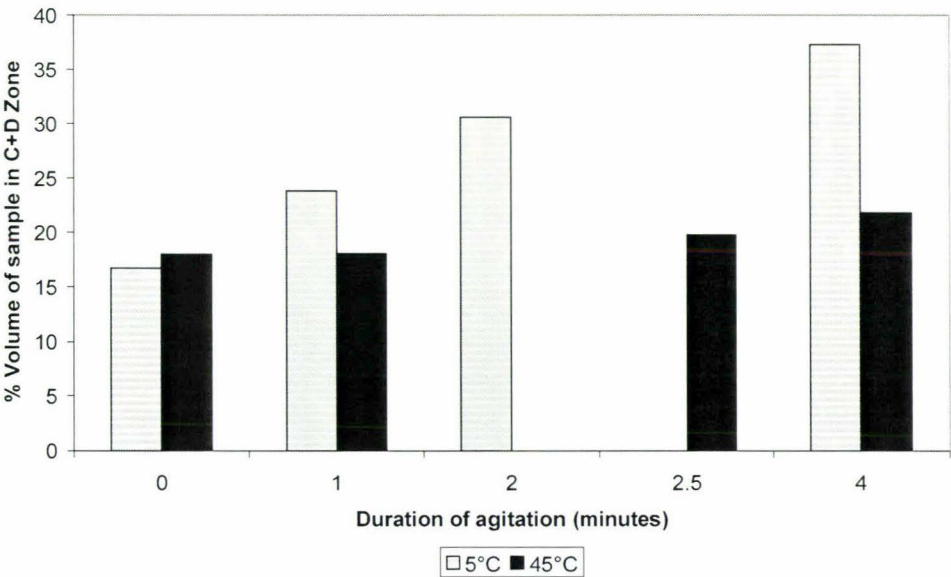
Figure 5.35: Effect of agitation time at two temperatures on C+D Zone of Farmhouse milk. Runs T19-22 and C54-56.

Effect of agitator speed

Figure 5.36 shows the changes in the A+B and C+D zones as a function of the agitator speed in runs C39-44. There was no change in PSD for agitator speeds up to roughly 800 rpm. The fat globules in the liquid milk tended to flocculate or coalesce as the agitator speed increased to 1300 rpm, shown by the increase in the C+D Zone and decrease in the A+B Zone. Upon further increases of speed to 2000 rpm the fat globules began to be disrupted, as shown by the increase in the A+B Zone and drop in the C+D Zone. These trends are well replicated in the later agitation experiment (runs C91-96) performed four years later, as shown in Figure 5.37. Those observations are compatible with the reasoning of Walstra et al. (1984) who argued that flocculation/coalescence was favoured at low shear and disruption at high shear. Metzner & Otto (1957) showed that the shear rate at the impeller tip is directly related to the impeller speed.
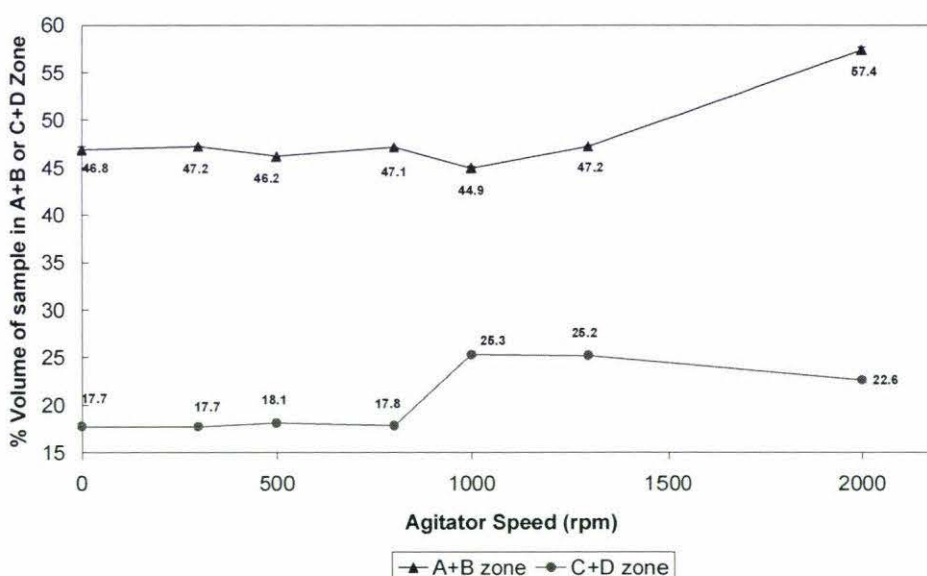
Figure 5.36: Effect of agitation speed on A+B and C+D zones of Farmhouse milk at 25°C. Run C39-44.
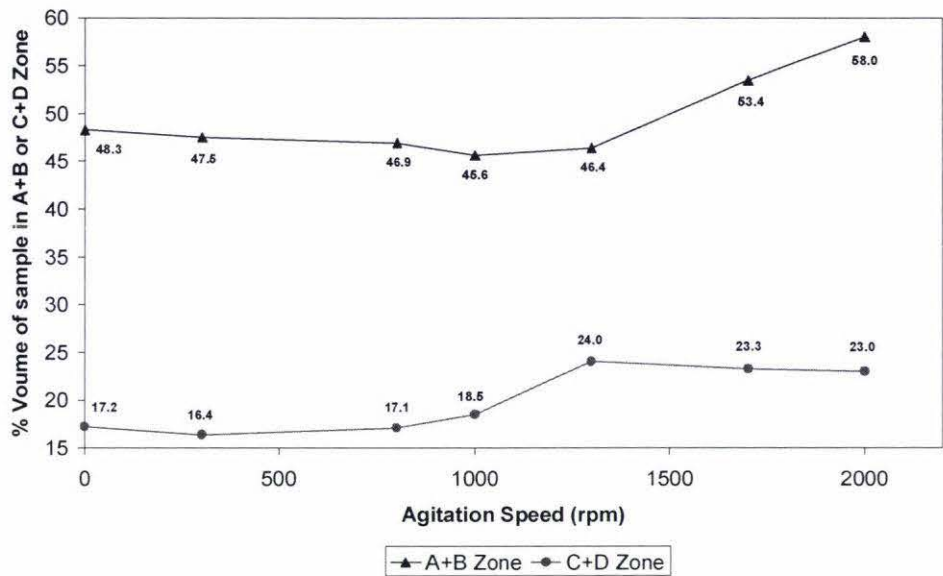
Figure 5.37: Effect of agitation speed on A+B and C+D zones of 25°C Farmhouse milk. Run C91-96.

At 25°C there was always a significant amount of churned fat produced at speeds of 1300 rpm and above. Churned fat was very difficult to emulsify in water. Any particle size measurement made by re-suspending it in water seemed to reflect the efficiency of the dispersion process. This is quite evident in the variability of the PSD curves shown in Figure 5.38, especially at the larger fat globule sizes. Thus the PSD of the liquid samples alone represents an underestimate of the amount of flocculation and cannot adequately assess the extent of NMFGM damage to the original milk when churned fat is produced.
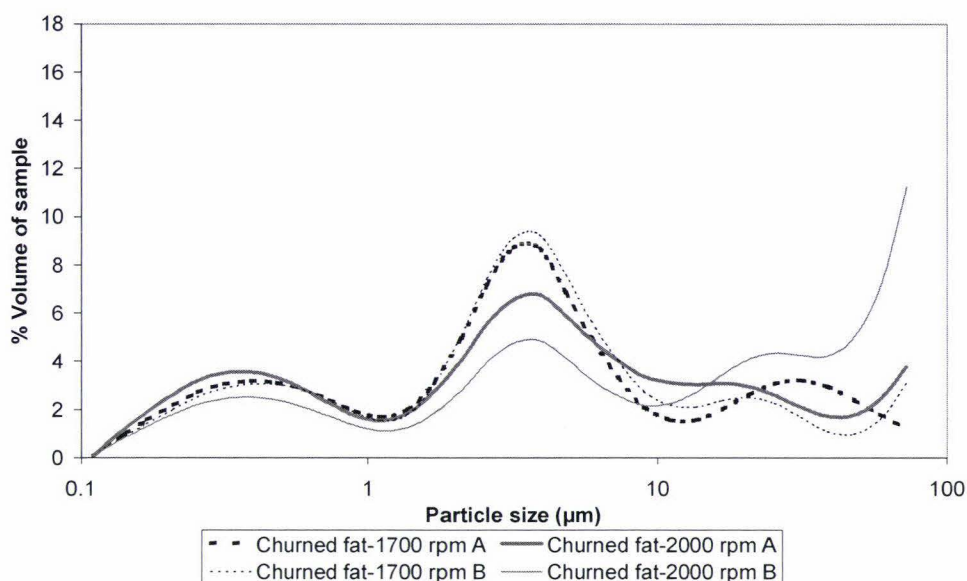
Figure 5.38: Particle size distributions (PSDs) for churned fat from 25°C Farmhouse milk agitated at 2000 rpm for 5 minutes (runs C95-96). Graph shows PSD for individual samples from duplicates.

The evolution of FFA values of the liquid samples (determined by the SL1 test) with agitator speed in runs C91-96 are shown in Figure 5.39. This shows an increase up to 1300 rpm before a significant drop at 1700 rpm and an increase again at 2000 rpm. The churned fat which started to appear at 1300 rpm was collected in the runs at 1700 rpm and 2000 rpm, weighed and its SLFA determined with the SL1 test after resuspending 2.5-5.0 grams of churned fat in 50 mL of sterilised distilled water and inverting by the technique described in the SL1 test methodology reported in Chapter 3. The mass of the churned fat produced from each run (C94-96) is reported in Table 5.8. The total SLFA value produced from the original milk sample was estimated by the weighted contribution of the liquid and churned fat samples (in grey colour, Figure 5.39). Clearly the total SLFA produced increased with agitator speed and the apparent peak shown by the liquid sample at 1300 rpm simply reflected the fact that a significant amount of NMFGM damage had moved from the liquid to the churned fat at higher agitator speeds.

Table 5.8: Effect of agitation speed on amount of foam and/or churned fat produced from a 400 mL batch of Farmhouse milk (runs C94-96).

Run #	Milk Temperature	Agitation Speed	Mass of foam plus churned fat	% mass of foam plus churned fat	Visual observation
C94	25°C	1300 rpm	6.9 g	1.7%	churned fat only
C95	25°C	1700 rpm	11.3 g	2.7%	churned fat only
C96	25°C	2000 rpm	17.1 g	4.2%	churned fat only
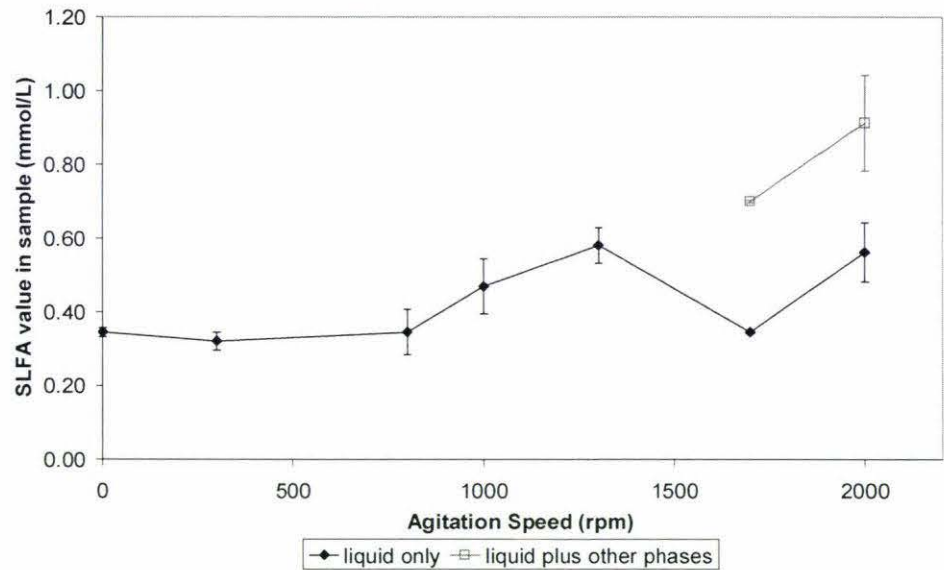
Figure 5.39: Effect of agitation speed on SLFA of 25°C Farmhouse milk (runs C91-96).

Deeth & Fitz-Gerald (1977) found that FFA levels increased when raw milk was agitated at 20°C at four speeds ranging from 4000 to 13000 rpm for 20 seconds. In a second set of experiments Deeth & Fitz-Gerald (1978) showed that the level of solvent-extracted FF was higher at 15000 rpm than at 4000 rpm.

Effect of agitation temperature

The effect of agitation temperature was first observed in the early runs (C50-52) performed at 5, 20 and 45°C. Churned fat was only observed at 25°C when the milk fat

was partly liquid and partly solid but not when the fat was completely solid (5°C) or completely liquid (45°C).

The shift in PSD on agitation for 4 minutes at 2000 rpm is summarised in Figure 5.40. The C+D Zone increased at all temperatures and was largest at 5°C and 20°C. In contrast, there was a small decrease in the A+B Zone at 5°C whilst the largest increase in the A+B Zone was at 20°C, similar to the increase in the C+D Zone. The biggest change in the liquid milk samples was attributable to flocculation and/or coalescence.
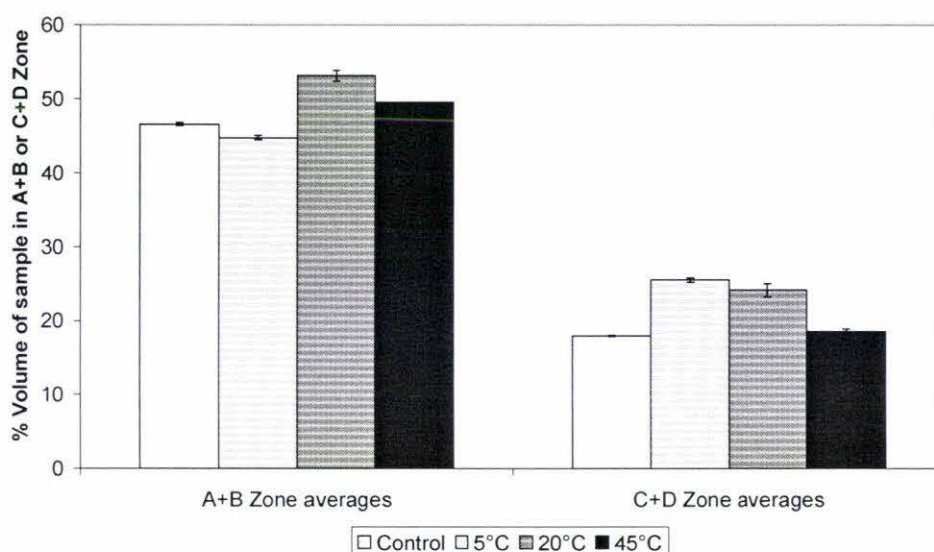
Figure 5.40: Effect of agitation temperature on A+B and C+D Zone of Farmhouse milk. Runs C50-52. Error bars indicate the uncertainty in duplicate samples.

A more detailed set of experiments was performed in runs C85-90 with agitation temperatures of 5, 10, 15, 20, 25 and 45°C at a speed of 2000 rpm for 4 minutes. The changes in the A+B and C+D Zones with temperature were similar to the earlier runs (C50-52, Figure 5.40), though the uncertainty of the 5°C sample was much higher. In general the replication of the results of the agitation experiments was good. The most notable new information was the peak of the C+D Zone, which occurred between 10 and 15°C (Figure 5.41).
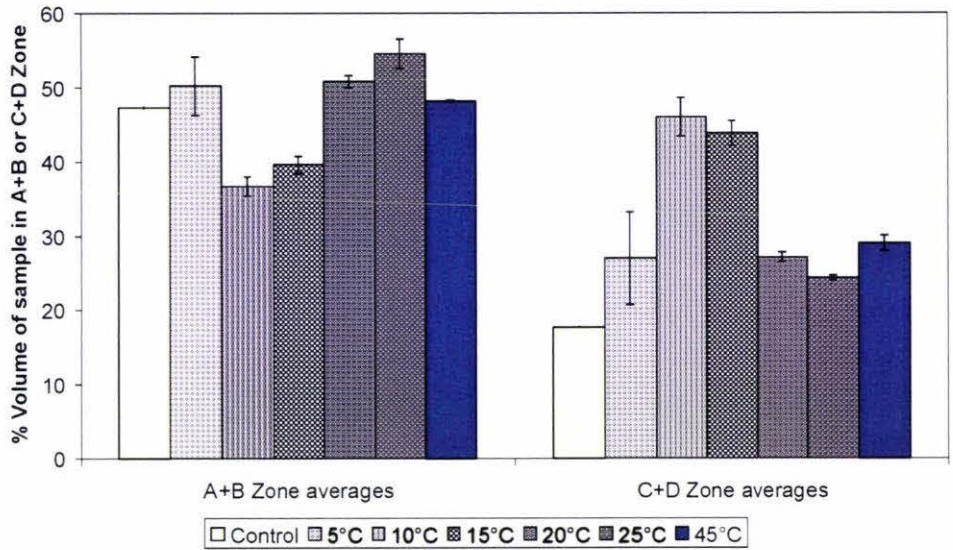
Figure 5.41: Effect of agitation temperature on A+B and C+D Zone of Farmhouse milk. Runs C85-90. Error bars indicate the uncertainty in duplicate samples.

Foaming was observed at the beginning of all runs (C85-90) but churned fat was observed only at 20°C and 25°C (runs 89-90). In runs C89-90 foaming ceased when churned fat began to appear whereas it persisted in all other runs until the end. These observations are illustrated in Figure 5.42. These are compatible with descriptions of the churning process in butter manufacture (Walstra et al., 1999). Essentially, air incorporated in the churning process is dispersed as bubbles. Parts of the NMFGM and the liquid fat spread over the surfaces of the bubbles, one bubble capturing several globules. As the air bubbles collide they coalesce and the adhering fat globules are driven towards each other. The liquid fat acts as a sticking agent and allows the fat globules to form small clumps. These clumps can be seen as small yellow patches incorporated in the foam as shown previously in Figure 5.11. The presence of liquid fat tends to destabilise the foam, thus when damage is very extensive, as occurs in the 20°C run, the foam disappears altogether and the churned fat gathers into a continuous mass (Figure 5.42B). The amount of air incorporated into the milk could not be monitored but special care was taken in the design of the tank (i.e. the presence of baffles) to minimise vortex formation, and at no time during the agitation experiments could a vortex be observed that extended down to the impeller.

(A)

(B)

(C)
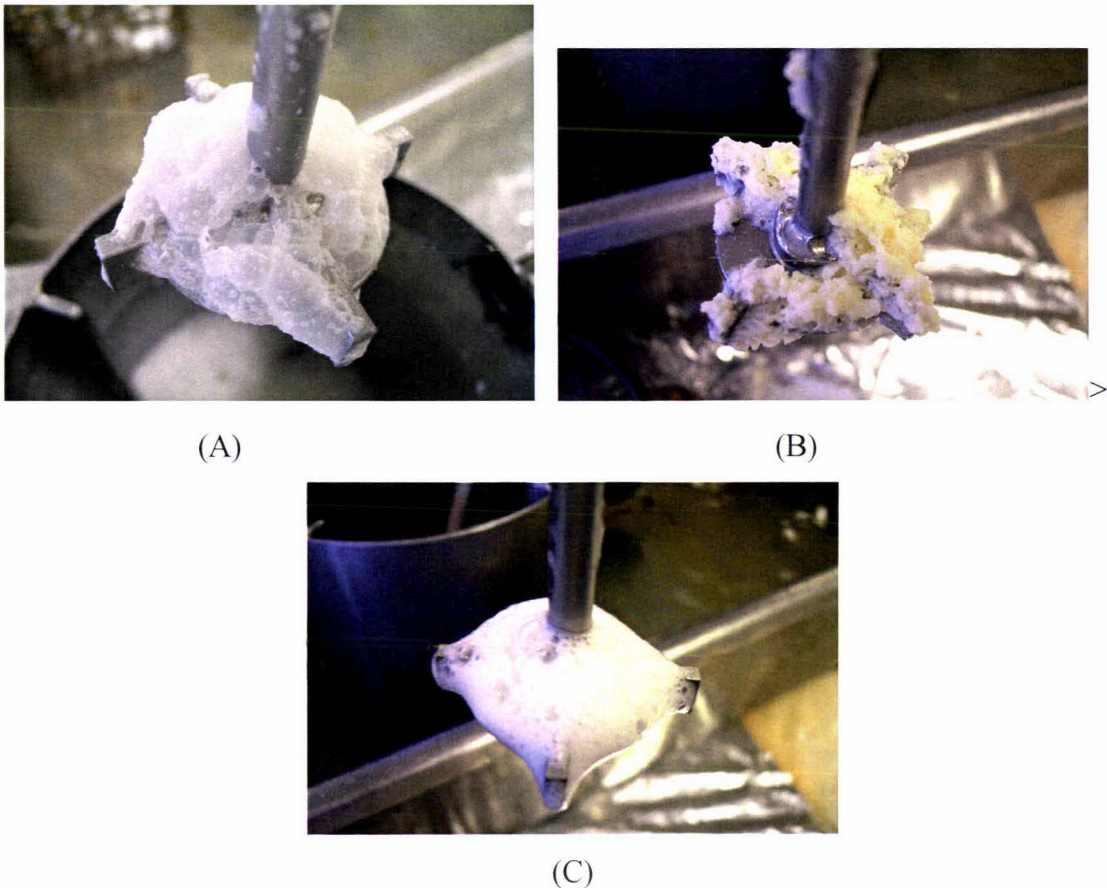
Figure 5.42: Photographs of phenomena observed after agitation in runs C85-90 and C97-102. Agitation at (A) 5°C, (B) 20°C, and (C) 45°C. Phenomena at temperatures of 10 and 15°C were similar to (A) and at 25°C were similar to (B).

Samples were taken from the liquid and foam/churned fat and analysed with the SL1 test. The masses of the different phases in runs C85-90 are reported in Table 5.9. The SLFA values (Figure 5.43) in the liquid milk samples go through a peak at 10°C as agitation temperature increased then fall to a minimum at 20°C before increasing again as the temperature was taken up to 45°C. Fitz-Gerald (1974) used the BDI method to measure FFA values in whole milk agitated at 7500 rpm in a Sorvall Omnimixer for 10 seconds and then incubated at 5°C for 20 hours. She found, similarly, a peak in the ADV values at approximately 15°C and a minimum at approximately 20°C with a second peak at 45°C. Deeth & Fitz-Gerald (1977) confirmed the pattern identified by Fitz-Gerald but the FFAs were now determined by the global FFA test developed by Deeth et al. (1975).

Because of time and equipment constraints only one sample of foam at 5°C and one sample of churned fat at 20°C could be analysed by the SL1 test within the duration of

this experiment (16 hours). The weighted contribution of the SLFA values from the foam and churned fat samples were added to the SLFA values of the liquid samples, shown by dark bars in Figure 5.43. The contribution of the foam amounted to approximately 16% of the liquid SLFA at 5°C and the contribution of the churned fat amounted to approximately 31% at 20°C.

Table 5.9: Effect of agitation temperature on amount of foam and/or churned fat produced from a 400 mL batch of Farmhouse milk (runs C85-90).

Run #	Milk Temperature	Agitation Speed	Mass of foam plus churned fat	% mass of foam plus churned fat	Visual observation
C85	5°C	2000 rpm	3.7g	0.9%	foam only
C88	10°C	2000 rpm	9.5g	2.3%	foam only
C86	15°C	2000 rpm	9.5g	2.3%	foam only
C89	20°C	2000 rpm	17.5g	4.2%	churned fat only
C90	25°C	2000 rpm	15.3g	3.7%	churned fat only
C87	45°C	2000 rpm	8.9g	2.2%	foam only
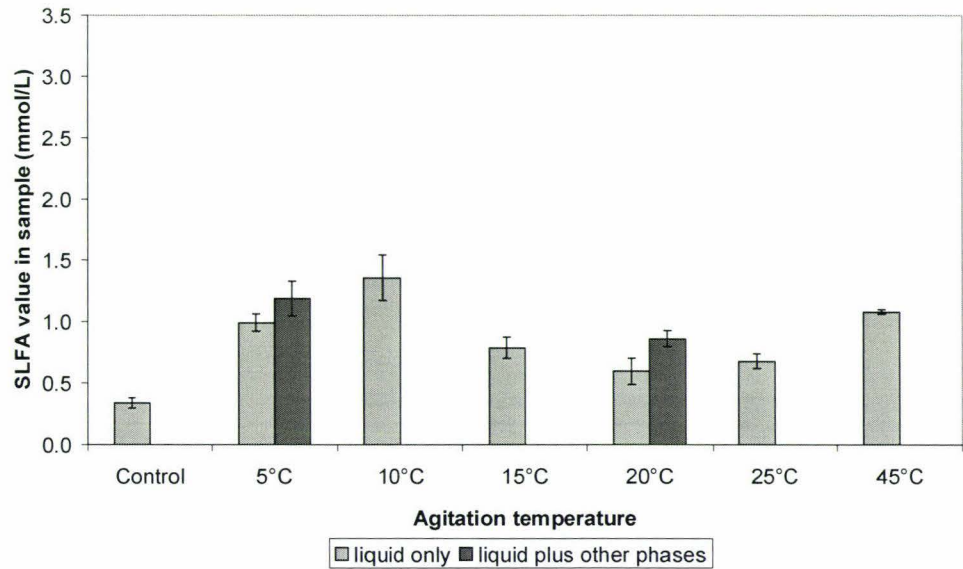
Figure 5.43: Effect of agitation temperature on SLFA from runs C85-90, reported in Figure 5.41. Error bars indicate the uncertainty in duplicate samples.

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It was noted during the above experiment that a number of fat/FFA droplets floated on top of the surface of the milk after incubation. This was particularly serious in the samples dealing with foam and churned fat, as was shown in Figure 4.32 of Chapter 4. The sample inversion technique of the SL1 test described in Chapter 3 was not able to give a uniform sample from which representative sub-samples could be taken for titration. A new version of the selective lipolysis test (SL2) was developed as described in Chapters 3 and 4 where the incubated samples were homogenised.

A new experiment on the effect of agitation temperature was performed in runs C97-102 where the SLFA values of the liquid and foam/churned fat at all temperatures (5, 10, 15, 20, 25, 45°C) were determined using the SL2 test. The FF content of all the liquid and four of the foam/churned fat samples (5, 15, 20, 45°C) were also measured. (The 10°C and 25°C samples were taken but were subsequently lost).

The evolution of the C+D and A+B Zones with temperature in runs C97-102 (Figure 5.44) was again similar to those obtained in runs C50-52 and C85-90, indicating that the replication of the trends obtained in the PSZ measurements was satisfactory.
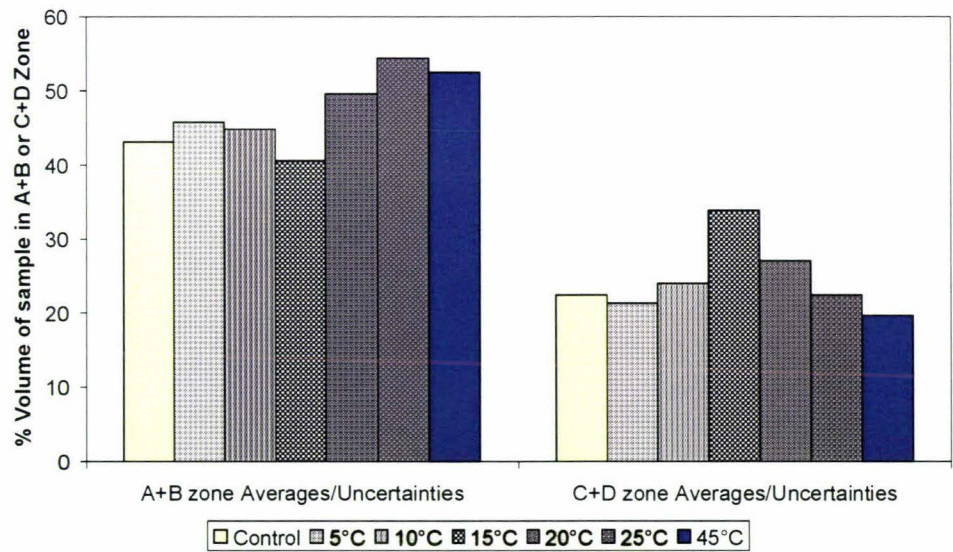
Figure 5.44: Effect of agitation temperature on A+B and C+D Zone of Farmhouse milk. Runs C97-102.

The SLFA values in the liquid samples measured with the SL2 test (runs C97-102, Figure 5.45) were substantially higher than those obtained by the SL1 test (Figure 5.43) but the trends were exactly the same. When the contribution of the foam and the

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churned fat to the total SLFA are taken into account according to the masses in Table 5.10 the trend remained the same (dark bars, Figure 5.45). Interestingly enough the contribution of SLFA from the foam at 5°C in run C85 was 29%, which is within the experimental error of the same conditions in run C97. Similarly the contributions of churned fat at 20°C to the total SLFA were respectively 52% and 44% of the SLFA value of the liquid samples. Thus as discussed in Chapter 4 the SL2 test simply results in a more representative sub-sample of the incubated milk sample but does not affect in a major way the trends in NMFGM damage observed.

Table 5.10: Effect of agitation temperature on amount of foam and/or churned fat produced from a 400 mL batch of Farmhouse milk (runs C97-102).

| Run #	Milk Temperature	Agitation Speed	Mass of foam plus churned fat	% mass of foam plus churned fat	Visual observation
C100	5°C	2000 rpm	4.8g	1.2%	foam only
C97	10°C	2000 rpm	3.0g	0.7%	foam only
C101	15°C	2000 rpm	5.0g	1.2%	foam only
C98	20°C	2000 rpm	16.6g	4.0%	churned fat only
C102	25°C	2000 rpm	12.6g	3.1%	churned fat only
C99	45°C	2000 rpm	8.9g	2.2%	foam only
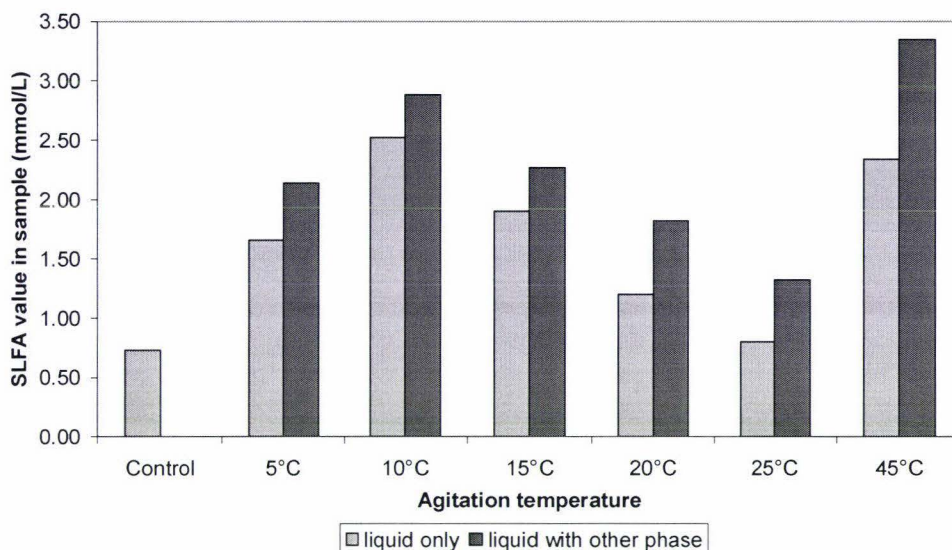
Figure 5.45: Effect of agitation temperature on SLFA (SL2 test) from runs C97-102, reported in Figure 5.44.

The changes in FF with agitation temperature in runs C97-102 are shown in Figure 5.46. The FF in the liquid samples peaked at 15°C compared with 10°C for the SLFA results but the major difference came at 45°C where very little FF was extracted whereas the SLFA values (Figure 5.45) were almost comparable to the peak at 10°C. When the weighted contribution of FF in the foam and churned fat samples (5, 15, 20 and 45°C) was added the pattern of FF against agitation temperature became substantially different from the pattern of SLFA (dark bars, Figure 5.46). The peak occurred at 20°C and there was no minimum beyond that. In fact the amount of FF obtained at 45°C was minimal. Kessler & Fink (1992) found that centrifugally recovered FF created by shearing with a paring disc separator peaked between 20 and 30°C and dropped significantly at higher temperatures. Similarly, Deeth & Fitz-Gerald (1978) observed a peak of solvent-extracted FF at 20°C when milk was agitated at 15000 rpm in a Sorvall Omnimixer for 20 seconds.
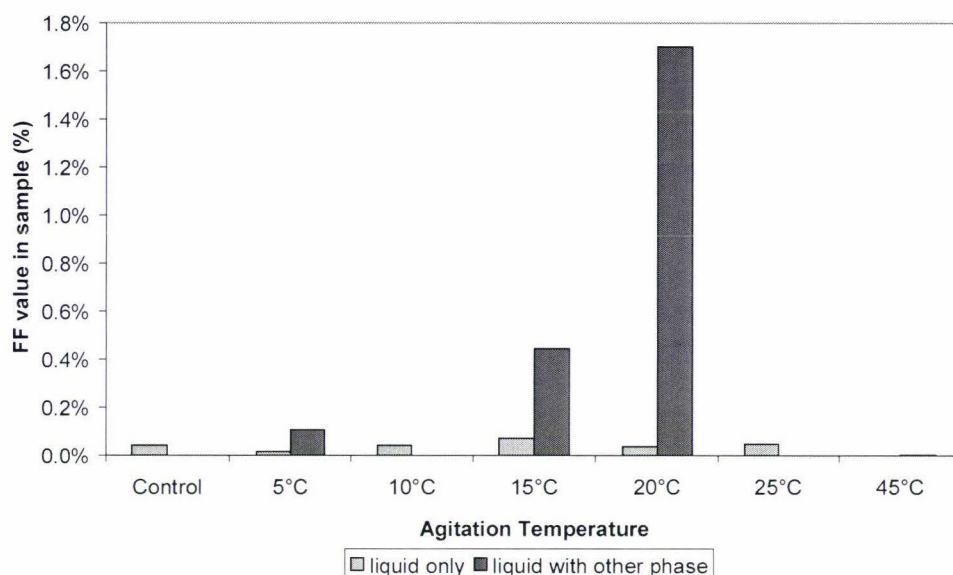
Figure 5.46: Effect of agitation temperature on FF from runs C97-102, reported in Figure 5.44. Note that values for the "liquid plus the other phases" were not available at agitation temperatures of 10°C and 25°C.

While the agreement between these observations and those of Kessler & Fink and Deeth & Fitz-Gerald is encouraging, these authors unfortunately did not discuss their method of sampling, which makes it difficult to know whether the FF was determined only from the liquid sample or whether their samples included liquid, foam and churned fat. Certainly, Deeth & Fitz-Gerald (1977) state clearly that marked change to induced lipolysis only occurs in the presence of foam formation. In fact Fitz-Gerald (1974) purposely bubbled oxygen-free nitrogen to "activate" the milk samples during agitation at 10, 15, 20 and 37°C.

The experiments just described show clearly that the picture of NMFGM damage presented by the selective lipolysis and FF tests are substantially different. Kessler & Fink (1992) also noted different patterns shown by FFA and centrifugal FF measurements when milk was agitated at different temperatures. Thus the FF and selective lipolysis tests measure different things. While both were designed to measure consequences of NMFGM damage it is not yet clear which one would give a more accurate picture. The almost total lack of FF at an agitation temperature of 45°C contrasts sharply with the significant amount of SLFA at that temperature, which seems to indicate that the solvent-extraction FF test measures exactly that, which is the amount

of fat that can be extracted from the milk with a non-polar solvent, but cannot measure the susceptibility of the fat to lipolysis by a selective enzyme.

In the SL2 test churned fat was essentially suspended in water by placing both in a sample container and inverting five times as described in the SL1 test procedure in Chapter 3. The idea was not to further damage any intact fat globules that might have been present in the churned fat. However the procedure did not result in very fine dispersion of fat. After addition of the PPL and during incubation the large fat globules tended to rise in the solution. It is probable that within the six hours of incubation the PPL would not have reached all the fat in the globules and would have acted only on a layer near the surface. Since the churned fat is almost entirely composed of FF the situation is not unlike lipolysis of anhydrous milk fat (AMF). The common practice in measuring the acidity of AMF is to disperse the AMF in an aqueous solution with strong agitation in the presence of guar gum. In retrospect that method should have been adopted in the application of the SL2 test for foam and churned fat because almost all of the fat would be unprotected by the NMFGM, and the test would probably have resulted in significantly higher values of SLFA. If all of the foam/churned fat was converted to SLFA the results would look like that shown in Figure 5.47. Clearly, the samples with churned fat produced very high SLFA values. In addition, there are similarities in the trend in Figure 5.47 with those in Figure 5.46, for FF. However, note that at 45°C there was virtually no FF extracted (Figure 5.46), whereas there was a high level of SLFA extracted at 45°C. This most likely points to differences in what the FF and selective lipolysis tests measure (i.e. fat and FFAs).
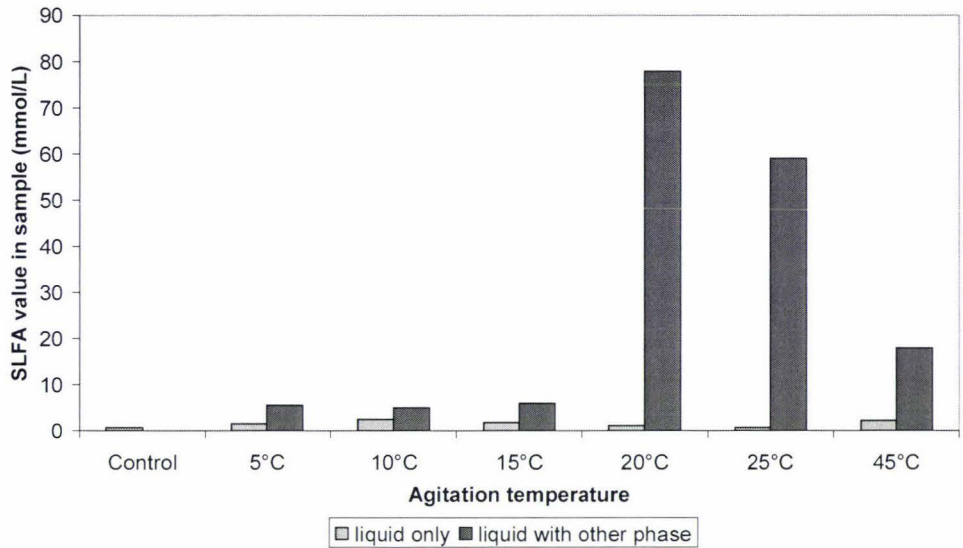
Figure 5.47: Modified SLFA values from agitation runs C97-102 reported in Figure 5.45. SLFA contribution from the other phase was calculated by assuming the total fat content of that phase could be converted to FFAs. Note that the maximum scale of the Y-axis in this graph is 26 times larger than the scale in Figure 5.45.

5.4 Evaporation experiments

Three runs were performed on a three-effect falling film evaporator located in the Massey University milk processing plant. The flow rate of the milk was 30 L/hr and the evaporator was allowed to reach steady state conditions before sampling, approximately two hours from the start of the run. Samples were taken in duplicate. For ease of comparison, all values of SLFA have been re-calculated to adjust for variations in fat content caused by the evaporation process. A sample calculation is shown in Appendix C2.

In the first run, the procedure for controlling the evaporator was being developed and the final concentration from the third effect was only 24% total solids (TS). With operating experience, a much higher TS of 52% was achieved, which is much closer to industrial practice in New Zealand. Thus the operating conditions of the two runs were not the same. Figure 5.48 shows the evolution of SLFA in the milk samples of run C71. SLFA increased steadily as it passed through the three effects of the evaporator. It was clear that damage in the evaporator was substantial. The SLFA profile in a second run (C73 – Figure 5.49) also showed an increase of SLFA as the milk passed through the

evaporator, but the trend was not as smooth as in the first run. No SLFA results were obtained for run C72 because of an error in operation.
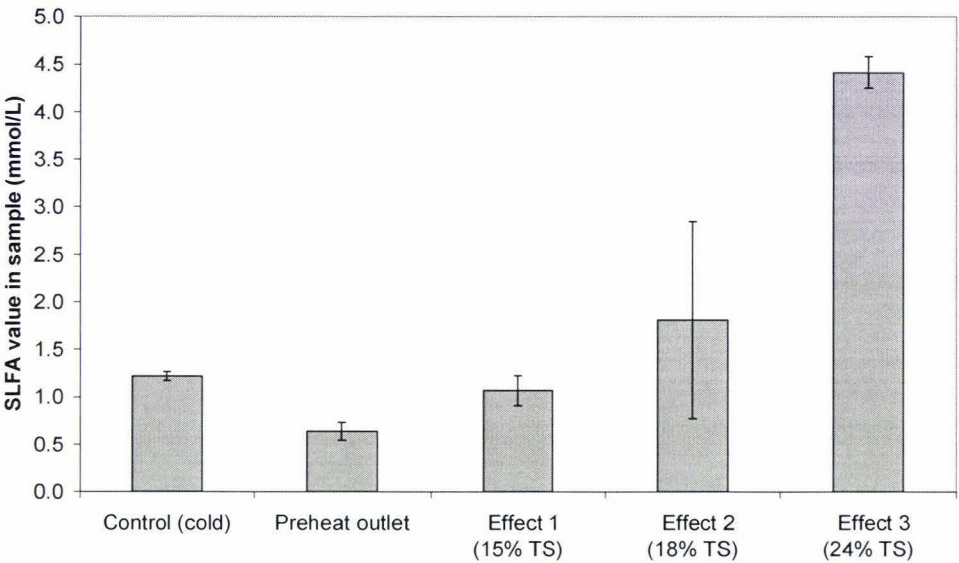
Figure 5.48: SLFA values for milk passed through a three-effect re-circulation evaporator running at 30 L/hr. Run C71.
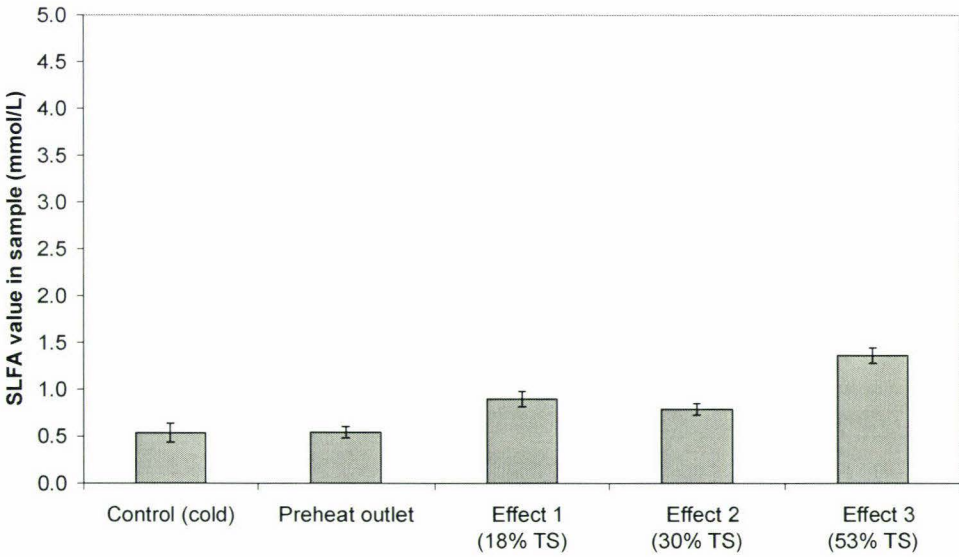
Figure 5.49: SLFA values for milk passed through a three-effect re-circulation evaporator running at 30 L/hr. Run C73.

The average PSD changes in the milk samples, from the milk vat to effect 3, are shown in Figure 5.50. There was a small increase in the C+D Zone during evaporation from the milk vat to the effect 2, but a slight dip in the third effect. The A+B Zone did not vary significantly from the milk vat to effect 2, but there was a slight increase in effect 3,

which complements the drop in the C+D Zone in that effect. Thus it appears that there was a particle size increase in the earlier parts of the evaporator at lower total solids, but a reduction in particle size in effect 3. This pattern of physical change was found in all three runs.

The value of SLFA found at the evaporator outlet (Figure 5.48) was very significant and was one of the largest values compared with those found in all previous operations that damage the NMFGM. Few reliable observations of NMFGM in evaporators are available for comparison. The only work using FFA measurement found in the literature on the topic used the BDI method without incubation (Evers et al., 2000b) and thus could not capture the effect of damage properly.

Because the pilot plant evaporator ran at a very low flow rate, there was always a danger of substantial fouling because of poor liquid coverage by the falling film around the tube wall. To ensure adequate coverage the milk was re-circulated around each pass at a flow rate of approximately 200 L/hr. Since the evaporator tubes were only 2.9 metres high the re-circulation of six passes was equivalent to a single pass of the milk falling down an industrial evaporator tube 18 metres high. Nonetheless the residence time of the milk in this small-scale evaporator was higher than in industrial evaporators. As a consequence there was more time for the particles to flocculate and/or coalesce.
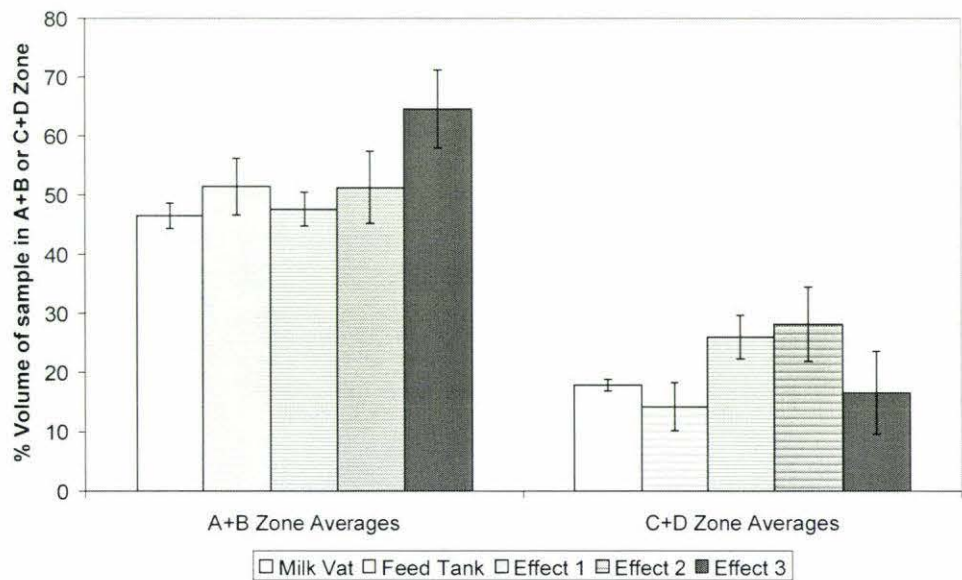
Figure 5.50: Average A+B and C+D Zone values of Farmhouse milk passed through a three-effect re-circulation evaporator in runs C71-73. Error bars indicate the variation in the three runs.

Inspection of the samples taken out of the evaporator showed that there was strong coalescence, which could be seen as a layer of yellowish fat (literally unprotected fat) floating on top of the milk upon storage in a beaker (Figure 5.51 – Richard Croy, unpublished results, 2003). As previously mentioned in Chapter 4, it is not certain that the inversion procedure used in the selective lipolysis test would have negated completely the effect of creaming and produced a homogeneous sample able to be transferred into an incubation container. This may have also affected the PSD determination. This may explain the unusual PSZ test results for effect 3, since the inversion procedure may not have remixed the layer of fat on top of the milk homogeneously into the sample, and smaller sub-samples for PSZ test measurements might have been unrepresentative.

Figure 5.51: Photograph showing rising of fat to surface of evaporated milk sample (Croy, unpublished results, 2003).

5.5   Conclusion

Four common operations within milk powder manufacturing processes were investigated in this chapter: pumping, heating, agitation and evaporation.

The controlled pumping experiments showed that air inclusion was a major factor in increasing the level of NMFGM damage. This was consistent with previous work in liquid milk samples (Aule & Worstorff, 1975; Miller & Puhan, 1986b). In addition,

pump speed, pipeline velocity and valve cavitation were shown to be contributing factors to an increase in NMFGM damage.

Heating using a plate heat exchanger was not found to change the particle size distribution of milk but direct steam injection was found to change the particle size by increases in the A+B and C+D Zones, suggesting that both disruption and coalescence were significant factors. This appears to be proportional to the required temperature increase provided by the DSI unit.

Agitation speed, duration and temperature were found to affect the incidence of NMFGM damage. Agitation temperature had a significant impact on the amount of NMFGM damage contributed in the liquid, foam and churned fat phases. NMFGM damage reached a maximum between 20-25°C. As the agitation speed increased, so did the value of NMFGM damage, though it was found that a minimum speed was required before NMFGM damage was detected.

Evaporation was clearly shown to cause disruption and coalescence of fat globules and give an increase in SLFA levels.

Chapter 6
Industrial Survey of NMFGM Damage in Milk Powder Plants

6.1   Introduction

Several industrial surveys were attempted during the course of this work, since the author was required by the rules of his fellowship (a Technology for Industry Education Fellowship provided by the New Zealand Government) to spend significant periods in industry. The first two attempts, made in the first two years of research at the Waitoa site of the then New Zealand Co-operative Dairy Company Limited, highlighted the very poor performance of previously accepted test methods and sampling techniques, and resulted in the extensive development of the new NMFGM damage measurement techniques and sample handling protocols described in Chapters 3 and 4.

Early concepts of monitoring NMFGM damage were ambitious because they began from the idea that milk handling from the cow at the farm to reception and standardisation at a factory were the major causes of damage (Anderson, 1983). It turned out that a serious analysis of the problem would be extremely complicated. First of all, it was realised that the determination of FFAs (OFA in the nomenclature used here) as recommended in the 1991 IDF publication (Anderson et al., 1991) could not truly reflect the amount of NMFGM damage since not all of the unprotected fat (UPF) in the sample would have been converted to FFAs without the addition of exogenous lipolytic enzymes.

Logistically, taking representative samples was a very difficult exercise. At that time the Waitoa site processed about 3 million litres of milk per day sourced from several hundred farms. Most of these farms were located within 100 kilometres of the factory, but some farms were up to 200 kilometres from the factory. Milk was collected by 20,000 litre capacity tankers that would typically travel for variable lengths of time depending on the most economical route to collect a full tanker-load. This would

therefore result in the milk temperature rising to varying extents before the milk reached the factory.

In order to follow the history of milk in a storage silo at the plant, a composite of samples would have been required from the different tankers that supplied milk to that silo. This would have required a very close interaction with the people who scheduled the tanker transport. Furthermore, to measure NMFGM damage in milk during transportation, a further composite sample of the quality of the milk at the farms that contributed to each tanker load would have been needed.

Thus the number of samples would have grown exponentially as one went back from the factory to the cow. Naturally since the milk taken from the cow is stored and then transported to the factory, the elapsed time between the milking and reception steps would change considerably according to local practice and the distance between the farm and the factory. It was extremely difficult under these circumstances to follow single batches of milk from the farm to the factory. Very large sample populations would have been required to give a reliable overall picture of NMFGM damage during milk handling. In the end the exercise proved to be too complex and too large for successful completion within the timeframe of the work reported here.

New surveys were made to analyse the damage to the NMFGM during milk processing in individual milk powder plants. This chapter reports the results of these surveys.

6.2   Results

The surveys were conducted by making measurements of SLFA (using the SL1 test), IFA and PSD in different sections of two Fonterra Co-operative Group milk powder plants located at Pahiatua, approximately 30 kilometres (1 hour's drive) away from Massey University. This site was selected because it was nearest to the laboratory facilities and equipment required for analysis, which were located at Massey University. A detailed protocol was carefully developed to ensure that the samples, especially the concentrated milk samples, did not change significantly during storage and transportation between the factory and the laboratory. This was very important, as

concentrated and heated milk solutions tend to age-thicken, and eventually gel, during storage. The detailed protocol for the surveys was described in Section 5 of Chapter 3.

The two milk powder plants at the Pahiatua site, identified as Powder 1 and Powder 2, shared the same reception and standardisation lines. The difference between Powder 1 and Powder 2 lay mainly in the designs of the evaporators: a 5-effect TVR (thermal vapour recompression) evaporator in Powder 1 and a 2-effect MVR (mechanical vapour recompression) evaporator in Powder 2.

Typically, milk was delivered at reception by milk tankers into waiting, cleaned raw milk silos. Milk from the silos was sent to a pasteuriser and a bank of separators, and then standardised for fat content and protein. Pasteurised, standardised milk was pumped from intermediate silos to an evaporator feed tank. From the feed tank, the milk was passed through a preheating system comprising a number of indirect heaters, and finally a direct steam injection unit. The milk then entered the evaporator, where it was taken from approximately 12% total solids up to 40-50% total solids depending on the product specifications of the milk powder. The concentrated milk was stored in a balance tank before being heated in a scraped surface heat exchanger, and homogenised, before being sent to the spray dryer.

Figure 6.1-2 shows the change in SLFA for Powder 1 from the cold feed balance tank of the evaporator to either the outlet of the evaporator or the outlet of the homogeniser (runs S10-11). Figure 6.3 shows results in Powder 2 from the cold feed balance tank to the outlet of the homogeniser. For ease of comparison, all values of IFA and SLFA have been re-calculated to adjust for variations in fat content due to the evaporation process. A sample calculation is shown in Appendix C2.

The level of IFA was low in all three runs though in two samples from both runs S11 and S12 the IFA value was higher than the SLFA value. Since the IFA test includes no added lipolytic enzymes a higher value than the SLFA must have been due to contamination of the samples in some form. Possible reasons could have been either contamination of the sample by microbial enzymes already present in the sample when collected from the evaporator, or contamination from the container used to incubate the samples in the IFA and SLFA tests. In the former, a systematic error would be expected

in all samples downstream of the microbial enzyme contamination. No systematic error was observed. Some incubation containers may have been randomly contaminated with microbes or microbial enzymes. This is possible though all containers were sterilised at the same time. However, it has been observed previously by other workers using the same autoclave to sterilise agar that some random contamination of agar samples occurred. The IFA values that were higher than the SLFA value have been omitted. The IFA values remained constant for run S10 and (apart from the anomalous results already mentioned) and also in run S12 (Figure 6.3). In run S11 the IFA values varied by 0.5 mmol/L, which is significant based on the typical uncertainty for the IFA test but was still not a large variation in terms of the SLFA values, and are therefore attributed to random error.

A large change in SLFA was found in each evaporator for both Powder 1 and Powder 2. This came as no surprise after the controlled experiments in the Massey University pilot plant evaporator reported in Section 4 of Chapter 5. However, the observation is new to the field, as previous surveyors, on the contrary, reported no change in FFAs over evaporators using the BDI method of measurement. Reported measurement of NMFGM damage in evaporators is extremely rare (Tolstukhina & Aristova, 1979; Evers et al., 2000b). The consequence of this damage in terms of both the mechanical design of evaporators and the process design of milk powder manufacturing lines has to be considered in the light of these results.
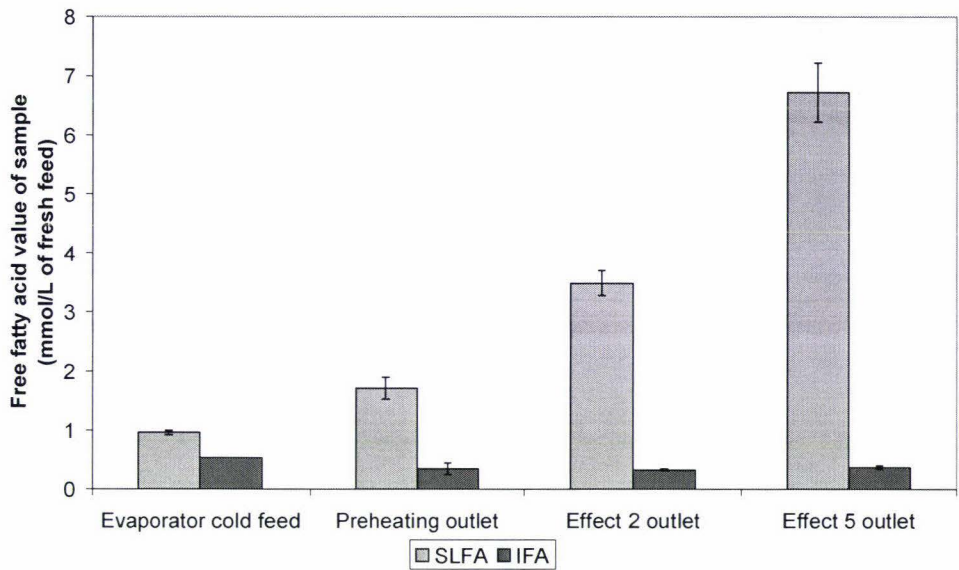
Figure 6.1: SLFA and IFA values from run S10 at Powder 1 at the Pahiatua dairy factory. Error bars indicate the uncertainty in duplicate samples.

In Powder 1 there was a continuous increase in SLFA from the evaporator feed tank to effect 5. In run S11 performed a week later (Figure 6.2) the specification of manufacture had changed, and, while there was the same type of increase across the evaporator up to effect 1, there was apparently a drop in the SLFA between effect 3 and effect 5.
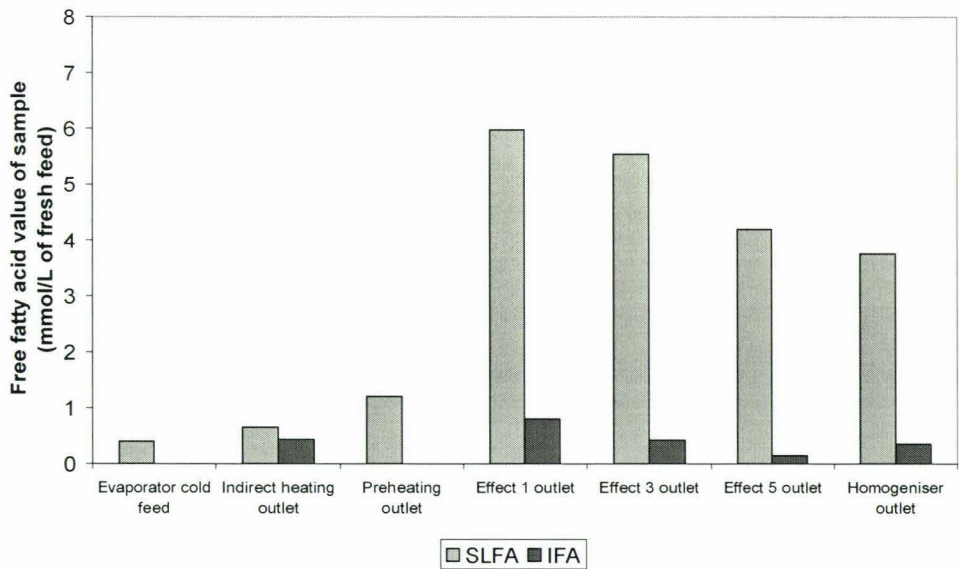
Figure 6.2: SLFA and IFA values from run S11 at Powder 1 at the Pahiatua dairy factory.

It is not clear whether the drop in SLFA in run S11 (Figure 6.2) was due to the changed powder specification, which resulted in a different total solids in that effect compared to

the total solids in run S10 (Figure 6.1), or whether it was due to errors in sampling and incubation.
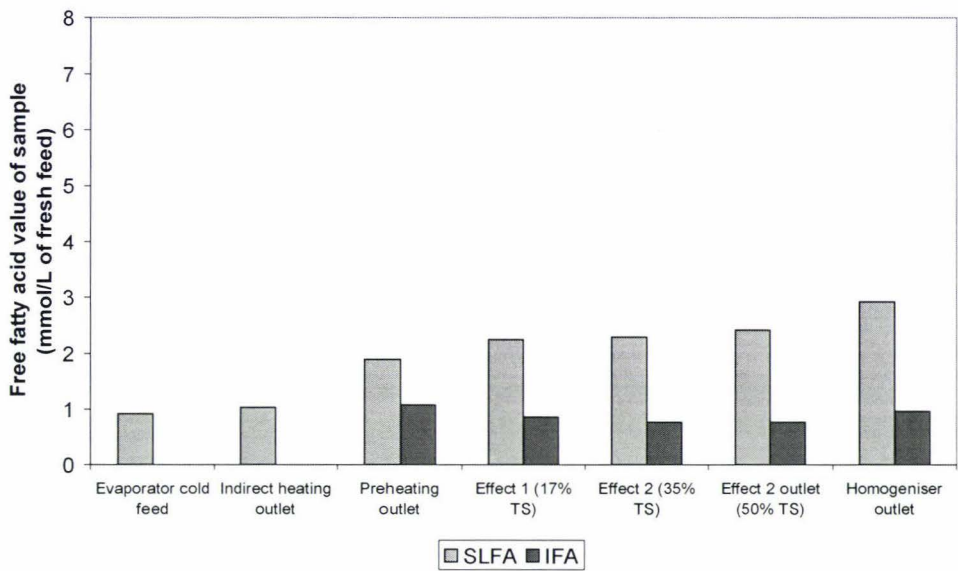
Figure 6.3: SLFA and IFA values from run S12 at Powder 2 at the Pahiatua dairy factory.

The different configurations of the two evaporators did result in different profiles for SLFA. In Powder 2, the MVR configuration resulted in an increase in SLFA from the feed tank to effect 1 but there was only a very small increase after that. Substantial repeat measurements in these two plants must be performed to confirm that the different trends observed between the two evaporator designs are real. One of the problems encountered was that the two plants ran on different milk powder products. Powder 1 normally ran on whole milk and Powder 2 on skim milk. Only on rare occasions, such as the need to produce large quantities of whole milk powder in a short time, would both plants (Powder 1 and Powder 2) draw on the same batch of whole milk and operate to the same manufacturing specification. Thus, in order to repeat the measurements performed here, one had to keep in constant contact with the plant scheduling team so as to be on site at the right time on these rare occasions.

Interestingly, the data from both Powder 1 and Powder 2 (Figures 6.2-6.3) indicate that there was little change in SLFA from the evaporator outlet to the homogeniser outlet. Particle size measurements reported below indicate, as expected, that the homogeniser resulted in massive disruption of fat globules. Ordinarily one would expect that this massive amount of disruption would create a substantial increase in NMFGM damage

and therefore a similarly substantial increase in SLFA. Since the lack of increase in SLFA upon homogenisation after the concentrating process was an observation that was quite repeatable, the inevitable conclusion is that heating milk at high concentrations (roughly 50% total solids) creates an artificial milk fat globule membrane that is much more impermeable to lipolytic enzymes than the artificial membrane created in milk of a natural total solids (approximately 12%). By contrast, Deeth (2002) showed that homogenised raw milk (therefore of a natural % total solids) became rancid almost immediately. The strong preheat treatment given to the milk before evaporation in the work described here (above 90°C) would have inactivated the endogenous lipolytic enzymes that Deeth encountered in raw milk, though not all of the lipolytic enzymes present in the milk. Thus when the casein micelles attached to the newly formed membrane in homogenised, concentrated and heated milk they would not bring with them active milk lipoprotein lipase. This does not explain the lack of effectiveness of the pig pancreatic lipase (PPL) upon incubation. The answer probably lies in observations made by Dalgleish & Banks (1991) and others (e.g. Ye, 2003) which suggest that when concentrated milk is heated and disrupted the amount of membrane protein, formed by the deposition mainly of casein on the fat-serum interface, tends to increase with increases in temperature and concentration. Electron micrographs, as shown for example in Figure 6.4, do show indeed that the fat globules are covered by very thick layers of casein that can sometimes show evidence of fusion of individual micelles (Trinh, 2004). It is proposed that this thick layer prevents access by PPL to the core fat.
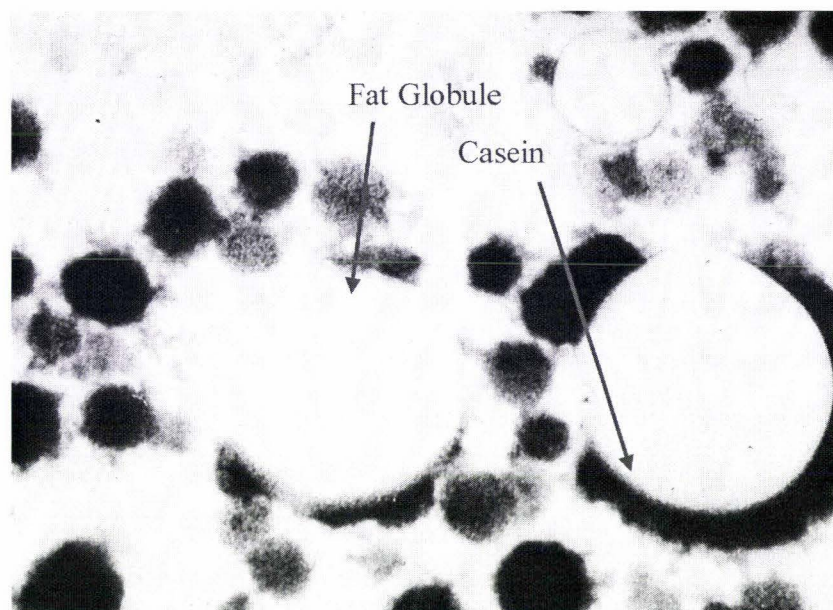
Figure 6.4: Electron micrograph of homogenised milk concentrate (Trinh, 2004).

The evolution of particle size in the two plants is shown in Figures 6.5 and 6.6. The graphs showing the changes in the O Zone show clearly that the O Zone decreased continuously from the evaporator balance tank to the concentrate heater (Figure 6.5B) or homogeniser outlet (Figures 6.5D, 6.6A). Thus, clearly, the fat globules were being continuously damaged during the milk powder manufacturing process. In Powder 1, a small drop was measured in the value of the C+D Zone as the milk went through the preheating section and this was accompanied by a significant increase in the A+B Zone (Figure 6.5A and 6.5C). This, together with the decrease in the O Zone, gives a picture of disruption not only of the native fat globules (O Zone) but also of those that had flocculated and/or coalesced (C+D Zone). This result was to have been expected from the results of the DSI experiments reported in Section 2 of Chapter 5.

(A)

(B)

(C)

(D)
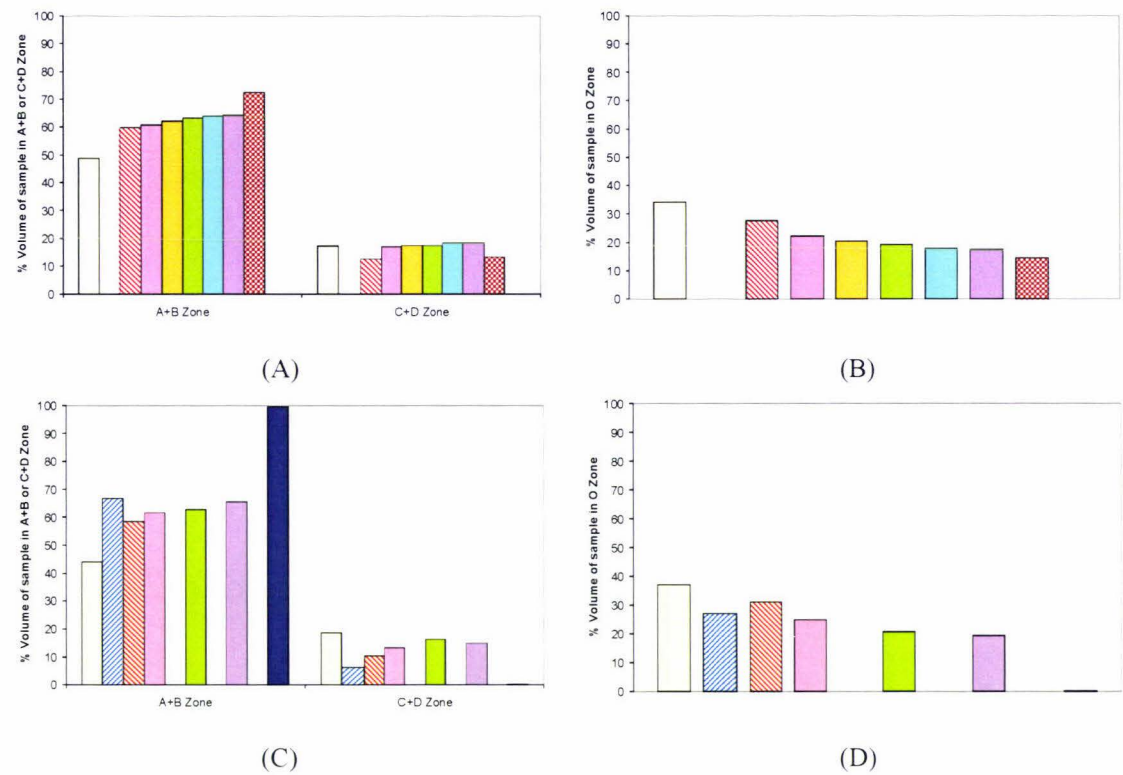
Figure 6.5: PSZ test results from Powder 1 (runs S10, S11) at Pahiatua dairy factory. A+B and C+D Zones are shown for runs S10 (A) and S11 (C). O Zone data are shown for runs S10 (B) and S11 (D).

Evaporator Balance Tank	Indirect preheat outlet	Total preheat outlet	Effect 1 outlet	Effect 2 outlet	Effect 3 outlet	Effect 4 outlet	Effect 5 outlet	Concentrate heater	Homogeniser outlet
☐	☒	☒	☐	☐	☐	☐	☐	☒	■

(A)   (B)

Figure 6.6: PSZ test results from Powder 2 (run S12) at Pahiatua dairy factory. A+B and C+D Zones are shown S12 (A). O Zone data are shown for run S12 (B).

Evaporator balance tank	Indirect preheat outlet	Total preheat outlet	Effect 1 outlet	Effect 2	Effect 2 outlet	Homogeniser outlet
☐	▧	▨	■	■	■	■

The changes in the milk as it passed through the evaporators were somewhat variable. There was a general increase in the C+D Zone over the first part of the evaporator (effect 1 to effect 3) in Powder 1 but the C+D Zone then tended to either increase (run S10, Figure 6.5A) or decrease (run S11, Figure 6.5C) slightly from effect 3 to effect 5. Extensive particle size measurements made by a fellow PhD candidate who collaborated with the author in a survey of the two evaporators at the Pahiatua site showed clearly that the C+D and A+B Zone data from the first three effects did not vary significantly with manufacturing specifications or the date of manufacture but that the C+D Zone data from the last two effects showed very wide variations between runs, with substantial fluctuations (Figures 6.7 and 6.8, by permission, Croy et al., 2004). This can be attributed either to the fact that the large fat aggregates were easily destabilised or the fact that the concentrated samples of whole milk creamed very fast, as reported in Chapter 5, thus making it impossible to collect representative samples.

Samples taken post-homogeniser showed complete disruption of large fat globules through a massive increase in the A+B Zone and the complete disappearance of the C+D Zone and O Zone (Figure 6.5 and 6.6). This again would have been expected from results presented in Chapter 5.
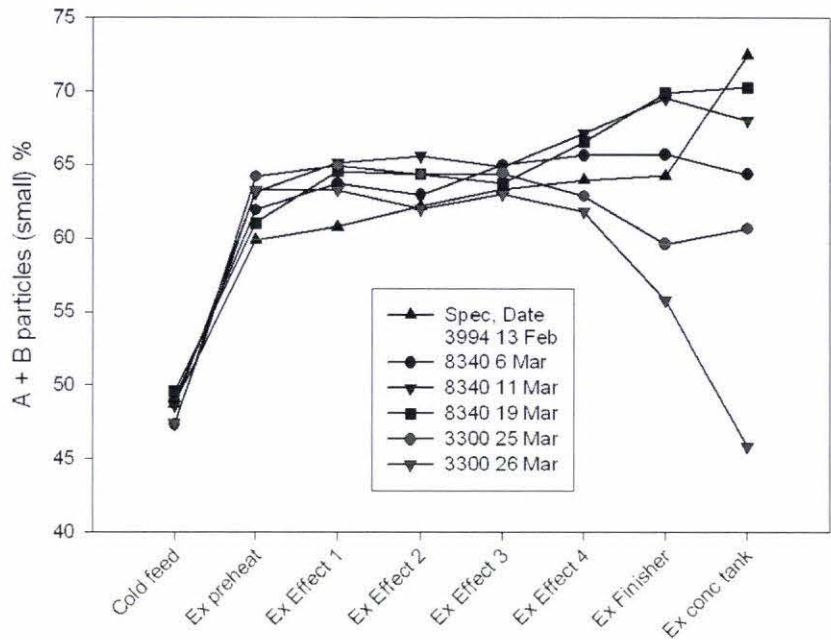
Figure 6.7: Effect of different specifications and dates of manufacture on PSD (A+B Zone) of milk concentrate from Powder 1 at Pahiatua dairy factory. Source: Croy et al. (2004).
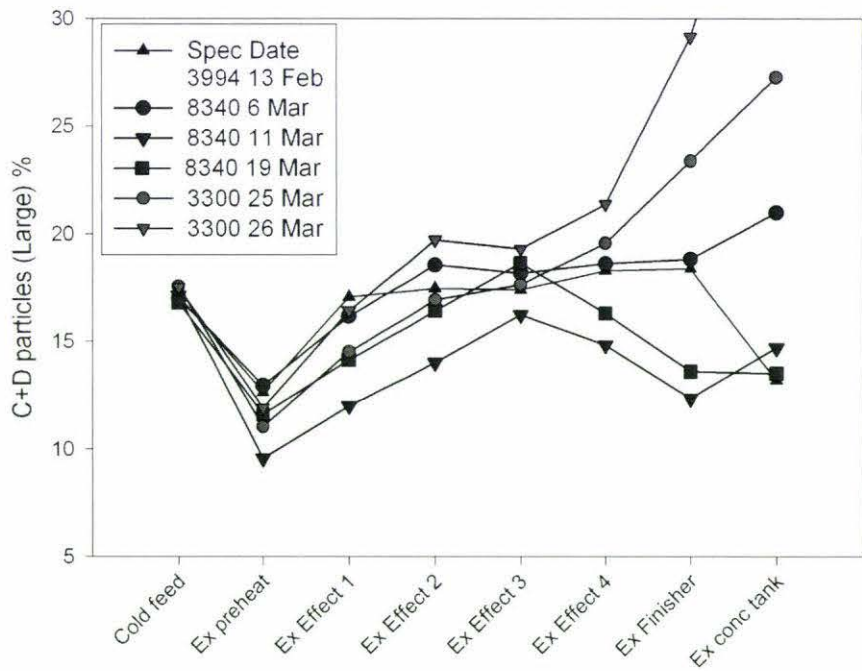
Figure 6.8: Effect of different specifications and dates of manufacture on PSD (C+D Zone) of milk concentrate from Powder 1 at Pahiatua dairy factory. Source: Croy et al. (2004).

6.3   Conclusion

These surveys of NMFGM damage in the dairy industry were confined to milk powder manufacturing plants only because of the difficulty of obtaining representative sample

populations of events from the farm to the factory. Only two plants at one site were surveyed adequately. The evidence shows that there was continuous damage to fat globules from the time raw milk entered the plant to the time the concentrated milk exited the homogeniser. In general the UPF level increased up to the homogeniser as indicated by measurements of SLFA. The homogeniser itself caused extensive disruption of fat globules but did not create further UPF. This is the only observation that was not predicted from the controlled experimental work performed at Massey University reported in Chapters 4 and 5. Since the actual details of the measured SLFA and PSD profiles varied so much with the design and operation of the plant it is recommended, now that the protocol for the survey has been successfully tested, that the survey be repeated many times, and at many other sites, if a more complete picture of NMFGM damage in the New Zealand dairy industry is required.

Chapter 7
Conclusions

Damage to the NMFGM in milk powder manufacturing operations was the focus of this work, which was divided into three objectives:

1. Development of techniques for measuring NMFGM damage.

2 Analysis of the factors that cause NMFGM damage using the measurement techniques devised.

3. Case studies of NMFGM damage occurring in a number of key operations or equipment found in milk powder manufacturing plants, where the factors studied in reaching objective 2 could be found.

7.1 Measurement of NMFGM damage

The literature review (Chapter 2) revealed clearly at the beginning of this work that no techniques for the direct measurement of NMFGM damage were available. The indirect tests then available appeared inadequate for measuring NMFGM damage reliably. For example, what is actually measured in the long-used solvent extractable free fat (FF) test is highly questionable (Evers et al., 2001). The use of particle size measurement to measure NMFGM damage had been applied only in a qualitative way. Traditional FFA measurement tests or the lipolysable fat (LF) test of Miller and Puhan (1986a) were inadequate for use in milk powder manufacturing plants, where the milk lipase had been largely inactivated by pasteurisation. The most promising approach appeared to be that of Fang (1998) who added a lipolytic enzyme, pig pancreatic lipase (PPL), that was reported to attack only the fat made available by disruption of the NMFGM but not attack the membrane itself. In the present study, the lipolysable free fat (LFF) test of Fang (1998) was found not to be reliable enough to allow accurate measurement of NMFGM damage in early surveys of industrial plants.

A major reason for inconsistencies was found to be microbial growth during the incubation of samples at 30°C for 24 hours. Two preservatives (bronopol and sodium azide) were tested in an attempt to reduce the microbial contamination, but both created

artefacts in the FFA determination. This was in agreement with previous research by Miller & Puhan (1986a) and Imhof & Bosset (1995).

A reduced incubation time of 6 hours at 37°C, and an increase in added lipase concentration from 5 µg/L to 20 µg/L, proved satisfactory in reducing the effects of microbial contamination. Because of the changes made to the LFF test and the confusing use of the term 'lipolysable fat' in the literature, the decision was made to give this test a new name, the selective lipolysis (SL1) test.

The reproducibility of the SL1 test was twice that of the LFF test. The SL1 test was found to be sensitive in measuring NMFGM damage in a number of milk processing operations including agitation, pumping and evaporation. Some levels of damage could not be determined. For example, milk passed 3 times through a pumping rig could not be differentiated from milk passed through 6 times. The effects of preheating operations were also difficult to measure if the incubation fatty acids (IFA) value changed between the inlet and outlet value, owing to a drop in the microbial and/or endogenous lipase activity. The measurement of IFA and of original fatty acids (OFA) was found to be insensitive to changes in NMFGM damage.

It was found that large amounts of NMFGM damage created separation of some of the fat from the milk emulsion, either in foam or as churned fat. Few authors mention the presence of foam in their experiments and very few authors have reported the measurement of NMFGM damage in foam (Miller & Puhan, 1986b). The measurement of FFA in foam and particularly in churned fat in the present study was found to be difficult. Liquid milk damaged at temperatures below 15°C was found to have fat floating on top of it, after the incubation period, in the SL1 test. Though a gentle inversion procedure was already used in the SL1 test, a far more vigorous agitation procedure (homogenisation for 15 seconds) was employed late in this work and formed the basis for version 2 of the selective lipolysis test – the SL2 test. This considerably improved the ability to take a representative sub-sample after a milk or foam sample had been incubated.

Measurements of the physical consequences of NMFGM damage reported in the literature have been mostly reliant on FF tests. These tests were heavily criticised by

authors such as Deeth & Fitz-Gerald (1978), Walstra et al. (1984), Miller & Puhan (1986b) and Evers et al. (2001). For this reason, measurement of particle size distributions (PSDs) of milk samples was used predominately in this work. The new particle size zoning (PSZ) test was designed to give quantitative measures of disruption and coalescence, by extracting information from the PSDs of milk samples.

The PSZ test was found to be very reproducible and easy to use. It was found that the test was sensitive in measuring changes in particle size, owing to either disruption or coalescence caused by agitation, pumping, preheating and evaporation. Samples could be stored for up to 24 hours at 5°C before measurement without deterioration. Difficulty was found in measuring samples with foam or churned fat.

The present study showed that measurements on foam and churned fat must be included in measurements of NMFGM damage. Both contained high fat contents and high levels of NMFGM damage.

Comparisons between the SL1 test, the PSZ test, and the solvent-extractable FF test of Fang (1998) showed that all three tests gave different measures of NMFGM damage. No one NMFGM damage test gives a full picture of NMFGM damage in a sample. The SL1 and PSZ tests yield a more comprehensive and complex picture of the damaged system than has been obtained with any single test in previous attempts to measure consequences of NMFGM damage.

7.2   Mechanical factors affecting NMFGM damage

The present study showed that a number of mechanical factors increase NMFGM damage as measured using the SL1, SL2 and PSZ tests. Air inclusion was a major factor in increasing the level of NMFGM damage at 10°C and 20°C. This was consistent with previous work on liquid milk samples (Aule & Worstorff, 1975; Miller & Puhan, 1986b).

Pump speed, pipeline velocity and valve cavitation were shown to be contributing factors to NMFGM damage. The increase in NMFGM damage with pump speed was consistent with the results of Rudzik (1987) and Escobar & Bradley (1990).

Preheating using a plate heat exchanger (PHE) was found not to change the particle size distribution of milk but direct steam injection was found to cause significant increases in the A+B and C+D Zones, suggesting that both disruption and coalescence occurred. These increases were found to be proportional to the temperature difference across the DSI unit, which in turn is proportional to the rate of steam injection.

Agitation time and temperature were found to affect the amount of NMFGM damage. As the agitation time increased, the C+D Zone also increased. This was more pronounced at 5°C than at 45°C. Agitation above 1000 rpm at 25°C was found to increase the C+D Zone, indicating coalescence, and, above 1300 rpm to increase the A+B Zone, indicating disruption. This is consistent with the argument of Walstra et al. (1984) that disruption begins at higher shear rates than does coalescence. NMFGM damage was a maximum at an agitation temperature of 20-25°C. This was consistent with the work of Deeth & Fitz-Gerald (1978). However, in the present study, foam and/or churned fat were visually observed and measured. The contribution of NMFGM damage from the foam and/or churned fat had a significant effect on the total level of NMFGM damage. This is a new finding.

Evaporation was shown to cause an increase in SLFA and to cause both coalescence and disruption of fat globules. This is in agreement with Ye (2003) who showed this qualitatively when milk was passed through a pilot plant evaporator.

7.3 Industrial survey of NMFGM damage in milk powder plants

The preliminary evidence collected in the present work suggests that there is continuous damage to fat globules from the time milk enters the plant to the time the concentrated milk exits the homogeniser. In general the extent of NMFGM damage increased up to

the homogeniser, as indicated by measurements of SLFA. Disruption was shown by an increase in the A+B Zone up to and including the homogeniser.

7.4 Strengths and limitations of present work

This work has provided a large amount of evidence to buttress and extend the current understanding of NMFGM damage. In particular, two new tests have been developed that together give a reasonably comprehensive picture of NMFGM damage in a range of milk processing operations.

A number of sample handling issues have been resolved or identified, for example the use of homogenisation in the SL2 test when fat separates from the liquid milk after incubation. Microbial contamination is still a problem and limits the application of the SL2 test to situations where the microbial and endogenous lipolytic activity is constant. The PSZ test is limited to liquid milk samples that do not contain churned fat.

The selective lipolysis test is based on the action of a selective enzyme. Pig pancreatic lipase was used in this work because of its similar action to milk lipoprotein lipase (LPL) and its ready availability.

The present work was done on pasteurised milk, which has rarely been studied. While the trends in NMFGM damage presented here are similar to those for raw milk and cream reported by previous authors, it cannot be assumed that the new tests developed will be suitable for these dairy products on this evidence alone.

Many processing variables were studied in this work, but these did not include the many compositional, chemical and physical factors that also affect NMFGM damage. Further research on the enzymes and microbes responsible for NMFGM damage is needed.

7.5 Further Studies

The original goal of this work was to determine whether an increase in the level of NMFGM damage to the milk during milk powder processing would reduce the quality

of the resulting milk powder and the efficiency of the manufacturing process. This was not possible within the timeframe of this Ph.D. due to deficiencies in the current measurement techniques of NMFGM damage. These measurement issues had to be addressed first.

More work is required to refine the new techniques of measuring NMFGM damage described and used in this work. The greater the flexibility and practicality of these new tests, the more likely they are to become widely used. Thus, it is very important that the sample handling and microbial contamination issues identified are dealt with in a systematic way that can be applied without greatly increasing the complexity of the tests.

The wide use of these tests will allow the many variables in other dairy manufacturing processes (and farm operations) that influence NMFGM damage to be studied.

It is suggested that more surveys of NMFGM damage in milk powder plants should be made to obtain a more complete picture. The profile of NMFGM damage in these plants should also be correlated with identifiable equipment design characteristics such as pump model, evaporator configuration and operation, and drier characteristics. Surveys should also be made of NMFGM damage in other dairy processes, particularly for cream and cheese products.

Attempts should be also made to correlate the extent of NMFGM damage with product quality measures such as flavour, milk powder solubility and other functional properties, so that process modifications can be made where they are deemed necessary.

References

Adhikari, A. K. and Singhal, O. P. (1992) Effect of heat resistant micro-organisms on the fatty acid profile and the organoleptic quality of UHT milk during storage, *Indian Journal of Dairy Science*, **45** (5), 272.

Ahrné, L. and Björck, L. (1985) Lipolysis and the distribution of lipase activity in bovine milk in relation to stage of lactation and time of milking, *Journal of Dairy Research*, **52** (1), 55.

Al-Shabibi, M. M. A., Langner, E. H., and Tuckey, S. L. (1964) Effect of added fatty acids on the flavor of milk, *Journal of Dairy Science*, **47**, 295.

Allen, J. C. and Joseph, G. (1985) Deterioration of pasteurized milk on storage, *Journal of Dairy Research*, **52** (3), 469.

Anderson, M. (1981) Inhibition of lipolysis in bovine milk by proteose peptone, *Journal of Dairy Research (abstract)*, **48** (2), 247.

Anderson, M. (1982) Factors affecting the distribution of lipoprotein lipase activity between serum and casein micelles in bovine milk, *Journal of Dairy Research (abstract)*, **49** (1), 51.

Anderson, M. (1983) Milk lipase and off-flavour development, *Journal of the Society of Dairy Technology*, **36** (1), 3.

Anderson, M. and Cheeseman, G. C. (1975) Stability of the fat globule membrane, *Annual Bulletin, International Dairy Federation*, **86**, 11.

Anderson, M., Cheeseman, G. C., and Knight, D. J. (1972) Location of proteins within the milk fat globule membrane, *Journal of Dairy Research*, **39**, 409.

Anderson, M., Heeschen, W., Jellema, A., Kuzdzal-Savoie, S., Needs, E. C., Suhren, G., and van Reusel, A. (1991) Monograph on determination of free fatty acids in milk & milk products, *Bulletin of the International Dairy Federation*, (No. 265), 52pp.

Antonelli, M. L., Curini, R., Scricciolo, D., and Vinci, G. (2002) Determination of free fatty acids and lipase activity in milk: quality and storage markers, *Talanta*, **58** (3), 561.

Ardo, Y. (1979) Bronopol as a preservative in milk samples, *Milchwissenschaft*, **34** (1), 14.

Ardo, Y. (1982) Bronopol as a preservative in milk samples for the determination of cell content using fossomatic, *Milchwissenschaft*, **37** (3), 139.

Aule, O. and Worstorff, H. (1975) Influence of mechanical treatment of milk on quantities of FFA and free fat in the milk, as well as on the separability of the milk, *Annual Bulletin, International Dairy Federation*, **86**, 116.

References

Ayers, C. W. (1956) *Analytical Chimie Acta*, **15**, 77.
Cited by: Koops, J. and Klomp, H. (1977) Rapid colorimetric determination of free fatty acids (lipolysis) in milk by the copper soap method, *Netherlands Milk and Dairy Journal*, **31**, 56

Balasubramanya, N. N., Bhavadasan, M. K., and Naryanan, K. M. (1988) Lipolysis of milk fat in cream, *Indian Journal of Dairy Science*, **41** (3), 269.

Beisson, F., Tiss, A., Rivière, C., and Verger, R. (2000) Methods for lipase detection and assay: a critical review, *European Journal of Lipid Science and Technology*, **102**, 133.

Benda, P. (1995) The effect of some preservatives on natural microflora in milk samples, *Veterinarni Medicina*, **40** (11), 359.

Bengtsson, G. and Olivecrona, T. (1982) Activation of lipoprotein lipase CH. Demonstration of an effect of the activator on the binding of the enzyme in milk fat globules, *FEBS Letters*, **47**, 18.

Bennett, H. A. E. (2000) "Whole milk fouling of heated surfaces". Masters Thesis, Massey University, Palmerston North, New Zealand.

Bernstein, B. A. (1977) "I. Inhibition of cholesterol biosynthesis and acetyl-coenzyme-a synthetase by bovine milk and orotic acid. II. Cytochromes in the fat globule membrane of bovine milk and in the microsomes of the lactating bovine mammary gland". Ph.D. Thesis, University of Wisconsin, Madison *Dissertation Abstracts International B* 38 No. 5256.

Bors, W., Saran, M., Lengfelder, E., Spottl, R., and Michel, C. (1974) *Current Topics in Radiation Research Quarterly*, **9**, 247.
Cited by: McPherson, A. V. and Kitchen, B. J. (1983) Reviews of the progress of dairy science - the bovine milk fat globule membrane - Its formation, composition, structure and behavior in milk and dairy products, *Journal of Dairy Research*, **50**, 107

Bosset, J. O., Imhof, I., and Steiger, G. J. (1990) Determination of free fatty acids in milk and cream. I. Development of an automatic potentiometric titration method in non-aqueous solutions and comparison with the Deeth Visual Titration Method, *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, **81** (3), 296.

Brockerhoff, H. and Jensen, R. G. (1974) In: *Lipolytic Enzymes,* Academic Press, New York, USA, pp. 118-129.

Brockman, H. L. (1984) In: *Lipases,* (Ed. Borgstrom, B. and Brockman, H. L.), Elsevier Science, Amsterdam, Netherlands, pp. 1-46.

Brooker, B. E., Anderson, M., and Andrews, A. T. (1986) The development of structure in whipped cream, *Food Microstructure*, **5**, 277.

Brunner, J. R. (1974) In: *Fundamentals of Dairy Chemistry,* 2nd edn, (Ed. Webb, B. H., Johnson, A. H., and Alford, J. A.), AVI Publishing, Westport, Conneticut, USA, pp. 474-602.

Buchanan, R. A. (1965) Lipolysis and the frothing of milk, *Australian Journal of Dairy Technology*, **20**, 62.

Buma, T. J. (1971) Free fat in spray-dried whole milk. 2. Evaluation of methods for determination of free-fat content, *Netherlands Milk and Dairy Journal*, **25** (1), 42.

Carter, R. S. (1990) Alfa-Laval Triovac Milking System., *Proceedings of the 7th Seminar - Dairy Cattle Society of the New Zealand Veterinary Association*, Hamilton, New Zealand.

Cartier, P. and Chilliard, Y. (1989a) Lipase redistribution in cows' milk during induced lipolysis. I. Activation by agitation, temperature change, blood serum and heparin, *Journal of Dairy Research*, **56** (5), 699.

Cartier, P., Chilliard, Y., and Bout, J. (1989b) Lipase redistribution in cows' milk during induced lipolysis. II. Activation by milk pH adjustment, *Journal of Dairy Research*, **56** (5), 711.

Celestino, E. L., Iyer, M., and Roginski, H. (1997a) The effects of refrigerated storage of raw milk on the quality of whole milk powder stored for different periods, *International Dairy Journal*, **7** (2-3), 119.

Celestino, E. L., Iyer, M., and Roginski, H. (1997b) Reconstituted UHT-treated milk: Effects of raw milk, powder quality and storage conditions of UHT milk on its physio-chemical attributes and flavour, *International Dairy Journal*, **7** (2-3), 129.

Chen, J. H. S. and Bates, C. R. (1962) Observations on the pipeline milker operation and its effects on rancidity, *Journal of Milk and Food Technology*, **25**, 176.

Chen, L. (2000) "Thermophilic enzymes and their impact on milk powder during storage". Ph.D. Thesis, University of Waikato, Hamilton.

Chen, L., Daniel, R. M., and Coolbear, T. (2003) Detection and impact of protease and lipase activities in milk and milk powders, *International Dairy Journal*, **13** (4), 255.

Chilliard, V. (1982) Variation physiologiques des activites lypasiques et de la lypolyse spontanee des laits de vache, de chevre et de femme, *Le Lait*, **62** (1), 126.

Choi, I. W., Jeon, I. J., and Smith, J. S. (1994) Isolation of lipase-active fractions from ultra-high temperature-processed milk and their patterns of releasing fatty acids from milk fat emulsion, *Journal of Dairy Science*, **77** (8), 2168.

Christen, G. L., Shen, N., and Maruri, J. L. (1993) Recovery of short chain-length fatty acids from milk by several methods, *Dairy Food & Environmental Sanitation*, **13** (12), 707.

Collomb, M. and Spahni, M. (1991) Potentiometric determination of the acidity of butterfat in non-aqueous medium, *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, **82** (2), 174.

References

Collomb, M. and Spahni, M. (1995) Review of the methods used for the determination of free fatty acids in milk and dairy products, *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie*, **28** (4), 355.

Connolly, J. F. (1978) Recent findings on lipolysis in Ireland - The impact of environment and nutrition on free fatty acids and flavour in milk and dairy products, *XX International Dairy Congress*, vol. 62 ST (B), Paris, France.

Croy, R. J. (2000) "Development of an in-line CIP sensor". Masters Thesis, Massey University, Palmerston North, New Zealand.

Croy, R. J., Trinh, K. T. Chen H., and Hemar, Y. (2004) The rheology and particle size of milk concentrate in industrial falling-film evaporators, *9th International Conference of Engineering and Food*, Montpellier, France.

Dalgleish, D. G. and Banks, J. M. (1991) The formation of complexes between serum proteins and fat globules during heating whole milk, *Milchwissenschaft*, **46** (2), 75.

Darling, D. F. and Butcher, D. M. (1978) Milk-fat globule membrane in homogenized cream, *Journal of Dairy Research*, **45**, 197.

Deeth, H. C. (1997) The role of phospholipids in the stability of milk fat globules, *Australian Journal of Dairy Technology*, **52** (1), 44.

Deeth, H. C. (2002) Lipolysis in Milk and Dairy Products - a Research Journey, *Food Australia*, **54** (10), 433.

Deeth, H. C. and Fitz-Gerald, C. H. (1976) Lipolysis in dairy products: a review, *Australian Journal of Dairy Technology*, **31** (2), 53.

Deeth, H. C. and Fitz-Gerald, C. H. (1977) Some factors involved in milk lipase activation by agitation, *Journal of Dairy Research*, **44**, 569.

Deeth, H. C. and Fitz-Gerald, C. H. (1978) Effects of mechanical agitation of raw milk on the milk-fat globule in relation to the level of induced lipolysis, *Journal of Dairy Research*, **45** (3), 373.

Deeth, H. C. and Fitz-Gerald, C. H. (1995) In: *Advanced Dairy Chemistry, Volume 2: Lipids*, (Ed. Fox, P. F.), Chapman and Hall, London, U.K., pp. 247-308.

Deeth, H. C., Fitz-Gerald, C. H., and Snow, A. J. (1983) A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products, *New Zealand Journal of Dairy Science and Technology*, **18**, 13.

Deeth, H. C., Fitz-Gerald, C. H., and Wood, A. F. (1975) A convenient method for determining the extent of lipolysis in milk, *Australian Journal of Dairy Technology*, **30** (3), 109.

Deeth, H. C., Khusniati, T., Datta, N., and Wallace, R. B. (2002) Spoilage patterns of skim and whole milks, *Journal of Dairy Research*, **69** (2), 227.

Diller-Zulauf, A. and Wirsekara, P. R. (1943-1944) *Deutsche Molkerei-Zeitung*, **92**, 46.

Cited by: Aule, O. and Worstorff, H. (1975) Influence of mechanical treatment of milk on quantities of FFA and free fat in the milk, as well as on the separability of the milk, *Annual Bulletin, International Dairy Federation*, **86**, 116

Dole, V. P. (1956) A relation between non-esterified fatty acids in plasma and the metabolism of glucose, *Journal of Clinical Investigation*, **35**, 150.

Dole, V. P. and Meinertz, H. J. (1960) Micro-determination of LCFA in plasma and tissues, *Journal of Biological Chemistry*, **235**, 2595.

Downes, T. E. H., Nieuwoudt, J. A., and Slabbert, E. A. (1974) The influence of the physical treatment of milk in milking machine systems, and during cream separation, on the lipolysis of milk fat, *South African Journal of Dairy Technology*, **6** (4), 215.

Downey, W. K. (1975) Identity of the major lipolytic enzyme activity of bovine milk in relation to spontaneous and induced lipolysis, *Annual Bulletin, International Dairy Federation*, **86**, 80.

Downey, W. K. (1980) Review of the progress of dairy science: Flavour impairment from pre- and post-manufacture lipolysis in milk and dairy products, *Journal of Dairy Research*, **47**, 237.

Downey, W. K. and Murphy, R. F. (1970) Association of lipases with micellar and soluble casein complexes, *Journal of Dairy Research*, **37**, 47.

Downey, W. K. and Murphy, R. F. (1975) Partitioning of the lipolytic enzymes in milk, *Annual Bulletin, International Dairy Federation*, **86**, 19.

Driessen, F. M., Jellema, A., van Luin, F. J. P. , Stadhouders, J., and Wolbers, G. J. M. (1977) The estimation of fat acidity in raw milk. An adaption of the BDI method, suitable for routine assays, *Netherlands Milk and Dairy Journal*, **31**, 40.

Driessen, F. M. and Stadhouders, J. (1974) A study of spontaneous rancidity, *Netherlands Milk and Dairy Journal*, **28** (2), 130.

Driessen, F. M. and Stadhouders, J. (1975) The lipolytic enzymes and co-factors responsible for spontaneous rancidity in cow's milk, *Annual Bulletin, International Dairy Federation*, **86**, 73.

Duncan, S. E. and G. L. Christen (1991) Sensory detection and recovery by acid degree value of fatty acids added to milk, *Journal of Dairy Science* **74**, 2855.

Duncan, S. E., Christen, G. L., and Penfield, M. P. (1990) Acid degree value - does it really predict rancid flavor in milk?, *Dairy & Food Sanitation*, **10** (12), 715.

Duncan, S. E., Christen, G. L., and Penfield, M. P. (1991) Rancid flavour of milk: relationship of acid degree value, free fatty acids, and sensory perception, *Journal of Food Science*, **56** (2), 394.

Duncombe, W. G. (1963) The colorimetric microdetermination of long chain fatty acids, *Biochemistry Journal*, **88**, 7.

Earley, R. R. and Hansen, A. P. (1982) Effect of process and temperature during storage of ultra-high temperature steam-injected milk, *Journal of Dairy Science*, **65** (1), 11.

Escobar, G. J. and Bradley, R. L. Jr. (1990) Effect of mechanical treatment on the free fatty acid content of raw milk, *Journal of Dairy Science*, **73** (8), 2054.

Evers, J. M. (2003) Determination of free fatty acids in milk using the BDI method - some practical and theoretical aspects, *International Dairy Journal*, **13** (2-3), 111.

Evers, J. M., Crawford, R. A., Robinson, N. P., Harding, D. R. K., and McCarthy, O. J. (2001) 'Free fat' solvent extraction methods damage milkfat globules, *Milchwissenschaft*, **56** (9), 504.

Evers, J. M., Luckman, M. S., and Palfreyman, K. R. (2000a) The BDI method - Part I: determination of free fatty acids in cream and whole milk powder, *Australian Journal of Dairy Technology*, **55** (1), 33.

Evers, J. M., Morris, A., Conaghan, E. F., and Palfreyman, K. R. (2000b) The BDI method - Part II: the effect of the whole milk powder manufacturing process on free fatty acid levels, *Australian Journal of Dairy Technology*, **55** (1), 37.

Evers, J. M. and Palfreyman, K. R. (2001) Free fatty acid levels in New Zealand raw milk, *Australian Journal of Dairy Technology*, **56** (3), 198.

Fang, L. (1998) "The effect of milk fat globule membrane damage in the absence of air on fouling in heat exchangers". Masters Thesis, Massey University, Palmerston North, New Zealand.

Fink, A. and Kessler, H. G. (1983) Determination of free fat in cream for the assessment of damage caused to fat globules by processing. *Milchwissenschaft*, **38** (6), 330.

Fink, A. and Kessler, H. G. (1985) Changes in the Fat Globule Membrane Produced by Heating, *Milchwissenschaft*, **40** (5), 261.

Fink, A. and Kessler, H. G. (1986a) Determination of free fat in cream in assessment of changes in fat-globule membrane caused by processing. I. Centrifugation method. *Deutsche Milchwirtschaft*, **37** (10), 256.

Fink, A. and Kessler, H. G. (1986b) Determination of free fat in cream in assessment of changes in fat-globule membrane caused by processing. II. Extraction method. *Deutsche Milchwirtschaft*, **37** (11), 296.

Fitz-Gerald, C. H. (1974) Milk lipase activation by agitation - influence of temperature, *Australian Journal of Dairy Technology*, **28**, 28.

Fitz-Gerald, C. H., Deeth, H. C., and Coghill, D. M. (1982) Low-temperature inactivation of lipases from psychrotrophic bacteria, *Australian Journal of Dairy Technology*, **37** (2), 51.

Fleming, M. G. (1979) Lipolysis in bovine milk as affected by mechanical and temperature activation - a review, *Irish Journal of Food Science and Technology*, **3** (2), 111.

Fox, P. F., Power, P., and Cogan, T. M. (1989) In: *Enzymes of psychrotrophs in raw food,* (Ed. Mckellar, R. C.), CRC Press, U.S.A., pp. 57-120.

Frankel, E. N. and Tarassuk, N. P. (1955) An extraction-titration method for the determination of free fatty acids in rancid milk and cream, *Journal of Dairy Science,* **38**, 751.

Gander, G. W. (1955) The occurrence of rancidity in milk from pipeline milkers (abstract), *Journal of Dairy Science,* **38**, 595.

Gander, G. W. and Jensen, R. G. (1960) Pipeline milker studies. Some factors affecting the development of lipolysis (abstract), *Journal of Dairy Science,* **43**, 1887.

Gholston, J. H., Gelpi, A. J. Jr., and Frye, A. B. Jr. (1966) Effect of high level and low level milk pipeline on milk fat acid degree values, *Journal of Milk and Food Technology,* **29**, 248.

Girardet, J. M., Linden, G., Loye, S., Courthaudon, J. L., and Lorient, D. (1993) Study of mechanism of lipolysis inhibition by bovine-milk proteose-peptone component-3, *Journal of Dairy Science,* **76** (8), 2156.

Goulden, J. D. S. and Phipps, L. W. (1964) Factors affecting the fat globule sizes during the homogenization of milk and cream, *Journal of Dairy Research,* **31**, 195.

Greenbank, G. R. and Pallansch, M. J. (1961) Migration of phosphatides in processing dairy products, *Journal of Dairy Science,* **44** (9), 1597.

Grindal, R. J. (1988) Influence of milking conditions and vacuum control on hydraulic milking performance, *Journal of Dairy Research,* **55** (1), 15.

Halter, N., Puhan, Z., and Schmutz, M. (1978) A simple and fast method for determining fat damage in milk and cream, *Nordeuropaeisk Mejeri-Tidsskrift,* **44** (7), 190.

Hamosh, M. (1984) In: *Lipases,* (Ed. Borgstrom, B. and Brockman, H. L.), Elsevier Science, Amsterdam, Netherlands, pp. 49-81.

Harper, W. J., Gould, I. A., and Hankinson, C. L. (1961) Observations on the free volatile acids in milk, *Journal of Dairy Science,* **44**, 1764.

Harper, W. J., Schwartz, D. P., and El Hagarawy (1956) A rapid silica gel method for measuring total free fatty acids in milk, *Journal of Dairy Science,* **39**, 46.

Heeschen, W., Suhren, G., and Tolle, A. (1975) Measurement of free fatty acids in milk in the Auto-analyzer II system, *Annual Bulletin, International Dairy Federation,* **86**, 178.

Hemingway, E. B., Smith, G. H., and Rook, J. A. F. (1970a) The pattern of release of free fatty acids from milk fat under the action of intrinsic and added lipases, *Journal of Dairy Research,* **37**, 83.

References

Hemingway, E. B., Smith, G. H., Rook, J. A. F., and O'Flanagan, N. C. (1970b) Lipase taint, *Journal of the Society of Dairy Technology*, **23** (1), 44.

Herbst, K. D., Roiner, F., and Thormahlen, H. (1984) Effect on milk quality of deep cooling and mechanical treatment, *Deutsche Milchwirtschaft*, **35** (19), 704.

Herrington, B. L. and Krukovsky, V. N. (1942) Studies of lipase action IV. The influence of the rate of cooling upon the subsequent rate of lipolysis in milk stored at low temperatures, *Journal of Dairy Science*, **25**, 241.

Hicks, C. L., Purba, A., and O'Leary, J. (1990) Effect of cycle pumping milk on cheese yield, *Cultured Dairy Products Journal*, **25** (1), 20.

Hinrichs, J. (1994) Stress limit for fat globules, *Milchwirtschaftlich Berichte*, **121**, 227.

Hinrichs, J. (1998) Flow rates for milk and cream in pipelines, *Milchwissenschaft*, **53** (3), 139.

Hisserich, D. and Reuter, H. (1984) Limits of the mechanical stress of raw milk for induced lipolysis, *Milchwissenschaft*, **39** (6), 333.

Hlynka, I., Hood, E. G., and Gibson, C. A. (1944) *Canadian Dairy and Ice Cream Journal*, **23** (3), 26

Hohe, K. A., Dimick, P. S., and Kilara, A. (1985) Milk lipoprotein lipase distribution in the major fractions of bovine milk, *Journal of Dairy Science*, **68** (5), 1067.

Houlihan, A. V. (1992) Enzymatic activity other than lipase, *Bulletin of the International Dairy Federation*, (No. 271), 21.

Houlihan, A. V., Goddard, P. A., Nottingham, S. M., Kitchen, B. J., and Masters, C. J. (1992) Interactions between the bovine milk fat globule membrane and skim milk components on heating whole milk, *Journal of Dairy Research*, **59**, 187.

Hunter, A. C. (1966) Lipolysis in milk in N.E. Scotland, *Dairy Industries*, **31**, 227.

Imhof, I. and Bosset, J. O. (1995) The determination of free fatty acids in milk and cream. V: Chemical and thermal stabilisation of samples, *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, **86** (2), 161.

Instrument Society of America (1995) "Considerations for evaluating control valve cavitation. Recommended practice (ISA-RP75.23)". North Carolina, U.S.A., 58pp.

International Dairy Federation Standard. 141C (2000), Whole milk: Determination of milkfat, protein and lactose content. Guidance on the operation of mid-infrared instruments. International Dairy Federation, Brussels, Belgium.

Jaeger, K.-E., Dijkstra, B. W., and Reetz, M. T. (1999) Bacterial biocatalysts: Molecular biology, three-dimensional structures, and biotechnological applications of lipases, *Annual Reviews Microbiology*, **53** (1), 315.

References

Jaeger, K.-E., Ransac, S., Dijkstra, B. W., Colson, C., van Heuvel, M., and Misset, O. (1994) Bacterial lipases, *FEMS Microbiology Reviews*, **15** (1), 29.

Jellema, A. and Schipper, C. J. (1975) Influence of physiological factors on the lipolytic susceptibility of milk, *Annual Bulletin, International Dairy Federation*, **86**, 2.

Jensen, R. G., Smith, A. C., MacLeod, P., and Dowd, L. R. (1958) Acid degree of milk obtained with pipeline and bucket milkers, *Journal of Dairy Science*, **41**, 341.

Joerin, M. M. and Bowering, A. (1973) The use of Rhodamine dyes in the estimation of free fatty acids in bovine milk, *New Zealand Journal of Dairy Science and Technology*, **8** (3), 112.

Johnson, B. C. and Gould, I. A. (1949a) Milk lipase system. II. Comparison of solvent extraction and churning methods for obtaining fat from milk for free fatty acid measurement, *Journal of Dairy Science*, **32**, 435.

Johnson, B. C. and Gould, I. A. (1949b) Milk lipase system. III. Further studies of the solvent extraction procedure for obtaining fat from milk for titration, *Journal of Dairy Science*, **32**, 447.

Johnson, P. E. and Von Gunten, R. L. (1962) A study of factors involved in development of rancid flavour in milk, *Oklahoma State University, Bulletin B-593*. Cited by: Fleming, M. G. (1979) Lipolysis in bovine milk as affected by mechanical and temperature activation - a review, *Irish Journal of Food Science and Technology*, **3**, 111

Jokay, L. and Jensen, J. M. (1956) Effects of variants in pipeline installations on the acid degree of milk (abstract), *Journal of Dairy Science*, **39**, 927.

Joshi, N. S. and Thakar, P. N. (1994) Methods to evaluate deterioration of milk fat - a critical appraisal, *Journal of Food Science and Technology*, **31** (3), 181.

Judge, F. J., Fleming, M. G., O'Shea, J., and Raftery, T. F. (1977) Effect of milking pipeline height and excessive air admission at the claw on free fatty acid development in raw milk, *Irish Journal of Agricultural Research*, **16**, 115.

Kammerlehner, J. and Kessler, H. G. (1980) Mechanical effects on cream during stirring and pumping, *Deutsche Milchwirtschaft*, **31** (43), 1746.

Kashket, S. (1971) An automated method for analysis of free fatty acids, *Analytical Biochemistry*, **41**, 166.

Kaw, A.K. (1998) "Properties of milks concentrated by reverse osmosis". Ph.D. Thesis, Massey University, Palmerston North, New Zealand.

Keenan, T. W., Morré, D. J., Olson, D. J., Yunghans, W. N., and Patton, S. (1970) Biochemical and morphological comparison of plasma membrane and milk fat globule membrane from bovine mammary gland, *Journal of Cell Biology*, **44**, 80.

Kelley, L. A. and Dunkley, W. L. (1954) Hydrolytic rancidity induced in pipeline milkers, *Journal of Milk and Food Technology*, **17**, 306.

References

Kessler, H. G. and Fink, A. (1992) Physico-chemical effects of pasteurization on cream properties, *Bulletin of the International Dairy Federation*, (No. 271), 11.

Kintner, J. A. and Day, E. A. (1965) Major free fatty acids in milk, *Journal of Dairy Science*, **48**, 1575.

Kirst, E. (1980) Lipolytic processes in milk and milk products. Review of literature and study of effects of stirring and pumping on milk fat, *Lebensmittelindustrie*, **27** (1), 27.

Kishonti, E. (1975) Influence of heat resistant lipases and proteases in psychrotrophic bacteria on product quality, *Annual Bulletin, International Dairy Federation*, **86**, 121.

Kitchen, B. J. and Aston, J. W. (1970) Milk lipase activation, *Australian Journal of Dairy Technology*, **25**, 10.

Klei, L. R., Lynch, J. M., Barbano, D. M., Oltenacu, P. A., Lednor, A. J., and Bandler, D. K. (1997) Influence of milking three times a day on milk quality, *Journal of Dairy Science*, **80**, 427.

Klungel, G. H., Slaghuis, B. A., and Hogeveen, H. (2000) The effect of the introduction of automatic milking systems on milk quality, *Journal of Dairy Science*, **83** (9), 1998.

Kobylka, D. and Carraway, K. L. (1973) Proteolytic digestion of proteins of the milk fat globule membrane, *Biochimica et Biophysica Acta*, **307**, 133.

Kon, H. and Saito, Z. (1997) Factors causing temperature activation of lipolysis in cow's milk, *Milchwissenschaft*, **52** (8), 435.

Koops, J. and Klomp, H. (1977) Rapid colorimetric determination of free fatty acids (lipolysis) in milk by the copper soap method, *Netherlands Milk and Dairy Journal*, **31** (1), 56.

Koops, J., Klomp, H., and van Hemert, H. (1990) Rapid enzymatic assay of free fatty acids (lipolysis) in farm tank by a segmented continuous-flow method. Comparison of the results with those obtained by the BDI procedure, *Netherlands Milk and Dairy Journal*, **44**, 3.

Kosikowski, F. V. (1959) Free volatile fatty acids in homogenized raw milk, *15th International Dairy Congress*, vol. 3, London, 1545-1550.

Krukovsky, V. N. and Herrington, B. L. (1939) Studies of lipase action II. The activation of milk lipase by temperature changes, *Journal of Dairy Science*, **22**, 137.

Krukovsky, V. N. and Sharp, P. F. (1938) Effect of shaking on lipolysis of cows milk, *Journal of Dairy Science*, **21**, 671.

Kuzdzal-Savoie, S. (1975) Is there a correlation between organoleptic and chemical procedures for the assessment of lipolysis?, *Annual Bulletin, International Dairy Federation*, **86**, 165.

Kuzdzal-Savoie, S. (1980) Determination of free fatty acids in milk and milk products, *Bulletin of the International Dairy Federation*, (No. 118), 53.

References

Kuzdzal-Savoie, S. and Petit, J. (1966) Fractionnement chromatographique des produits de la lipolyse dans les fromages, *17th International Dairy Congress*, vol. D2, Munich, 329-334.

Lagoni, H. and Peters, K. H. (1959) *Kieler Milchwissenschaft Forschugberichte*, **11**, 291.
Cited by: Aule, O. and Worstorff, H. (1975) Influence of mechanical treatment of milk on quantities of FFA and free fat in the milk, as well as on the separability of the milk, *Annual Bulletin, International Dairy Federation*, **86**, 116

Law, B. A., Sharpe, M. E., and Chapman, H. R. (1976) The effect of lipolytic Gram-negative psychrotrophs in stored milk on the development of rancidity in Cheddar cheese, *Journal of Dairy Research*, **43** (3), 459.

Lindqvist, B., Roos, T., and Fujita, H. (1975) Autoanalyzer determination of free fatty acids in farm milk. Modification of present methods to simplify transportation of the sample, *Annual Bulletin, International Dairy Federation*, **86**, 171.

Mahieu, H. (1985) Rapid method for determination of free fatty acids in milk, *Deutsche Molkerei-Zeitung*, **106** (14), 397.

Mather, I. H. and Keenan, T. W. (1998) Origin and secretion of milk lipids, *Journal of Mammary Gland Biology and Neoplasia*, **3** (3), 259.

Matta, H. and Punj, V. (1999) Isolation and identification of lipolytic, psychrotrophic, spore forming bacteria from raw milk, *International Journal of Dairy Technology*, **52** (2), 59.

McCabe, W. L., Smith, J. C., and Harriott, P. (1993) "Unit operations of chemical engineering". 5th edn, McGraw-Hill, New York, U.S.A.

McCarthy, R. D. and Duthie, A. H. (1962) A rapid quantitative method for the separation of free fatty acids from other lipids, *Journal of Lipid Research*, **3**, 117.

McDonald, S. T., Spurgeon, K. R., and Gilmore, T. M. (1986) Flavour and other properties of cheddar cheese made from rancid milk, *Cultured Dairy Products Journal*, **21** (3), 16.

McKay, D. B. and Beacham, I. R. (1995) The effect of temperature on the degradation of triglycerides by a pseudomonad isolated from milk: free fatty acid accumulation as a balance between rates of triglyceride hydrolysis and fatty acid consumption, *The Society for Applied Bacteriology*, **79**, 651.

McKellar, R. C. (1989) "Enzymes of psychrotrophs in raw food". CRC Press, Florida, USA, 310pp.

McPherson, A. V. and Kitchen, B. J. (1983) Reviews of the progress of dairy science - the bovine milk fat globule membrane - Its formation, composition, structure and behavior in milk and dairy products, *Journal of Dairy Research*, **50** (1), 107.

Metzner, A. B. and Otto, R. E. (1957) Agitation of non-newtonian fluids, *A.I.Ch.E. Journal*, **3**, 3.

References

Michalski, M. C., Michel, F., and Geneste, C. (2002a) Appearance of submicronic particles in the milk fat globule size distribution upon mechanical treatments, *Lait*, **82** (2), 193.

Michalski, M. C., Michel, F., Sainmont, D., and Briard, V. (2002b) Apparent ζ-potential as a tool to assess mechanical damages to the milk fat globule membrane, *Colloids and Surfaces B-Biointerfaces*, **23** (1), 23.

Miller, B. and Puhan, Z. (1986a) 'Lipolysable' fat as an indicator of damage to fat in raw milk, *Schweizerische Milchwirtschaftliche Forschung*, **15** (1), 10.

Miller, B. and Puhan, Z. (1986b) Fat damage during milking and milk handling, *Schweizerische Milchwirtschaftliche Forschung*, **15** (3), 69.

Miller, E. J. (1979) Preliminary design investigation into milk agitation, *New Zealand Journal of Dairy Science and Technology*, **14** (3), 265.

Miller, E. J. (1981) The design and operation of agitators for use in whole milk storage vessels, *New Zealand Journal of Dairy Science and Technology*, **16** (3), 221.

Mouillet, L., Luquet, F. M., Nicod, H., Boudier, J. F., and Mahieu, H. (1981) Rapid method for measuring lipolysis in milk, *Le Lait*, **61**, 171.

Mulder, H. and Walstra, P. (1974) "The milk fat globule: emulsion science as applied to milk products and comparable foods". Centre for Agricultural Publishing and Documentation, Netherlands, 296pp.

Murphy, J. J., Connolly, J. F., and Headon, D. R. (1979) A study of factors associated free fatty acid development in milk, *Journal of Food Science and Technology*, **3**, 131.

Needs, E. C., Anderson, M., and Morant, S. V. (1986) Interaction of factors which influence the extent of lipolysis during milking and storage of raw milk, *Journal of Dairy Research*, **53**, 203.

Needs, E. C., Ford, G. D., Owen, A. J., Tuckley, B., and Anderson, M. (1983) A method for the quantitative determination of individual free fatty acids in milk by ion exchange resin adsorption and gas-liquid chromatography, *Journal of Dairy Research*, **50** (3), 321.

Standard Laboratory Methods - Chemistry. (1980), Free fatty acid content for all milk, 1.12.New Zealand Dairy Division of Ministry of Agriculture and Fisheries, New Zealand.

Chemical Methods Manual. NZTM 3 (2000), Section 7: Fat degradation analysis. Issue 7,New Zealand Dairy Industry.

O'Brien, B., O'Callaghan, E., and Dillon, P. (1998) Effect of various milking machine systems and components on free fatty acid levels in milk, *Journal of Dairy Research*, **65** (2), 335.

References

O'Halloran, J. C., Fleming, M. G., and Raftery, T. F. (1975) Induced hydrolytic rancidity in milk in relation to farm production, storage and assembly procedures, *Annual Bulletin, International Dairy Federation*, **86**, 127.

Olivecrona, T. (1980) Biochemical aspects of lipolysis in bovine milk fat, *International Dairy Federation Bulletin*, (No. 118), 19.

Olivecrona, T. (1984) In: *Lipases*, Elsevier, Amsterdam, Netherlands, pp. 205-261.

Patton, S. (1964) Flavor threshold of volatile fatty acids, *Journal of Food Science*, **29**, 679.

Perrin, D. R. and Perrin, D. D. (1958) The determination of free fatty acids in milk, *Journal of Dairy Research*, **25**, 221.

Perry, R. H., Green, D. W., and Maloney, J. O. (1997) "Perry's chemical engineers' handbook". 7th (international) edn, McGraw-Hill, U.S.A., (Chapter 2).

Pillay, V. T., Myhr, A. N., and Gray, J. I. (1980) Lipolysis in milk. I. Determination of free fatty acids and threshold value for lipolyzed flavor detection, *Journal of Dairy Science*, **63** (8), 1213.

Pillay, V. T. T., Myhr, A. N., and Gray, J. I. (1978) Milking systems and their influence on lipolysis, *XX International Dairy Congress*, vol. E, 131.

Puhan, Z. (1989) Influence of cold storage on milk - chemical aspects, *Tejipar*, **39** (1), 6.

Ramsey, J. A. and Swartzel, K. R. (1984) Effect of ultra high temperature processing and storage conditions on rates of sedimentation and fat separation of aseptically packaged milk, *Journal of Food Science*, **49**, 257.

Rerkrai, S., Jeon, I. J., and Bassette, R. (1987) Effect of various direct ultra-high temperature heat treatments on flavor of commercially prepared milks, *Journal of Dairy Science*, **70** (10), 2046.

Richardson, G. H. (1985) "Standard Methods for the Examination of Dairy Products". 15th edn, American Public Health Association, Washington D.C., USA.

Robson, E. W. and Dalgleish, D. G. (1984) Coagulation of homogenized milk particles by rennet, *Journal of Dairy Research*, **51**, 417.

Ross Valve Manufacturing Company Incorporated (accessed: 2004) http://www.rossvalve.com/PDF/cavitation.pdf

Rothwell, J. (1962) Liquid cream processing, *Journal of the Society of Dairy Technology*, **15** (4), 190.

Rudzik, L. (1987) Detection of product damage by pumping of milk. Studies with a cavitation-controlled pump., *Deutsche Milchwirtschaft*, **38** (35), 1211.

References

Salih, A. M. A., Anderson, M., and Tuckley, B. (1977) The determination of short- and long-chain free fatty acids in milk, *Journal of Dairy Research*, **44**, 601.

Salvatierra, S., Stannard, D. J., and Parkinson, R. D. J. (1978) The effect of two types of milking claws on foaming and fat globule damage in milk, *New Zealand Journal of Dairy Science and Technology*, **13**, 111.

Scanlan, R. A., Sather, L. A., and Day, E. A. (1965) Contribution of free fatty acids to the flavour of rancid milk, *Journal of Dairy Science*, **48**, 1582.

Schmidt, D., Cromie, S. J., and Dommett, T. W. (1989) Effect of pasteurization and storage-conditions on the shelf- life and sensory quality of aseptically packaged milk, *Australian Journal of Dairy Technology*, **44** (1), 19.

Senyk, G. F., Murphy, S. C., Barbano, D. M. and Shipe, W. F. (1985) Sources of high acid degree values in raw milk supplies, *Journal of Dairy Science*, **68** (Suppl. 1), 73.

Shamsuzzaman, K., Modler, W., and Mckellar, R. C. (1987) Survival of lipase during manufacture of nonfat dry milk, *Journal of Dairy Science*, **70** (4), 746.

Shelley, A. W., Deeth, H. C., and MacRae, I. C. (1987) A numerical taxonomic study of psychrotrophic bacteria associated with lipolytic spoilage of raw milk, *Journal of Applied Bacteriology*, **62**, 197.

Shimizu, M., Yamauchi, K., and Kanno, C. (1979) Proteolytic digestion of milk fat globule membrane proteins, *Milchwissenschaft*, **34** (11), 666.

Shimizu, M., Yamauchi, K., and Kanno, C. (1980) Effect of proteolytic digestion of milk fat globule membrane proteins on stability of the globules, *Milchwissenschaft*, **35** (1), 9.

Shipe, W. F., Senyk, G. F., and Fountain, K. B. (1980) Modified copper soap solvent extraction method for measuring free fatty acids in milk, *Journal of Dairy Science*, **63** (2), 193.

Snoeren, T. H. M., Damman, A. J., and Klok, H. J. (1983) The effect of homogenisation on the viscosity of whole milk concentrate and on the qualities of whole milk powder, *Zuivelzicht*, **76** (3), 64.

Stannard, D. J. (1975) The use of marker enzymes to assay the churning of milk, *Journal of Dairy Research*, **42**, 241.

Stetina, B. and Lück, H. (1976) Determination of the free fatty acids in milk by means of the rhodamine method, *South African Journal of Dairy Technology*, **8** (2), 111.

Suhren, G. (1989) In: *Enzymes of psychrotrophs in raw food*, (Ed. Mckellar, R. C.), CRC Press, U.S.A., pp. 3-34.

Suhren, G., Heeschen, W., and Tolle, A. (1975) Free fatty acids in milk and bacterial activity, *Annual Bulletin, International Dairy Federation*, **86**, 51.

Sundheim, G. (1988) Spontaneous lipolysis in bovine milk: combined effects of cream, skim milk, and lipoprotein lipase activity, *Journal of Dairy Science*, **71** (3), 620.

Sundheim, G. and Bengtsson-Olivecrona, G. (1985) Lipolysis in milk induced by cooling or by heparin: comparisons of amount of lipoprotein lipase in the cream fraction and degree of lipolysis, *Journal of Dairy Science*, **68**, 589.

Sundheim, G. and Bengtsson-Olivecrona, G. (1987a) Methods to assess the propensity of milk fat globules toward lipolysis and the ability of skim milk to inhibit lipolysis, *Journal of Dairy Science*, **70** (10), 2040.

Sundheim, G. and Bengtsson-Olivecrona, G. (1987b) Isolated milk fat globules as substrate for lipoprotein lipase: Study of factors relevant to spontaneous lipolysis in milk, *Journal of Dairy Science*, **70**, 499.

Sundheim, G. and Bengtsson-Olivecrona, G. (1987c) Hydrolysis of bovine milk fat globules by lipoprotein lipase: Inhibition by proteins extracted from milk fat globule membrane, *Journal of Dairy Science*, **70**, 1815.

Swarna, C., Prabha, R., and Shankar, P. A. (1997) Effect of added lipases of psychrotrophic Yersinia, Enterobacter and Citrobacter spp. on the keeping quality of milk and fat rich milk products, *Indian Journal of Animal Sciences*, **67** (7), 611.

Sweetsur, A. W. M. and Muir, D. D. (1982) Manipulation of the heat stability of homogenized concentrated milk, *Journal of the Society of Dairy Technology*, **35** (4), 126.

Sweetsur, A. W. M. and Muir, D. D. (1983) Effect of homogenization on the heat stability of milk, *Journal of Dairy Research*, **50**, 291.

Tamsma, A., Edmondson, F., and Vettel, H. E. J. (1959) Free fat in foam-dried whole milk, *Journal of Dairy Science*, **42**, 240.

Tarassuk, N. P. and Frankel, E. N. (1955) Technical notes on the mechanism of activation of lipolysis and the stability of lipase systems of normal milk, *Journal of Dairy Science*, **38**, 438.

Tarassuk, N. P. and Frankel, E. N. (1957) The specificity of milk lipase IV. Partition of the milk lipase in milk, *Journal of Dairy Science*, **40**, 418.

Te Whaiti, I. E. and Fryer, T. F. (1975) Factors that determine the gelling of cream, *New Zealand Journal of Dairy Science and Technology*, **10**, 2.

Te Whaiti, I. E. and Fryer, T. F. (1976a) Factors influencing the release of free fat in raw milk, *New Zealand Journal of Dairy Science and Technology*, **11**, 91.

Te Whaiti, I. E. and Fryer, T. F. (1976b) The influence of free fat on lipolysis in milk, *New Zealand Journal of Dairy Science and Technology*, **11** (4), 273.

Thomas, E. L., Nielsen, A. J., and Olson, J. C. (1955a) Hydrolytic Rancidity in milk: a simplified method for estimating the extent if its development, *American Milk Review*, **17**, 50.

Thomas, E. L., Nielsen, A. J., and Olson, J. C. (1955b) Observations on the extent of lipolysis in milk supplies as related to various milk handling procedures (abstract), *Journal of Dairy Science*, **38**, 596.

Thomas, R. H., Holgren, C. J., Jokay, L., and Bloch, I. (1957) A rapid method for determining free fat in dry milks, *Journal of Dairy Science*, **40**, 605.

Thompson, M. P., Brunner, J. R., Stine, C. M., and Lindquist, K. (1961) Lipid components of the fat globule membrane, *Journal of Dairy Science*, **44** , 1589.

Tolstukhina, L. S. and Aristova, V. P. (1979) Changes in fat phase stability during milk drying, *Molochnaya Promyshlennost' (abstract)*, (No. 2), 15.

Trinh, B., Haisman, D., and Trinh, K. T. (2002) Design of recombination system for time-dependent food systems, *Asia Pacific Confederation of Chemical Engineering*, Christchurch, New Zealand, #402.

Trinh, K. T. (2004) "Massey University milk powder technology course notes". Palmerston North, New Zealand.

Ubben, E. H., Heeschen, W., and Reichmuth, J. (1997) Somatic cell counting in milk: performance of the Anadis Scc and comparison with the Fossomatic 360 system, *Milchwissenschaft*, **52** (1), 10.

Ukeda, H., Wagner, G., Weis, G., Miller, M., Klostermeyer, H., and Schmid, R. D. (1992) Application of a microbial sensor for determination of short-chain fatty acids in raw milk samples, *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung*, **195** (1), 1.

van Boekel, M. A. J. S. and Folkerts, T. (1991) Effect of heat treatment on the stability of milk fat globules, *Milchwissenschaft*, **46** (12), 758.

van Boekel, M. A. J. S. and Walstra, P. (1989) Physical changes in the fat globules in unhomogenised and homogenised milk, *Bulletin of the International Dairy Federation*, (No. 238), 13.

Walstra, P. (1965) Light scattering by milk fat globules, *Netherlands Milk and Dairy Journal*, **19** (2).

Walstra, P. (1969a) Studies on milk fat dispersion. I. Methods for determining globule-size distribution, *Netherlands Milk and Dairy Journal*, **23**, 12.

Walstra, P. (1969b) Studies on milk fat dispersion. II. The globule-size distribution of cow's milk, *Netherlands Milk and Dairy Journal*, **23**, 99.

Walstra, P. (1969c) Studies on milk fat dispersion. III. The distribution function of globule size in cow's milk and the process of milk fat formation, *Netherlands Milk and Dairy Journal*, **23**, 111.

Walstra, P. (1983) In: *Developments in dairy chemistry - 2. Lipids,* (Ed. Fox, P. F.), Applied Science Publishers, London, pp. 119-158.

Walstra, P., Geurts, T. J., Noomen, A., Jellema, A., and van Boekel, M. A. J. S. (1999) "Dairy technology: principles of milk properties and processes". Marcel Dekker, New York.

Walstra, P., Jenness, R., and Badings, H. T. (1984) "Dairy chemistry & physics". John Wiley & Sons, New York, USA, 467pp.

Walstra, P. and Oortwijn, H. (1982) The membranes of recombined fat globules. 3. Mode of formation, *Netherlands Milk and Dairy Journal*, **36**, 103.

Wang, L. and Randolph, H. E. (1978) Activation of lipolysis I: Distribution of lipase activity in temperature activated milk, *Journal of Dairy Science*, **61** (7), 874.

Webb, B. H. and Hall, S. A. (1935) Some physical effects of freezing upon milk and cream, *Journal of Dairy Science*, **18** (5), 275.

Wiking, L., Bjorck, L., and Nielsen, J. H. (2003) Influence of feed composition on stability of fat globules during pumping of raw milk, *International Dairy Journal*, **13** (10), 797.

Wirtz, N., Oechtering, K., Tholen, E., and Trappmann, W. (2002) Comparison of an automatic milking system to a conventional milking parlour, *First North American Conference on Robotic Milking*, Toronto, Canada.

Woo, A. H. and Lindsay, R. C. (1980) Method for the routine quantitative gas chromatographic analysis of major free fatty acids in butter and cream, *Journal of Dairy Science*, **63**, 1058.

Woolford, M. W., Sherlock, R. A., and Bryant, A. M. (1993) On-farm practices and milkfat flavour, *Milk Fat Flavour Symposium*, 101-106.

Woorstoft, H., Heeschen, W., Reichmuth, J., and Telle, A. (1972) Free fatty acids in milk in relation to flow and conditions in milking machine, *Milchwissenschaft*, **27**, 477.

Ye, A. (2003) "Behaviour of fat globules and membrane proteins under different processing environments as related to milk powder manufacture". Ph.D. Thesis, Massey University, Palmerston North, New Zealand.

Zadow, J. G. (1969) Studies on the ultra-heat treatment of milk. Part 1-Comparison of direct and indirect heating of whole milk, *Australian Journal of Dairy Technology*, **24** (2), 44.

Appendices

Appendix A– Procedures

<u>A1: Mojonnier Total Fat Test – (AACC 30-10)</u>

Reagents

Ethanol (95%)

Diethyl Ether

Petroleum Ether (boiling point below 60°C)

35% Ammonium Hydroxide

2% Phenolphthalein

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).

2.   Add 2 mL ammonium hydroxide and mix well in the lower bulb. Place in a 60°C water bath for about 5 minutes and swirl occasionally. Cool. Add 2-4 drops phenolphthalein.

3.   Add 10mL of ethanol 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, stopper the tube and rock gently for about 1 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 rinsings to run into the tube.

6.   Replace the stopper again, and rock carefully for 1 minute.

7. Centrifuge Mojonnier tube for 2 minutes at 600 rpm.

8. Remove the stopper and carefully decant as much as possible of the organic solvent layer into a pre-weighed short-necked flask by gradually bringing the cylindrical bulb into a horizontal position.

9. Add 5 mL of ethanol to the Mojonnier tube and mix. This helps prevent emulsions forming and is in accordance with the AOAC method.

10. Repeat the extraction using 15 mL of diethyl ether and 15 mL of petroleum ether (steps 5 to 8). Add the second extract into the same flask as used in step 8.

11. Distil carefully the solvents from the flask using a steam bath at 100°C and dry the flask in a drying oven at 100°C for 90 minutes, taking precautions to remove all traces of solvent vapour prior to placing in the oven.

12. Allow the flask to cool to room temperature. Do not use a desiccator.

13. Weigh the flask and record the fat content of the sample.

14. At the same time as the above procedure is being carried out, conduct a blank determination with 10mL 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 of % fat

$$\% \text{ free fat in sample} = \frac{W_3 - W_1 - W_b}{W_2} \times 100\% \qquad (A.1)$$

A2: Solvent Extraction Free Fat Test – based on Fang (1998), used in runs T06-09

1. Place four metal dishes into an oven at 100°C and 20 kPa vacuum for 5 minutes.
2. Transfer Dishes to a cooling oven for 5 minutes.
3. Weigh the metal dishes immediately (W_1).
4. Ensure sample reaches room temperature before proceeding.
5. Invert milk sample container 10 times to ensure homogeneity of milk samples
6. Weigh out accurately about 20g of sample (4DP balance), and place into a Mojonnier tube (W_2).
7. Add 50mL of petroleum ether.
8. Tilt tube back and forth 10 times during a 40 second period to promote mixing and extraction. Make every effort to avoid foaming of the milk. Above all else, ensure a consistent mixing method.
9. Centrifuge for 5 minutes (600 rpm).
10. Carefully pour the ether layer into one of the pre-weighed dishes (3).
11. Evaporate ether in dishes on a water bath (boiling) or hot plate. Ensure sufficient ventilation to expel vapour.
12. Heat the dishes in the oven at 100°C and 20 kPa vacuum for 5 minutes. Ensure there is no ether smell left coming from the flask before placing them into the oven.
13. Transfer dishes to a cooling oven for 5 minutes.
14. Weigh the dishes immediately (W_3).

Calculation:

$$\% \text{ free fat in total fat} = \frac{\% \text{ free fat in sample} \times 100}{\% \text{ Total fat}} \qquad (A.2)$$

$$\% \text{ free fat in sample} = \frac{W_3 - W_1 - W_b}{W_2} \times 100\% \qquad (A.3)$$

Accuracy: Agreement within 0.5% (for equation A.3).

A3: Solvent Extraction Free Fat Test – Fang (1998), used in runs T28-30, C97-106

1. Heat 100mL flasks in the oven for 30 minutes at approximately 100°C.
2. Cover the flasks, and allow them to cool for 1 hour.
3. Invert milk sample container 10 times to ensure homogeneity of milk samples
4. Temper sample at 40°C for 10 minutes.
5. Weigh out accurately about 20g of sample, and place in Mojonnier flask (W_2).
6. Add 50mL of petroleum ether.
7. Place in water bath at 40°C for 30 seconds.
8. Tilt tube back and forth 10 times during a 40 second period to promote mixing and extraction. Make every effort to avoid foaming of the milk.
9. Centrifuge for 5 minutes.
10. Weigh the 100mL flasks (W_1).
11. Carefully pour the ether layer into one of the pre-weighed flasks (10).
12. Evaporate ether on a water bath (boiling).
13. Wipe the outside of the flask with a clean, dry towel.
14. Heat the flask in the oven at approximately 100°C for 1 hour. Ensure there is no ether smell left coming from the flask before placing them into the oven.
15. Cover and cool the flask for 1 hour
16. Weigh the flask (W_3).

Calculation:

$$\% \text{ free fat in total fat} = \frac{\% \text{ free fat in sample} \times 100}{\% \text{ Total fat}} \tag{A.2}$$

$$\% \text{ free fat in sample} = \frac{W_3 - W_1 - W_b}{W_2} \times 100\% \tag{A.3}$$

Accuracy: Agreement within 0.5% (for equation A.3)

A4: Lipolysable Free Fat test - based on Fang (1998)

1. Add external lipase solution (50mg lipase to 100mL water) at a ratio of 1mL per 100mL of milk.

2. Incubate sample at 30°C for 24 hours.

3. Perform Free Fatty Acid test (New Zealand Dairy Division of Ministry of Agriculture and Fisheries, 1980) as follows:

A. Pipette 10 mL of well-mixed sample at 20°C±2°C into a Mojonnier flask.

B. Carry out blank using 10mL of water into a Mojonnier flask.

C. Add 3 drops of Bromophenol blue indicator to each flask.

D. Add sulfuric acid dropwise until colour changes to greenish/yellow (usually only one drop needed for blank).

E. Add 5mL of neutralised ethanol, stopper and shake vigorously for 1 minute.

F. Add 15mL of mixed solvent, stopper and shake vigorously for 1 minute.

G. Centrifuge for 5 minutes.

H. Place 10mL of the clear supernatant ether layer into a 50mL flask/container.

I. Add 10mL of neutralised methanol (or the same volume of ether added).

J. Titrate against 0.01M alcoholic KOH to the first sign of a greenish tinge in the pale yellow solution.

Notes: Neutralise the alcohols by adding 0.5 mL of Alpha-naphthophthalein to 100mL of alcohol, neutralise by pipetting against alcoholic KOH to the first sign of a greenish tinge.

Standardise the alcoholic KOH regularly against standardised hydrochloric acid (with borax).

Calculation:

$$\boxed{\text{Free fatty acid content} = 1.35(T - B)}$$

(mmol per litre), to the nearest 0.05 mmol/L

Accuracy: Agreement within 0.1 mmol/L

Appendix B – Reagents and Equipment used

B1: Technical Characteristics of Pig Pancreatic Lipase

Supplied by SIGMA Chemical Co. (Australia)

Synonyms: PPL, triacylglycerol acylhydrolase, triacylglycerol lipase

Enzyme Commission (EC) Number

3.1.1.3

The product is Type II, Crude grade, from Porcine Pancreas

It contains approximately 25% protein (remainder is sucrose). One unit will hydrolyse 1.0 microequivalent of fatty acid from a triglyceride in one hour at the indicated pH at 37°C. 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).

Contains amylase and protease activity

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

B2: Walstra Solution A preparation method (Walstra, 1965)

1. Add 800 mL of reverse osmosis (R.O.) water to 3.94 g of ethylene diamine tetra acetate (EDTA) salt. This weight allows for water of crystallisation.

2. Add 1.27 g of polyoxyethylene sorbitan monolaurate (Tween-20)

3. Note the pH of the solution.

4. Adjust pH to 10.00 with 1N sodium hydroxide.

5. Make up to 1 litre with R.O. water. Mix well.

Store at 5°C.

B3: Bronopol preservative specifications

Supplied by BASF plc, Cheadle Hulme, Cheshire SK8 6QG

Product Name: Myacide Pharma BP

Chemical Name: 2-bromo-2nitropropane-1,3-diol (>99%)

EINECS-No. 200-143-0, INDEX-No. 603-085-00-8

Hazards: Harmful in contact with skin and if swallowed.

        Irritating to respiratory system and skin.

        Risk of serious damage to eyes.

Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment.

Handling and Storage:

Ensure thorough ventilation and protection against sources of ignition.

Keep tightly closed in a dry and cool place.

Hazardous fumes released at temperatures above 90°C.

Properties:

Crystalline, white powder

Melting Point 128-132°C

Bulk density 1.1 g/cm^3

Solubility in water at 23°C 280 g/L

pH value (at 10g/L, 20°C) 5-7

B4: Sodium Azide preservative specifications

Chemical Name: Sodium Azide (90-100%)

Common Names: Azium, Smite, Hydrazoic acid, sodium salt, Kazoe

Cas-No. 26628-22-8

Hazards:

Exceptional health hazard - harmful if swallowed, inhaled, or absorbed through skin.

Combustible.

Risk of serious damage to eyes.

Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment.

Handling and Storage:

Keep away from heat, sparks, flame.

Do not get in eyes, on skin, on clothing.

Do not breathe dust. Use with adequate ventilation. Keep in tightly closed container.

Keep tightly closed in a dry and cool place.

In case of fire, do not use water. Use dry sand or soda ash.

Do not store near acids or in metal containers

Properties:

Crystalline, odourless, colourless solid

Melting Point 275°C

Specific gravity 1.84 g/cm^3

Solubility in water: more than 10%

B5: Pilot plant direct steam injection unit (cross section)
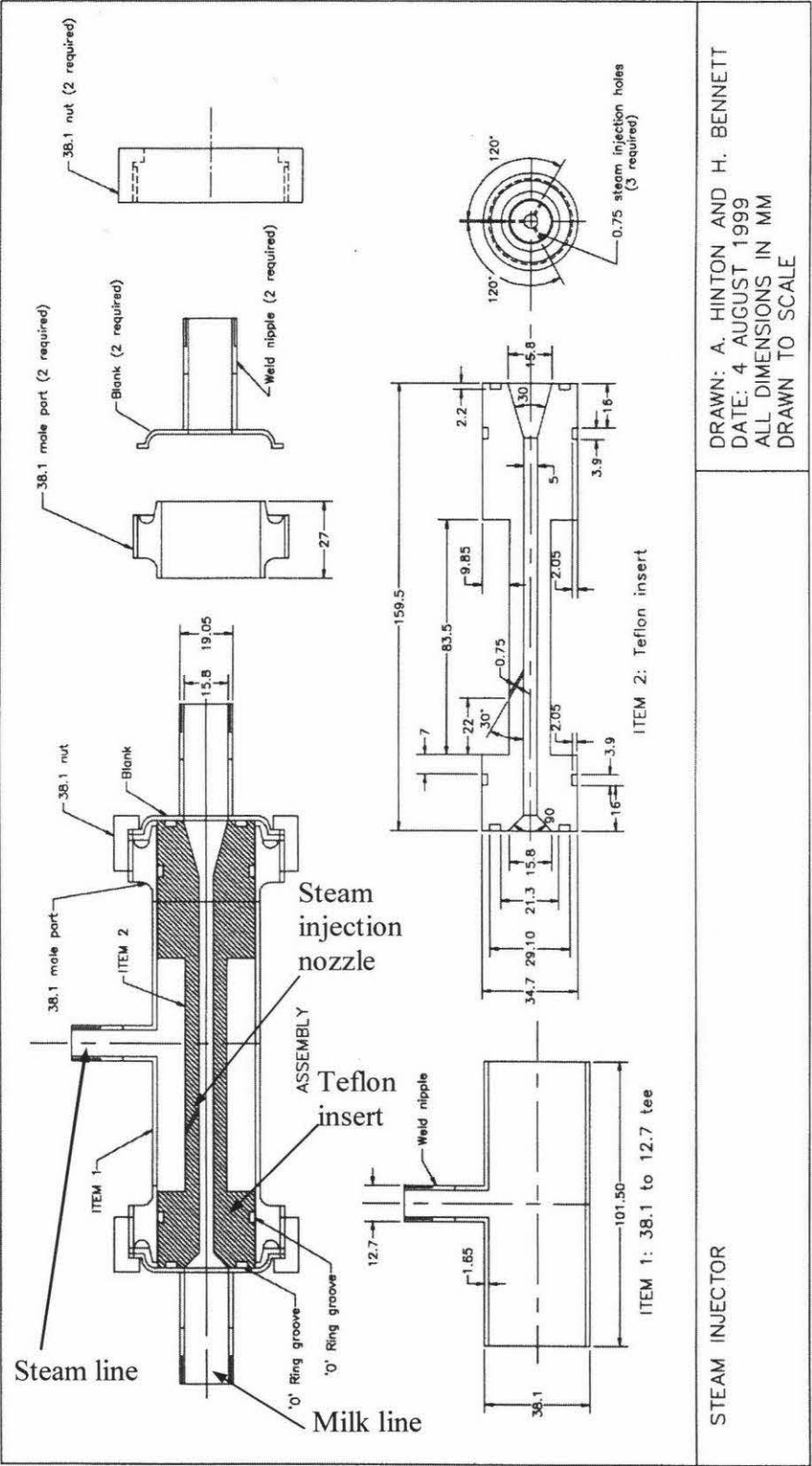
Figure B.1: Direct steam injection unit specifications for Massey University milk processing pilot plant.
From Bennett (2000).

Appendix C – Sample Calculations

C1: Sample calculation for the selective lipolysis test

Calculation of free fatty acids (FFAs) in a milk sample

$$\text{FFA content} = C \times 1.35 \times \frac{(T - B)}{1000} \times \frac{1000}{V} \tag{3.6}$$

where: C = actual concentration of KOH (mol/L)

T = titrated volume of KOH (mL)

B = blank titration volume (mL)

V = volume of the original sample (usually 10 mL)

(Example: control sample from run T29 - duplicates)

Variable	Control 1	Control 2
KOH concentration, C	0.01061 mol/L	0.01061 mol/L
Volume of KOH titrated from burette, T	0.48 mL	0.58 mL
Blank titration volume, B	0.17 mL	0.17 mL
Volume of sample, V	10 mL	10 mL

Using Equation 3.6, FFA content of control sample 1:

1. = 0.01061 x 1.35 x (0.31÷1000) x (1000÷10)
2. = 0.44 mmol/L

FFA content of control sample 2:

1. = 0.01061 x 1.35 x (0.58÷1000) x (1000÷10)
2. = 0.59 mmol/L

Calculation of Uncertainties

Half-range uncertainty calculated for samples of 3 replicates or less.

= 0.5 x (maximum replicate value-minimum replicate value) $\qquad$ (C.1)

(Example from control samples from run T28, using Equation C.1)

   = 0.5 x (0.59-0.44)

   = 0.07 mmol/L

FFA value reported as average of replicates ± half-range uncertainty

For example, in run T29 = 0.52± 0.07 mmol/L

C2: Sample calculation for adjusting basis of selective lipolysis test (e.g. for concentrated milk samples)

By performing a simple mass balance on the system, the basis of the system can be changed from the amount of FFAs in the measured dilute concentrate sample to the amount of FFAs in the feed sample.

Nomenclature: P = Sub-sample from dilute sample used in titration.

             I   = Dilute sample used in incubation.

             C = Undiluted concentrate sample.

             F = Feed sample.

             a = Free fatty acids content

             b = Solids content

The amount of FFAs in the feed sample is expressed as:

$$\frac{m_F^a}{V_F} \tag{C.2}$$

Where: m_F^a = moles of FFAs in the feed sample (mmol).

      V_F = Volume of the feed sample (L).

From the mass balance of FFAs the concentration of FFAs in the sub-sample from the incubated sample, P is the same as that in the incubated sample, I.

$$\frac{m_I^a}{V_I} = \frac{m_P^a}{V_P} = \frac{m_F^a}{V_F} \tag{C.3}$$

Where: m_P^a = moles of FFAs in the sub-sample of the incubated sample used in the titration (mmol).

       m_I^a = moles of FFAs in the incubated sample (mmol)

       V_P = Volume of the sub-sample of the incubated sample used in the titration (L).

       V_I = Volume of the incubated sample (L).

In addition:

$$m_I^a = m_P^a = m_F^a \tag{C.4}$$

Therefore using Equation C.3 gives:

$$m_F^a = m_I^a = m_P^a \cdot \frac{V_I}{V_P} \tag{C.5}$$

From the mass balance of total solids it is clear that the mass of total solids (b) in the undiluted concentrate, C is equal to the mass of total solids in the feed sample, F.

$$M_C^b = M_F^b$$
$$\therefore x_C^b \cdot M_C = x_F^b \cdot M_F \tag{C.6}$$
$$\therefore x_C^b \cdot \rho_C \cdot V_C = x_F^b \cdot \rho_F \cdot V_F$$

Where: M_C^b = mass of total solids in the undiluted concentrate sample (g).

M_F^b = mass of total solids in the feed sample (g).

x_C^b = mass fraction of total solids in the undiluted concentrate sample.

x_F^b = mass fraction of total solids in the feed sample.

M_C = total mass of the undiluted concentrate sample (g).

M_F = total mass of the feed sample (g).

Therefore by rearranging Equation C.6:

$$\therefore V_F = \frac{x_C^b \cdot \rho_C \cdot V_C}{x_F^b \cdot \rho_F} \tag{C.7}$$

Substituting Equations C.5 and C.7 into Equation C.2 gives:

$$\frac{m_F}{V_F} = \frac{m_I^a \cdot V_I}{V_P} \div \frac{x_C^b \cdot \rho_C \cdot V_C}{x_F^b \cdot \rho_F} = \frac{m_P^a}{V_P} \cdot \frac{V_I}{V_C} \cdot \frac{x_F^b}{x_C^b} \cdot \frac{\rho_F}{\rho_C} \tag{C.8}$$

Example from run S10

Variable	Feed Sample	Effect 2 exit sample
SLFA of diluted sample (mmol/L), m^a/V_P	0.96	3.97
Total Solids (%), x^b	13.5	24.7
Density of sample based on TS (g/mL), ρ	1.035	1.07
Volume of dilute sample (mL), V_I	200	250
Volume of concentrate in dilute sample (mL), V_C	0	150

Calculation for feed sample, from Equation C.8:

Stream C = Stream F and $V_I=V_F$, therefore:

$$\frac{m_F}{V_F} = \frac{m_P^a}{V_P} \cdot \frac{V_F}{V_F} \cdot \frac{x_F^b}{x_F^b} \cdot \frac{\rho_F}{\rho_F} = \frac{m_P^a}{V_P} \quad\quad (C.9)$$

= 0.96 mmol/L

Calculation for concentrated sample, from Equation C.8:

= 3.97 x (250 ÷ 150) x (13.5 ÷ 24.7) x (1.035 ÷ 1.07)

= 3.97 x   1.67   x   0.55   x   0.97

= 3.50 mmol/L feed

C3: Sample calculation for combining liquid and churned fat/foam phase portions measured by the selective lipolysis test

By performing a simple mole balance on the system the SLFA measure of the liquid phase of the damaged milk sample and that of the corresponding churned fat/foam phase can be combined under the same basis, mmol FFAs per litre of original milk sample.

Nomenclature: I   = Incubated sample

                P   = Sub-sample of incubated sample used for analysis by titration

                T   = Tank sample (i.e. entire amount of sample from tank)

                (cf) = Churned fat/foam

                (l)   = Liquid milk

                a   = Free fatty acids

The amount of FFAs in the tank sample, T, is expressed as:

$$m_T^a = m_T^a(cf) + m_T^a(l) \tag{C.10}$$

Where: $m_T^a =$ moles of FFAs from the milk in the tank (mmol).

        $m_T^a(cf) =$ moles of FFAs from the churned fat/foam in the tank (mmol).

        $m_T^a(l) =$ moles of FFAs from the liquid milk in the tank (mmol).

Liquid Milk

From the mole balance of FFAs the concentration of FFAs in the liquid milk from the incubated sample, I, is the same as that for the liquid milk from the tank.

$$\frac{m_P^a(l)}{V_P} = \frac{m_I^a(l)}{V_I} = \frac{m_T^a(l)}{V_T} \therefore m_T^a(l) = m_I^a(l)\frac{V_T}{V_I} \tag{C.11}$$

Where: $V_T =$ volume of milk in the tank (L).

        $V_I =$ volume of milk in the incubated sample (L).

        $V_P =$ volume of milk in sub-sample of incubated sampled (L).

Churned Fat and Foam

From the mole balance of FFAs the concentration of FFAs in the churned fat/foam phase from the sub-sample of the incubated sample, P, is the same as that from the incubated sample.

$$\frac{m_I^a(cf)}{V_I} = \frac{m_P^a(cf)}{V_P} \quad \therefore \quad m_I^a(cf) = m_P^a(cf)\frac{V_I}{V_P} \tag{C.12}$$

Where: V_P = volume of distilled water in the portion of the incubation sample used for the titration (L).

In addition the mass of sample in the incubated and tank samples are known, therefore:

$$\frac{m_I^a(cf)}{M_I(cf)} = \frac{m_T^a(cf)}{M_T(cf)} \quad \therefore \quad m_T^a(cf) = m_I^a(cf)\frac{M_T(cf)}{M_I(cf)} \tag{C.13}$$

Where: $M_I(cf)$ = mass of churned fat in the incubated sample (g).

  $M_T(cf)$ = mass of churned fat in the tank (g).

Combining Equations C.12 and C.13 yields:

$$m_T^a(cf) = m_P^a(cf)\frac{V_I}{V_P}\frac{M_T(cf)}{M_I(cf)} \tag{C.14}$$

Substituting Equations C.11 and C.14 into Equation C.10 gives the milli-moles of FFAs in the tank. By dividing this by the total volume of milk in the tank, V_T, the FFA value can be expressed in mmol/L of milk.

$$m_T^a = m_P^a(cf)\frac{V_I}{V_P}\frac{M_T(cf)}{M_I(cf)} + m_I^a(l)\frac{V_T}{V_I} \tag{C.15}$$

Example from run C104 (20 minutes of pumping with air inclusion)

Variable	Value	Units
SLFA of liquid milk from portion of incubated sample used in titration, m^a_P (l)	0.01375	mmol
Volume of portion of incubated sample used in titration, V_P	0.01	L
Volume of incubated sample, V_I	0.05	L
Volume of milk in tank, V_T	50	L
SLFA of churned fat/foam sample from incubated sample, m^a_I (cf)	0.0101	mmol
Mass of churned fat/foam in incubated sample, M_I (cf)	2.688	g
Mass of churned fat/foam in tank sample, M_T (cf)	744	g

From equation C.11, liquid SLFA in tank sample:

$= 0.01375 \times (50 \div 0.01)$

$= 68.8$ mmol

From equation C.14, churned fat/foam SLFA:

$= (0.0101 \times (0.05 \div 0.01)) \times (744 \div 2.688)$

$= 14.0$ mmol

From equation C.15, total SLFA:

$= (68.8 + 14.0) \div 50$

$= 82.7$ mmol $\div 50$ L

$= 1.65$ mmol/L

C4: Sample calculation for combining liquid and churned fat/foam phase portions measured by the free fat test

A mass balance similar to the mole balance for the selective lipolysis test, previously described can be used to combine the liquid milk and churned fat/foam portions into one free fat measurement.

Nomenclature: S   = Sample taken from the tank

              P   = Portion of the Incubated sample used for the free fat analysis

              T   = Tank sample (i.e. entire amount of sample from tank)

              cf   = Churned fat/Foam

              l   = Liquid milk

The amount of free fat (FF) in the tank sample, T, is expressed as:

$$M_T^f = M_T^f(cf) + M_T^f(l) \tag{C.16}$$

Where: M_T^f =   mass of FF from the milk in the tank (g).

       $M_T^f(cf)$ =   mass of FF from the churned fat/foam in the tank (g).

       $M_T^f(l)$ =   mass of FF from the liquid milk in the tank (g).

The churned fat/foam and liquid milk portions can be treated in the same way using a mass balance. From the mass balance of FF the following relationship can be obtained between the sample (* either liquid or churned fat/foam) from the tank, S, and the total tank sample, T:

$$\frac{M_P^f(*)}{M_P(*)} = \frac{M_S^f(*)}{M_S(*)} = \frac{M_T^f(*)}{M_T(*)} \quad \therefore \quad M_T^f(*) = M_P^f(*)\frac{M_T(*)}{M_P(*)} \tag{C.17}$$

Where: M_T =   mass of sample in the tank (g).

       M_S =   mass of sample in the sample from the tank (g).

       M_P =   mass of sample in the portion of sample from the tank used to assess the FF content (g).

By combining the liquid and churned fat/foam portions the mass of FF in the tank (Equation C.16) can be calculated (Equation C.18). By dividing this by the mass of milk in the tank the value can be expressed as % FF in the sample.

$$M_T^f = M_P^f(cf)\frac{M_T(cf)}{M_P(cf)} + M_P^f(l)\frac{M_T(l)}{M_P(l)}$$ (C.18)

Example from run C104

Variable	Value	Units
FF of liquid milk from sub-sample, M_P^f (l)/M_P (l)	0.0371	g
Mass of liquid milk from sub-sample, M_P (l)	19.08	g
FF of churned fat/foam from sub-sample, M_P^f (cf)	0.1687	g
Mass of churned fat/foam in tank sample, M_P (cf)	0.3319	g
Mass of milk in tank, M_T	51500	g
Mass of churned fat/foam in tank sample, M_T (cf)	744	g

From Equation C.17, liquid FF:

= 0.0371 x ((51500-744)÷19.08)

= 98.7 g

From Equation C.17, churned fat/foam FF:

= 0.1687 x (744÷0.3319)

= 378 g

Combining the liquid and solid portions in Equation C.18 gives:

= (98.7 + 378) ÷ 51500

= 477 g ÷ 51500 g

= 0.93%

C5: Sample calculation for converting the total churned fat/foam phase into FFAs

Four assumptions were made in order to complete this calculation:

1. All of the fat in the churned fat/foam was unprotected fat.
2. The fat content of churned fat (~70%) or foam (~20%) was independent of the damage treatment (e.g. agitation, pumping).
3. The molecular weight of the fat is on average 780 g/mol.
4. The fat was entirely triacylglycerols. Therefore each mole of fat was equivalent to 3 moles of FFAs. Note that in practice, the PPL enzyme removes only 2 of the FFA chains from the glycerol molecule. Therefore the following equation reflects the action of the PPL enzyme.

The conversion from churned fat/foam to FFAs (mmol/L) is as follows in Equation C.19:

$$\frac{m_T^a(cf)}{V_T} = \frac{2M_T(cf)F(cf)}{WV_T} \tag{C.19}$$

Where: M_T (cf)   = mass of churned fat/foam in the tank (g).

         F (cf)   = percentage fat in the churned fat/foam (Table 4.7) (%).

         W   = average molecular weight of milkfat (g/mol)

                   (Halter et al., 1978)

         V_T = volume of milk in the tank (L)

         2   = Number of FFA chains per molecule of fat

Sample calculation for churned fat (run C102):

Variable	Value	Units
Mass of churned fat in tank sample, M_T (cf)	16.6	g
Fat content of churned fat in tank sample, %F (cf)	72	%
Average molecular weight of milkfat (MW)	780	g/mol
Volume of milk in tank, V_T	0.4	L

Using Equation C.19 gives the following:

= (2 x 16.6 x 72%) ÷ (780 x 0.4)

= 0.077 mol/L

= 77 mmol/L

Sample calculation for foam (run C99):

Variable	Value	Units
Mass of foam in tank sample, M_T (cf)	13.5	g
Fat content of churned fat in tank sample, %F (cf)	18	%
Average molecular weight of milkfat (MW)	780	L
Volume of milk in tank, V_T	0.4	L

Using Equation C.19 gives the following:

= (2 x 13.5 x 18%) ÷ (780 x 0.4

= 0.016 mol/L

= 16 mmol/L

C6: Sample particle size distribution analysis from Mastersizer E

Version 1.1   Thu. 5 Dec 2002 09:12AM

	mt02b2	:Run Number 1

Milk damaged with 50Hz pumping & splashing - upto 40 min.
Unhomogenised, pasteurised, 4% fat milk, 9°C initially
Control 1

Sample File Name: MILKD , Record: 476   Source: Analysed
Measured on: Thu, 5 Dec 2002 09:12AM   Last saved on: Thu, 5 Dec 2002 09:12AM

Presentation: (2NAD) 1.330, 1.456 + i 0.00000
Polydisperse model   Volume Result   Focus = 45 mm.

Residual = 0.366 %   Concentration = 0.009 %   Obscuration = 19.98 %
d (0.5) =   3.27 µm   d (0.1) =   0.34 µm   d (0.9) =   5.89 µm
D [4, 3] =   3.17 µm   Span =   1.70   Standard Deviation =   2.11 µm
Sauter Mean (D[3,2]) =   1.05 µm   Mode =   3.90 µm
Specific Surface Area = 5.7099 sq. m. / gm   Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %	Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.10	0.05	0.12	0.05	2.83	13.47	3.49	54.80
0.12	0.81	0.15	0.86	3.49	17.30	4.30	72.11
0.15	1.55	0.19	2.41	4.30	13.56	5.29	85.67
0.19	2.20	0.23	4.61	5.29	7.75	6.52	93.42
0.23	2.73	0.28	7.34	6.52	4.97	8.04	98.39
0.28	3.11	0.35	10.45	8.04	1.51	9.91	99.90
0.35	3.30	0.43	13.75	9.91	0.10	12.21	100.00
0.43	3.28	0.53	17.03	12.21	0.00	15.04	100.00
0.53	3.03	0.66	20.06	15.04	0.00	18.54	100.00
0.66	2.54	0.81	22.60	18.54	0.00	22.84	100.00
0.81	1.89	1.00	24.49	22.84	0.00	28.15	100.00
1.00	1.10	1.23	25.59	28.15	0.00	34.69	100.00
1.23	1.16	1.51	26.75	34.69	0.00	42.75	100.00
1.51	2.30	1.86	29.06	42.75	0.00	52.68	100.00
1.86	4.36	2.30	33.41	52.68	0.00	64.92	100.00
2.30	7.93	2.83	41.34	64.92	0.00	80.00	100.00

Figure C.1: Typical output from the Mastersizer E. A control sample (run T29).

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %	Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.10	0.05	0.12	0.05	2.83	10.60	3.49	49.98
0.12	0.78	0.15	0.83	3.49	12.17	4.30	62.15
0.15	1.49	0.19	2.32	4.30	11.28	5.29	73.43
0.19	2.10	0.23	4.42	5.29	9.26	6.52	82.69
0.23	2.59	0.28	7.02	6.52	7.16	8.04	89.85
0.28	2.92	0.35	9.94	8.04	4.67	9.91	94.52
0.35	3.05	0.43	12.98	9.91	2.67	12.21	97.19
0.43	2.95	0.53	15.94	12.21	1.38	15.04	98.57
0.53	2.62	0.66	18.56	15.04	0.75	18.54	99.33
0.66	2.10	0.81	20.66	18.54	0.45	22.84	99.77
0.81	1.50	1.00	22.16	22.84	0.23	28.15	100.00
1.00	1.11	1.23	23.27	28.15	0.00	34.69	100.00
1.23	1.32	1.51	24.59	34.69	0.00	42.75	100.00
1.51	2.53	1.86	27.12	42.75	0.00	52.68	100.00
1.86	4.67	2.30	31.79	52.68	0.00	64.92	100.00
2.30	7.58	2.83	39.37	64.92	0.00	80.00	100.00

Figure C.2: Damaged sample output from Mastersizer E (run T29).

C7: Sample calculation for particle size zoning (PSZ) test

Control sample (run T29 used as an example – data from Appendix C6):

Step 1 – Calculate control sample particle size A, D, O Zones

A Zone = sum of volumes between 0.1 μm and 1 μm (24.49 volume %)

D Zone = sum of volumes between 9.91 μm and 80 μm (0.10 volume %)

Primary Region = sum of volumes between 1 μm and 9.91 μm (75.41 volume %)

B Zone = 0.25 x Primary Region = 0.25 x 75.41 = 18.85 volume%

C Zone = 0.25 x Primary Region = 0.25 x 75.41 = 18.85 volume%

O Zone = 0.50 x Primary Region = 0.50 x 75.41 = 37.71 volume %

Step 2 – Calculate control particle size boundaries for B-O Zone and O-C Zone boundaries using linear interpolation.

Linear interpolation was used to find the B-O and O-C boundary when it was located within a particle size range. The full list of size ranges is shown in Figure C.1. It is assumed that within a size range the data follows a linear trend. Therefore:

$$x = \frac{x_1 - x_0}{(y_1 - y_0) \div PR} \cdot \left(\frac{y - y_0}{PR} \right) + x_0 \tag{C.20}$$

Where: x = Particle size boundary corresponding to upper 25% (B-O boundary) or lower 25% (O-C boundary) of primary region (μm).

y = Equivalent to 25% of total volume within primary region (volume %).

x_0 = Lower limit of particle size range incorporating 'x' (μm).

x_1 = Upper limit of particle size range incorporating 'x' (μm).

y_0 = Cumulative percentage volume of sample within primary region at lower limit of particle size range incorporating 'y' (volume %).

y_1 = Cumulative percentage volume of sample within primary region at upper limit of particle size range incorporating 'y' (volume %).

PR = Percentage volume of sample within primary region (volume %).

Table C.1 highlights the size ranges selected for linear interpolation. Each size range was first converted to a cumulative value beginning from both ends of the primary region. The boundary with the O Zone was defined as the point when 25% of the primary region was accounted for. Linear interpolation was therefore used on the size range in which the cumulative distribution exceeded 25%.

Table C.1: Primary region portion of PSD result from a control sample (run T29, Figure C.1, page Appendix C6) analysed with the Mastersizer E. A cumulative PSD was also calculated. The primary region summed to 75.41 volume %. Border and shading indicates size range where linear interpolation was carried out.

Size (Lo) in µm	Size (Hi) in µm	Result in - control in volume %	Cumulative total in volume % (fraction of P.R.)	
			from 1.00 µm	from 9.91µm
1.00	1.23	1.10	1.10 (0.01)	
1.23	1.51	1.16	2.26 (0.03)	
1.51	1.86	2.30	4.56 (0.06)	
1.86	2.30	4.36	8.92 (0.12)	
2.30	2.83	7.93	16.85 (0.22)	
2.83	3.49	13.47	30.35 (0.40)	
3.49	4.30	17.30		
4.30	5.29	13.56		27.79 (0.37)
5.29	6.52	7.75		14.23 (0.19)
6.52	8.04	4.97		6.48 (0.09)
8.04	9.91	1.51		1.51 (0.02)

Analysis for B-O Zone boundary (Equation C.20):

$x = (3.49-2.83) \div ((30.35-16.85) \div 75.41) \times (0.25-16.85 \div 75.41) + 2.83$

$x = 2.93$ µm.

Analysis for O-C Zone boundary (Equation C.20):

$x = (5.29-4.30) \div ((14.23-27.79) \div 75.41) \times (0.25-27.79) + 4.30$

$x = 4.95$ µm.

Step 3 – Calculate B and C Zones for treatment sample using linear interpolation.

$$y* = \frac{y_1 - y_0}{x_1 - x_0}(x - x_0) + y_0 \qquad\qquad\qquad (C.21)$$

Where: $y*$ = Percentage volume of sample within the B or C Zone

Table C.2 shows how the particular size range of a damaged sample is selected for linear interpolation. Whatever boundaries are selected using the control sample (in this case 2.93 and 4.95 µm), the particle size range of the damaged sample that these fall into are analysed with liner interpolation to find the % volume between the boundary and the upper or lower boundary of the primary region.

Table C.2: Primary region portion of PSD result from a damaged sample (run T29, Figure C.2, Appendix C6) analysed with the Mastersizer E. A cumulative PSD was also calculated. The primary region summed to 72.35 volume %. Shaded rows indicate size range where linear interpolation was carried out.

Size (Lo) (µm)	Size (Hi) (µm)	Result in - treatment (volume %)	Cumulative total from 9.91 µm (volume %)	Cumulative total from 1.00 µm (volume %)
1.00	1.23	1.11		1.11
1.23	1.51	1.32		2.43
1.51	1.86	2.53		4.96
1.86	2.30	4.67		9.63
2.30	2.83	7.58		17.21
2.83	3.49	10.60		27.81
3.49	4.30	12.17		
4.30	5.29	11.28	32.37	
5.29	6.52	9.26	21.09	
6.52	8.04	7.16	11.83	
8.04	9.91	4.67	4.67	

Analysis for B Zone (Equation C.21):

y* = (27.81-17.21) ÷ (3.49-2.83) x (2.93-2.83) + 17.21

$y* = 18.79$ volume %.

Analysis for C Zone (Equation C.21):

$y* = (21.09-32.37) \div (5.29-4.30) \times (4.95-5.29) + 21.09$

$y* = 24.94$ volume %.

Analysis for O Zone: the difference between the primary region and the sum of the B and C Zones was calculated:

$= 72.35 - (18.79+24.94) = 28.63$ volume %

The A and D Zones are analysed as for the control sample:

A Zone = sum of volumes between 0.1 μm and 1 μm (22.15 volume %)

D Zone = sum of volumes between 9.91 μm and 80 μm (5.48 volume %)

C8: Sample spreadsheet for PSZ test.

Equation C.23 = SUM(C9:C19)

Equation C.22 = SUM(G8,G9,G13)

Equation C.26 =IF(H16<2.3,SUM(D20:D22)+(H16-$A23)/($B23-$A23)*D23,IF($H$16<2.83,SUM(D20:D23)+($H$16-$A24)/($B24-$A24)*D24,IF(H16<3.49,SUM(D20:D24)+(H16-$A25)/($B25-$A25)*D25,"!!!")))

Equation C.27 =IF(H17>5.29,SUM(D29:D30)+($B28-$H$17)/($B28-$A28)*D28,IF($H$17>4.3,SUM(D28:D30)+($B27-H17)/($B27-$A27)*D27,IF(H17>3.49,SUM(D27:D30)+($B26-$H$17)/($B26-$A26)*D26,"!!!")))

Equation C.24 =IF((SUM(C20:C23)/G8)>0.25,($B23-$A23)/C23*(0.25*G8-SUM(C20:C22))+$A23,IF((SUM(C20:C24)/G8)>0.25,($B24-$A24)/C24*(0.25*G8-SUM(C20:C23))+$A24,IF((SUM(C20:C25)/G8)>0.25,($B25-$A25)/C25*(0.25*G8-SUM(C20:C24))+$A25,"!!!")))

Equation C.25 =IF((SUM(C28:C30)/G8)>0.25,($A28-$B28)/C28*(0.25*G8-SUM(C29:C30))+$B28,IF((SUM(C27:C30)/G8)>0.25,($A27-$B27)/C27*(0.25*G8-SUM(C28:C30))+$B27,IF((SUM(C26:C30)/G8)>0.25,($A26-$B26)/C26*(0.25*G8-SUM(C27:C30))+$B26,"!!!")))

PARTICLE SIZE ZONING TEST SAMPLE SPREADSHEET
To show how the five zones were calculated within a Microsoft Excel spreadsheet

Size (Lo) (µm)	Size (Hi) (µm)	Control	Treatment		F	Control	Treatment
0.00	0.10	0.00	0.00		% vol. in Primary Region	75.41	72.35
0.10	0.12	0.05	0.05		% vol. in Zone A	24.49	22.15
0.12	0.15	0.81	0.78		% vol. in Zone B	18.85	18.79
0.15	0.19	1.55	1.49		% vol. in Zone O	37.71	28.63
0.19	0.23	2.20	2.10		% vol. in Zone C	18.85	24.94
0.23	0.28	2.73	2.59		% vol. in Zone D	0.10	5.48
0.28	0.35	3.11	2.92		% vol. in Zone C+D	18.95	30.42
0.35	0.43	3.30	3.05		% vol. in Zone A+B	43.34	40.94
0.43	0.53	3.28	2.95		B-O zone boundary (µm)	2.93	2.93
0.53	0.66	3.03	2.62		O-C zone boundary (µm)	4.95	4.95
0.66	0.81	2.54	2.10			100.00	99.98
0.81	1.00	1.89	1.50				
1.00	1.23	1.10	1.11				
1.23	1.51	1.16	1.32				
1.51	1.86	2.30	2.53				
1.86	2.30	4.36	4.67				
2.30	2.83	7.93	7.58				
2.83	3.49	13.47	10.60				
3.49	4.30	17.30	12.17				
4.30	5.29	13.56	11.28				
5.29	6.52	7.75	9.26				
6.52	8.04	4.97	7.16				
8.04	9.91	1.51	4.67				
9.91	12.21	0.10	2.67				
12.21	15.04	0.00	1.38				
15.04	18.54	0.00	0.75				
18.54	22.84	0.00	0.45				
22.84	28.15	0.00	0.23				
28.15	34.69	0.00	0.00				
34.69	42.75	0.00	0.00				
42.75	52.68	0.00	0.00				
52.68	64.92	0.00	0.00				
64.92	80.00	0.00	0.00				

Calculation Procedure

1. Manually type in particle size information from datasheet (e.g. Appendix C6) into spreadsheet. Equation C.22 is used to check that all of the numbers have been typed in correctly (tabulated numbers correctly sum up to between 99.97-100.03 volume %).

2. Calculate A Zone (sizes below 1µm - shaded light grey), D Zone (sizes above 9.91µm - shaded dark grey) and primary region (P.R. - no shading) for control sample by summing particle size data within particular ranges. An example formula A Zone is shown in Equation C.23.

3. Calculate B and C Zones for control sample by multiplying the P.R. (cell G8) by 0.25, and for O Zone by multiplying the P.R. by 0.5.

4. The data above can be used to calculate the B-O and C-O Zone particle size boundaries by applying linear interpolation formulae described in Equations C.24 and C.25. These formulae (also Equations C.26 and C.27) are set up to account for large variations in the particle size distribution data of a control sample.

5. For the damaged sample (and all other samples damaged using the same batch of milk) repeat steps 1 and 2.

6. Apply Equations C.26 and C.27 to the damaged sample data. These equations use the linear interpolation in reverse and use the same B-O and C-O Zone boundaries (cells G16 and G17) calculated from the control sample.

C9: Reproducibility of pumping rigs - Repeated measures analysis of variance

Example program in SAS (bold type shows information specific to the runs used in the experiments):

```
PROC IMPORT OUT= WORK.expt2_2
      DATAFILE= "C:\Documents and Settings\mcdowney\My Documents\
My SAS Files\Titrations2.2.xls"
      DBMS=EXCEL2000 REPLACE;
   GETNAMES=YES;
RUN;

proc glm data=expt2_2;
      class runs;
      model T1 T2 T3= runs;
      repeated titrations;
run;
```

In runs J03-06, there were only two titrations for each sample, so the program code for data from those runs would contain only 'T1' and 'T2'

An example output is shown below for the four control samples from runs J17-20:

Source	Degrees of Freedom	Type 3 sum of squares	Mean square value
titrations	2	0.00065	0.000325
titrations*runs (interaction term)	6	0.04001667	0.00666944

The mean square value from the interaction term can be used as an estimate of uncertainty. This value is the square of the standard error. Therefore the 95% confidence interval is:

t statistic (for six degrees of freedom) x square root of the mean square value.

$= \pm 2.45$ x square root of (0.00666944)
$= \pm 2.45$ x 0.082
$= \pm 0.20$ mmol/L

Appendix D – Experimental Runs

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