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EXUDATION OF WHEY FROM CHEESE DURING STORAGE

**A thesis presented
in partial fulfilment
of the requirements for the
degree of Doctor of Philosophy
in Food Technology at Massey University**

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1991

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ABSTRACT

Cheeses of low pH, such as Feta, Blue, Cream and Cheshire, often exude whey after manufacture. This exudation lowers the yield and reduces product acceptability. Virtually no scientific study has been undertaken on this subject. Investigations were therefore undertaken to determine the factors affecting exudation and to elucidate the underlying mechanism. Cream cheese made by the hot-pack method and recombined Feta cheese made by the traditional method, representing unripened and ripened varieties of cheese respectively, were studied.

In Cream cheese the amount of exudate increased with decreased protein to fat (P/F) ratio, decreased homogenisation pressure, decreased pasteurisation temperature, decreased pH at cooking, decreased cooking temperature, increased storage temperature and increased storage time. Within the selected limits of variation of P/F ratio, fat did not affect exudation. However, an increase of moisture in non-fat substance resulted in an increased amount of exudate. The effect of homogenisation pressure appears to be due to the increase in the fat globule surface area and the increase in the coating of fat globule with casein. The partial heat-denaturation of the whey proteins in the cheesemilk was effective in reducing the rate of exudation, possibly due to the complex formation between β -lactoglobulin and κ -casein that prevented fusion of casein micelles.

Residual lactose and pH did not change, and proteolysis was not detected up to 16 weeks in Cream cheese stored at 5°C. It is concluded that exudation from Cream cheese does not occur due to any gross chemical changes during storage.

Manufacture of Feta cheese involved the use of recombined cow's milk and vacuum packaging of cheese after brining. A storage study of Feta cheese up to 6 months showed steady proteolysis, slow metabolism of residual lactose and a gradual decrease of pH. The water activity of the cheese depended on the salt-in-moisture concentration.

In Feta cheese the amount of exudate increased with increased P/F ratio, increased pH at draining, increased residual rennet, packaging cheese without vacuum, increased storage temperature and increased storage time. Variation of priming time, with a constant curd pH at draining, did not affect exudation. Unlike Cream cheese, an increase in protein and a decrease in fat content in Feta correlated with increase in the amount of exudate. The effects of change in pH and calcium (within a range expected in normal Feta) on exudation were minor.

Homogenisation was effective in reducing the rate of exudation in Feta cheese. However, a variation in the homogenisation pressure had no effect. The type of material adsorbed to the fat globule surface influences syneresis during manufacture as well as subsequent exudation during storage. The effect of a reduction in the size of fat globules on exudation appears to be less important.

In Feta cheese the incorporation of heat-denatured whey proteins did not affect exudation. However, there was a substantial increase in yield.

Proteolysis is the dominant factor affecting exudation. Its influence is apparently due to the disintegration of the casein network and the release of water physically held in the capillaries. Exudation is also substantially influenced by the gradient in NaCl concentration in Feta cheese following brining.

Denaturation of whey proteins in Cream cheese; and homogenisation, controlled proteolysis, decreased salt gradient, use of vacuum packaging in Feta cheese appear to be the main factors available for reducing the extent of exudation. Based on the findings of the investigation a hypothesis is proposed to explain the exudation from cheeses.

ACKNOWLEDGEMENTS

I wish to express my appreciation and deep sense of gratitude to my supervisors: Dr John Lelièvre, for providing guidance and the basic framework for the project during the initial phase of study; Mr Rod J. Bennett, for the general supervision and guidance, encouragement and the generous help in preparation of the manuscript; Dr Kevin N. Pearce, for providing the major impetus to the investigation, for his invaluable scientific critique, and for his patience during numerous sessions of discussions and editing of the thesis; and Mr Frank P. Dunlop, for arranging the facilities for cheesemaking trials and making available his wealth of experience in cheese technology.

I am grateful to the New Zealand Dairy Research Institute (NZDRI) for the facilities made available for carrying out the experimental work and the opportunity to work with the distinguished scientists.

I am indebted to Dr Frank G. Martley, Dr Harjinder Singh, Mr Keith A. Johnston, Dr Krish R. Aiyar, Dr Reyad R. Shaker (and their families) for the continued guidance, support and friendship.

I am extremely grateful to the following staff members at the NZDRI:

- Miss E. Jenni Madwick, for the help in designing the experiments and statistical analysis of all the data.
- Mr Arran D. Breslin, for the help with analyses involving PAGE and HPLC.
- Dr Alastair K.H. MacGibbon, for useful discussions and help in DSC study.
- Mr John Gilles, Dr Vaughan L. Crow, Dr R. C. Lawrence, Dr Lawrie C. Creamer, Dr Rose L. Motion and Mr Gerhard K. Hoppe, for helpful discussions.
- Mr David C. W. Reid, Miss Nicky J. Maxwell, Miss Yvonne E. van der Does, Miss Jillian M. Smith, Ms Mary-Jo Ray and Mrs Christina J. Coker for the assistance received with some specific analyses.

- Mr O. J. Freese, Mrs. Joan M Bennett, Mr Keith Montgomerie, Mr G. Steve Boleyn, Mr Malcolm J. Montgomerie, Mr Michael F. Lawson, and Mr Derek C. Goodwin, for the help during cheesemaking.
- Mr Errol F. Conaghan and his staff, for the help in chemical analysis of some of the samples.
- Mr Paul J. Le Ceve, for the preparation of the photographs.
- Mr Peter J. Rykenberg and Mr Dan F. Legg, for assistance with the word processor.

I extend my thanks to many friends, at Massey University (Food Technology Department, in particular) and NZDRI, who have been of help in many little ways.

The financial support in the form of a Commonwealth scholarship, awarded by the Government of New Zealand, is gratefully acknowledged. Thanks are due to the Government of India, for having nominated me for this award; and the National Dairy Development Board, Anand, India, for having granted study leave.

I am grateful to my parents and family members for providing the moral support and encouragement for this study.

Finally, many thanks to my wife, Dolly, who was most understanding and readily shared the stress and strain during the course of the study. Thanks are also due to our daughter, Deepika, who had to put up without me for most of the time in her second year.

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ABBREVIATIONS

ANOVA	Analysis of variance
A_w	Water activity
BSA	Bovine serum albumin
cm	Centimetre
d	Day
DDM	Dairy division manual
DM	Dry matter
DTE	Dithioerythritol
EDTA	Ethylene diamine tetra-acetic acid
F	F ratio
FDM	Fat in dry matter
FFMR	Fresh frozen milkfat for recombining
FGS	Fat globule size
f.p.	Freezing point
g	Gram
GMS	Glycero mono stearate
h	Hour
HPLC	High performance liquid chromatography
HTST	High temperature short time
IDF	International Dairy Federation
kg	Kilogram
kPa	Kilopascal
L	Litre
LHSMP	Low heat skim milk powder
LSM	Least square mean
m	Metre
M	Molar concentration
MFGM	Milk fat globule membrane
mg	Milligram
min	Minute (time)
ml	Millilitre
mm	Millimetre
mMol	Millimole
MNFS	Moisture in non-fat substance
mo	Month
m.p.	Melting point
nm	Nanometre

ns	Not significant
NSLAB	Non starter lactic acid bacteria
NZDRI	New Zealand Dairy Research Institute
PAGE	Poly acrylamide gel electrophoresis
P/F	Protein/fat
ppm	Parts per million
psi	Pounds per square inch
rpm	Revolutions per minute
RSM	Reconstituted skim milk
RU	Rennet unit
s	Second (time)
S.D.	Standard deviation
SDS	Sodium dodecyl sulphate
SES	Simulated external solution
S/M	Salt/moisture
SMP	Skim milk powder
SNF	Solids-not-fat
TN	Total nitrogen
TS	Total solids
UF	Ultrafiltration
UHT	Ultra-high temperature
wk	Week
WPC	Whey protein concentrate
WPNI	Whey protein nitrogen index
wt	Weight
w/v	Weight/volume
v/v	Volume/volume
w/w	Weight/weight
α -	Alpha-
β -	Beta-
κ -	Kappa-
°C	Degree Celsius (Centigrade)
μm	Micrometre
%	Per centum
>	Greater than
$\geq$	Greater than or equal to; not less than
<	Less than
$\leq$	Less than or equal to; not greater than

CHAPTER 1

INTRODUCTION

Gels are two-phase systems with a continuous network of solid material forming a matrix and enmeshing or holding an aqueous or other solvent phase which may also be continuous or finely dispersed (Matz, 1965; Glicksman, 1969). Gels show resistance under pressure and are capable of retaining a firm structural form.

Gels are frequently encountered in polysaccharide and protein based food products, *eg.* jams, jellies, custards, yoghurts, condensed milks and cheeses. Some of these gels, particularly those in the intermediate and high moisture foodstuffs, tend to contract in volume during storage resulting in the expression of part of the weakly held water or liquid phase. This phenomenon is termed syneresis.

The formation of gels from milk proteins is irreversible in contrast to gels of most other foods. The gels from casein include gels of renneted milk, yoghurt, stored heated milk and whey proteins. Gels formed from mainly casein, such as junket and yoghurt, are soft and brittle, and tend to show syneresis.

Syneresis is undesirable in finished dairy products such as yoghurt, sour cream, Cream cheese, Cottage cheese and quarg. However, in the manufacture of most cheese varieties syneresis (draining of whey) is an essential processing step and has a vital role in determining the quality. Syneresis involving contraction of the protein gel and concomitant expulsion of an aqueous whey phase is a complex and poorly understood process (Pearse *et al.*, 1984) although there is considerable information on factors that influence syneresis (Fox, 1987a). Reports in the literature describe efforts to reduce syneresis in yoghurts but the mechanism involved is not fully understood.

Cheeses of low pH and/or high moisture, such as Blue, Cheshire, Cream and Feta, often exude whey (moisture with soluble material) during storage or ripening. This exudation leads to considerable yield loss. Handling of product with exudate in the

bulk packs is inconvenient and difficult as the surface of the product is slippery. At times exudation is also detected in consumer packs. This is unacceptable to the consumer and brings disrepute to the image of the company or 'brand'.

The present investigation was undertaken to determine the factors affecting exudation of whey from cheese during storage and to study the underlying mechanism. It was of interest to find practical solutions for preventing exudation of whey from Cheshire, Cream and Cast Feta cheeses exported from New Zealand.

In many food products the exudation is controlled by the addition of stabilisers or hydrocolloids. However, this often has a detrimental effect on the flavour, body and textural attributes of the product. Recent trends indicate that consumers are demanding 'all natural or no additive' foods, and food processors are actively attempting to attain the 'clean' ingredient label for their products (Best, 1990). The use of stabilisers and emulsifiers is restricted by the food regulations in the individual countries. Moreover, in a complex food system as in cheese, particularly with the ripened varieties, the usefulness of stabilisers is uncertain. The use of stabilisers was therefore not considered in this study.

Very little is reported in the literature about the exudation of whey from cheese during storage or ripening. In the absence of guidance from the literature, studies on exudation were undertaken with hot-pack Cream cheese and Feta cheese made from recombined milk by the traditional method of manufacture, representing unripened and ripened varieties of cheese, respectively. It was hoped that the study of exudation from these cheeses would provide the basic information applicable to related varieties of cheese.

For the sake of convenience, some of the expressions used in the text have the following connotations, unless specified otherwise.

Syneresis:	The expression of whey during manufacture of cheese.
Exudation:	The expression of whey/liquid from cheese after manufacture.
Exudate:	The material that is expressed due to exudation.
Storage of cheese:	Includes the ripening process where appropriate.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

Very little has been reported on the exudation of whey from cheese during storage. The survey of literature has been therefore extended to include similar processes (syneresis of whey during cheese manufacture, salt diffusion during brining of cheese) and to a related product (yoghurt) that also shows exudation. Attention is given to milk proteins and their water-binding capacity, and the structure of milk gels. Reference is also made to Cream and Feta cheese since manufacture of these cheeses was undertaken during the course of the present study. Aspects related to emulsification of fat, material adsorbed to fat globule surface and incorporation of denatured whey protein in cheese are also included since they were expected to provide an insight into mechanisms of exudation. This review consists of the following sections:

- (a) Gels
 - Rennet-induced gels in milk and cheese
 - Acid-induced gels in yoghurt
- (b) Emulsions
- (c) Incorporation of whey protein in cheese
- (d) Cream cheese
- (e) Feta cheese
- (f) Salt diffusion
- (g) Changes in cheese during storage

2.2 Gels

2.2.1 Introduction: Gels display the properties of both solids and liquids: resembling solids in structural rigidity and elastic response when distorting forces are applied; and liquids in vapour pressure, compressibility, and electrical conductivity (Glicksman, 1969). Gel formation from solutions of long-chain

polymers occurs as adjacent molecules cross-link to form a continuous network possessing mechanical stability in the final gelled state. A continuous liquid phase consisting of the solvent and the solutes is entrapped, some of which may include non-cross-linked polymeric materials.

The elastic properties of a gel depend on the number and character of the cross-links at a given stage of strain and would largely depend upon what linkages have been broken and what linkages have been formed (Glicksman, 1969). The gel is usually elastic when the degree of cross-linking is low and more rigid when the degree of cross-linking is high and closely spaced.

The phenomenon of syneresis, which refers to exudation of solvent and some of the solutes from a gel system, is often encountered. Syneresis takes place in freshly prepared gels which tend to contract and occurs in both macromolecular gels with water as well as in those with organic liquids as solvents (Hermans, 1963). The degree of syneresis depends upon the concentration, temperature and the addition of salts and other factors. Syneresis is a complex process but in general, added compounds which favour swelling diminish syneresis and vice-versa.

Aging of gels leads to contraction and exudation of a portion of the liquid (Glicksman, 1969). The occurrence of syneresis in a gel during aging is due to the formation of additional intermolecular bonds with a consequent reduction in the number of loci available for solvent binding and to a decrease in the dimensions of the intermolecular spaces in which the solvent is contained (Matz, 1965).

2.2.2 Rennet-induced gels in milk and cheese

Aspects related to chemistry of curd making and syneresis of whey from curd during cheese manufacture have been comprehensively reviewed (Patel *et al.*, 1972; Dijk, 1982; Walstra *et al.*, 1985; Dijk & Walstra, 1986; Walstra & Vliet, 1986; Fox, 1987a; Pearse & Mackinlay, 1989).

Casein is a protein polymer composed of large molecules, each consisting of a large number of repeating units (amino acids) joined by covalent bonds (peptide bonds).

The casein micelles in milk consist mainly of protein (α_{s1} -, α_{s2} -, β - and κ -caseins), calcium phosphate and water (Walstra & Jenness, 1984). The casein molecules are present in small aggregates (submicelles) each containing different casein species and having a predominantly hydrophobic core and predominantly hydrophilic outer layer. The submicelles are clustered into spherical aggregates with interstitial moisture and most probably kept together by undissolved and colloidal calcium phosphate (Walstra *et al.*, 1985).

Lin *et al.* (1972) suggested a model for the structure and dissociation of the casein micelle: Ca^{2+} removal initially dissociates weakly bound caseins from the micelle, while a size-determining micellar framework remains intact. This protein framework was found to contain mainly α_s -casein, while the dissociable protein contained mainly β - and κ -casein. When the Ca^{2+} fell below a critical level, the micelle completely dissociated.

Addition of appropriate enzymes to milk leads to the gelation of casein. First the partial proteolysis of κ -casein occurs and in the presence of ionic calcium, casein micelles interact to form chains, which eventually cross-link to form a gel-matrix (Carlson *et al.*, 1986). The reaction rate increases with temperature as long as the enzyme is stable, peaks with an optimum pH around 5.0, and is partly influenced by salts probably by promoting or reducing the binding between the enzyme and the substrate. A model proposed by Carlson *et al.* (1986) states that the rate of gel firming is controlled by two reactions, the enzymatic hydrolysis of κ -casein to expose cross-linking sites and the reaction of exposed sites to form such cross-links.

The enzymatic action of rennet is of the first order leading to flocculation of micelles without the casinomacropetide; gradual increase in flocculation leads to the formation of a gel with a continuous network and the micelles gradually fuse, increasing the contact area between them with a possible rearrangement of calcium phosphate and submicelles (Walstra, 1985).

Green *et al.* (1977) reported that the gel formed by the rennet coagulation of milk is the result of linkage of groups of micelles together. The gel has an open,

irregular structure in which micelles are loosely packed, enabling much serum to be entrapped. The casein micelles in rennet-treated separated (skim) milk form a network type of gel, with similar structure in all dimensions (Green *et al.*, 1978a).

The aggregation of casein micelles starts after about 60% of the rennet-clotting time (visual clotting time of milk), when the enzymatic action is complete. The micelles form chains which then link into a close network. Micelles, initially linked by bridges, later on contract and bring the particles into contact and eventually cause partial fusion (Green *et al.*, 1978b). The rate of micellar aggregation is proportional to rennet concentration, even after κ -casein hydrolysis is complete. The increase in viscosity of rennet-treated milks can be considered as a measure of aggregation of casein micelles (Green, 1980).

A rennet coagulated gel formed undisturbed in a vessel of vertical walls, usually does not show syneresis because the gel is constrained in the vessel and can not shrink (Walstra *et al.*, 1985). The gel does not show syneresis at the surface because of being covered by a thin lipid-rich layer so that capillary forces prevent the serum from leaving the matrix. Cutting the gel disrupts this network and causes it to shrink, resulting in the expulsion of the aqueous phase (Pearse & Mackinlay, 1989). Syneresis is due to the shrinkage of the three-dimensional curd network, which had been formed from continued aggregation of the casein following coagulation (Casiraghi *et al.*, 1987). The network therefore tends to shrink and becomes more compact.

The continued action of chymosin (rennet) and the loss of calcium phosphate caused by acid conditions are two of the factors which bring about the fusion of the casein micelles (Brooker, 1987). The fusion of micelles continues until the protein phase forms continuous tracts which bear little resemblance to the curd at cutting.

Kimber *et al.* (1974) reported that the gel formed by adding rennet to milk does not reach a final rigidity, but continues getting firmer over several hours, by which time considerable syneresis has occurred. Firming appears to involve the formation

of more linkages between micelles. The overall curd structure may be visualised as a casein sponge in which fat globules are entrapped.

Cheese consists of a continuous protein matrix, throughout which a discontinuous, discrete fat phase can be observed and it is only the casein which is involved in the formation of the basic structure of cheese (Lawrence *et al.*, 1983). The role of casein is therefore expected to be of prime importance in the gel structure.

In low pH cheeses casein fractions form compact aggregates as they are close to their iso-electric point. These are held together with strong ionic and hydrophobic intra-aggregate forces while the inter-aggregate forces are weaker. In this most of the water is in an inert interstitial state, and not distributed evenly throughout the curd mass. The low pH cheeses may be considered as porous masses of casein and fat particles (Creamer & Olson, 1982).

(i) Syneresis in renneted milk gels

Walstra *et al.* (1985) described syneresis as a rate process and hypothesised that syneresis results from a contraction of the gel network by a gradual process of re-alignment and bond interchange to conformations of lower energy. The rate of syneresis is directly proportional to the pressure in the system (pressure exerted by the network on the moisture) and the permeability (resistance against flow through the matrix or the average cross section of the pores) of the network (Dijk, 1982); and inversely proportional to the viscosity of the continuous phase and the dimensions of the gel.

Syneresis in renneted milk gels is attributed to the increase in the degree of cross-linking of polymer networks, the change in the charge on polymer chains and the variation in solvent-polymer interaction coefficients (Lelievre, 1977). The increase in the number of junction points due to the casein-casein interactions are likely to be partly responsible for syneresis (Lelievre & Creamer, 1978).

Pearse & Mackinlay (1989) have suggested that the chemical interactions inducing syneresis of the curd network are in part an extension of the interactions that give

rise to curd formation. In the conversion of milk to cheese, casein micelles aggregate to form a network that entraps the aqueous phase. Any alteration in the composition of the casein micelles which form this curd network might be expected to affect the coagulation and subsequently the syneresis. Specific and non-specific interactions involving protein occur during curd formation and affect syneresis.

(ii) Factors affecting syneresis from milk coagulum and cheese curd

It is proposed that the exudation of whey during storage of low pH and high moisture cheeses is an extension of the syneresis process that started during cheese manufacture. The factors influencing syneresis during manufacture may therefore be expected to have an effect on exudation during storage. There is general agreement on a number of the factors affecting syneresis from renneted milk gels. This review is intended to serve as a guide in selecting the manufacturing variables for study of factors affecting exudation from cheese. Evidence for the likelihood of manufacturing variables affecting exudation was supported by the findings that the starter culture, rate of salt addition, temperature of overnight-drainage and the whitening treatment for the cream all significantly influenced exudation from 60-day-old Blue cheese (Pederson *et al.*, 1971). However, the above study did not take into account the moisture lost during the initial 60 days, and the method used to measure exudation was based on a temperature-stressed effect on cheese. The need to determine more clearly the effects of manufacturing variables on exudation is thus evident. Indeed, varying the manufacturing conditions may be the most effective practical tool to control exudation. Once the cheese is made there are not many options to regulate its properties during storage or ripening.

Concentration of milk or casein: Increase in protein concentration of milk by ultrafiltration decreased syneresis (Peri *et al.*, 1985) which may be attributed to much more evenness of the gel and the increase in the number of bonds per junction (Walstra & Vliet, 1986). Pearse & Mackinlay (1989) reported that the syneresis is sensitive to the concentration of β -casein and also to low levels of dephosphorylation of β -casein.

Role of fat: Syneresis decreased with increase in fat content in milk (Storry *et al.*, 1983; Lawrence & Gilles, 1987), although the total amount of fat lost in the whey increased (Fox, 1987a). Fat mechanically blocks the casein-casein interactions (Lelievre & Creamer, 1978) with an increased number of interstices within the reticulum of the coagulum (Storry *et al.*, 1983). This effect of fat on milk gels is consistent with a lowering of attractive forces between the protein molecules forming the gel structure (Johnston & Murphy, 1984).

Increasing the fat content in homogenised reconstituted non-fat dry milk resulted in a reduction of curd firmness, hardness, rate of firming, and syneresis (Kebary & Morris, 1990).

Role of whey protein: Syneresis is reduced in rennet-induced gels made from milk in which whey proteins have been denatured by heat treatment (Fox, 1987a).

Gelling capacity of whey protein gels is approximately proportional to the sulphhydryl content of the whey protein powders. The whey protein gels are stabilised by disulphide linkages and can be markedly influenced by small changes such as salt concentration (Green, 1980). Moisture loss and coarseness of whey protein gels decreased with increasing net charge on the proteins as pH was altered away from the iso-electric point (Hermansson, 1983).

Effect of temperature: Syneresis increased significantly with temperature (Patel *et al.*, 1972; Dijk, 1982; Walstra *et al.*, 1985; Walstra & Vliet, 1986) though the rate of heating during cooking did not seem to have a significant effect (Patel *et al.*, 1972).

Effect of homogenisation: Cheese curd is composed primarily of a network structure of casein in which fat globules are entrapped. In normal milk which has not been homogenised, the fat globules behave as an inert filler and there is no cross-linking between the fat globules and the casein network. However, when the cream (fat) fraction of the milk has been homogenised, the casein is adsorbed onto the fat-water interface (Mulder & Walstra, 1974). In this situation fat globules

participate in the formation of the casein matrix in the cheese (Vliet & Dentener-Kikkert, 1982; Walstra & Vliet, 1986).

Syneresis is retarded by homogenisation (Vaikus *et al.*, 1970; Emmons *et al.*, 1980; Storry *et al.*, 1983; Fox, 1987a; Kebary & Morris, 1990), probably because of alteration in coagulum structure and modification in the entrapment of fat globules within the coagulum (Storry *et al.*, 1983). In unhomogenised milk the fat globules are presumably passively enmeshed in the reticulum of the coagulum as gelation takes place. Homogenised milks will have fat dispersed into a greater number of smaller globules, the surfaces of which will be modified by the presence of adhering casein particles; and the stronger coagulum of the homogenised milk must reflect a denser reticulum of finer lattice structure (Storry *et al.*, 1983). The combined effect of finer lattice and increased number of smaller fat globules together with the possibility that the latter could be more tightly bound within the reticulum owing to coagulation of adhering casein, could result in slower drainage of whey.

Green *et al.* (1983) observed that when Cheddar cheese was made from homogenised milk, casein micelle aggregation occurred more slowly, protein network in the curd was less coarse, curd fusion was poor and the rate of whey loss was reduced.

Vaikus *et al.* (1970) concluded that homogenisation of whole milk has a different effect on milk proteins compared to when only cream is homogenised and added back to milk. They observed that syneresis from renneted milk gels was inversely proportional to the extent of homogenisation of the whole milk or only the cream. Further, syneresis was least when entire whole milk was homogenised, moderate when only cream was homogenised, and maximum when milk was unhomogenised.

Effect of pH: Syneresis increases significantly with decreasing pH (Zittle *et al.*, 1957; Patel *et al.*, 1972; Dijk, 1982; Walstra *et al.*, 1985; Peri *et al.*, 1985; Walstra & Vliet, 1986). As acid is produced by the conversion of lactose to lactic acid, the pH is lowered, more and more colloidal calcium phosphate goes into solution and syneresis is greatly enhanced (Walstra & Vliet, 1986; Casiraghi *et al.*, 1987).

The effect of lowering the pH may be explained by the decreased hydration of casein as the isoelectric pH is approached, and the release of colloidal calcium phosphate from the casein micelle (Patel *et al.*, 1972).

Role of calcium: Syneresis increases with an increase of CaCl_2 (Dijk, 1982). Factors that affect the state of colloidal and soluble fractions of calcium in milk, such as acidification, pH, citrate addition, influence syneresis (Zittle *et al.*, 1957; Casiraghi *et al.*, 1987).

Zoon *et al.* (1988) reported that a minimum amount of calcium is required for the clotting of rennet-induced gels to occur and at constant calcium ion activity, a lower micellar calcium phosphate concentration resulted in a longer clotting time.

Waugh *et al.* (1970) noted that one of the important primary reactions in rennet coagulation of milk is the binding of calcium ions to the casein proteins which renders the α_{s1} - and β -caseins liable to precipitation.

Miscellaneous: Czulak *et al.* (1969) found that during Cheddar cheese manufacture, while the curd particles remain in contact with the whey, the lactose fermented in the curd is replaced by the lactose diffusing from whey. Lactic acid produced in the curd diffuses into the whey rather slowly. When the acid production during cheese manufacture is too fast or too slow, the cheese becomes defective apparently due to the lack of optimum distribution of lactose and lactic acid between the curd and whey. When a high level of lactic acid was reached slowly, the cheese became acid and crumbly. Liquid separation (exudation) from the cheese occurred at 2 months of age. The authors suggest that the rate of development of acidity and the time the curd is in whey were critical factors in controlling Cheddar cheese quality.

During cheese manufacture the operations of cutting the coagulum, stirring and cooking affect syneresis (Walstra & Vliet, 1986; Walstra *et al.*, 1987). Increasing the speed of stirring increases syneresis to a small extent (Patel *et al.*, 1972). In addition, a number of interactions occurring during manufacture, such as between

pH and agitation, between pH and CaCl_2 , between temperature and agitation, have significant effects on syneresis (Patel *et al.*, 1972). Syneresis remains unaffected by variations in concentration of rennet used (Dijk, 1982).

2.2.3 Acid-induced gels in yoghurt

Introduction: Acid-induced gels in cheese could be considered similar in many ways to the acid-induced gels in yoghurt. This review is intended to serve as a guide in selecting the manufacturing variables for study of factors affecting exudation from Cream cheese, a unripened and non-rennet cheese, where few bio-chemical changes are expected during storage.

Most authors have referred to whey separation from yoghurt during storage as syneresis. Accordingly, in this section, exudation of whey from yoghurt during storage is being referred as syneresis.

Characteristics of acid gels

The progressive acidification of milk by lactic acid starter bacteria (Brooker, 1987) or by addition of mineral acid leads to the non-enzymic gelation of milk. The branched network of micelles differs from that of rennet gels in their ultra-structural characteristics. The fusion of micelles leads to contraction of the protein network of the gel and expulsion of water from its interstices.

The casein gels induced by rennet and acid are both viscoelastic (Vliet *et al.*, 1989). However, the protein-protein bonds are less mobile in acid-induced gels.

During fermentation of yoghurt coalescence of the casein micelles improves the texture of yoghurt (Mottar *et al.*, 1989). In comparison to rennet gels, the changes in the stages of acid gel formation are much slower and it is easier to distinguish the individual 'micelles' for a longer period because of the absence of colloidal calcium phosphate (Walstra & Vliet, 1986). In acid gels the permeability of the gel network is of the same order of magnitude as renneted gels and shows little change with time (Dijk, 1982).

In acid gels, particularly those produced by lactic acid bacteria, change in solubility of curd particles may be a factor contributing to syneresis from sour milk products (Walstra *et al.*, 1985). In yoghurt (an acid-induced milk gel) syneresis is undesirable (Harwalkar & Kalab, 1986), in contrast to renneted milk gels where controlled syneresis is desirable. Controlled syneresis is also desired in some acid gels, *e.g.* cottage cheese and quarg, and is brought about by cooking or salting the curd during manufacture. Cottage cheese and quarg possess entirely different ultrastructures compared to that of yoghurt (Brooker, 1987).

The microstructure of yoghurt is affected by the heat treatment of milk, bacterial starter cultures, total solids content, and the presence of thickening agents (Kalab *et al.*, 1983), all of which affect the firmness of the gel and its susceptibility to syneresis (Kalab *et al.*, 1976; Davies *et al.*, 1978).

Role of milk solids: The water-holding properties of the gel are improved and the mechanical properties of the gel are changed by increasing the levels of milk solids (Brooker, 1987). Weak body due to improper formulation and culturing may cause syneresis in yoghurt (Harwalkar & Kalab, 1983).

Cultured milks were prepared using whey protein concentrates from ultrafiltered acid whey, ultrafiltered rennet whey and industrial spray-dried process (Jelen *et al.*, 1987). The viscosity of soured milk decreased with increase in the amounts of whey protein. Upon standing for several days the finished product with pH varying between 4.4 and 4.55 showed whey syneresis. Syneresis increased in proportion to the increase in whey protein content in the cultured milks.

The viscosity of yoghurt is almost wholly dependent on the protein content of the milk. One way of increasing the protein level without increasing the lactose is to fortify the milk with caseinate powders. In addition to improving the consistency, added caseinates enhance the hydrophilic nature of protein and act as a stabiliser, thereby decreasing the problem of syneresis (Tamime & Deeth, 1980).

Increasing the total solids also increases the density of the protein matrix and this decreases syneresis (Harwalkar & Kalab, 1986).

While the rate and extent of syneresis appears to be related to total solids, not all types of milk solids have a similar effect. Besides, it is not always easy to vary a single solid component in milk. Therefore, parameters such as 'casein to fat ratio' or 'protein to fat ratio', which are commonly used to standardise milk, need to be carefully monitored.

Effect of heat treatment: Heat treatment of milk is an important step since it determines the body and texture of yoghurt as well as syneresis. The usual heat treatment of 85 °C/30 min or 90-95 °C/5-10 min to yoghurt milk results in minimal syneresis and maximal firmness of the yoghurt coagulum (Tamime & Deeth, 1980). This relatively severe heat treatment results in the binding of β -lactoglobulin to κ -casein which prevents fusion of the casein micelles (Brooker, 1987). Milk which has not been sufficiently heated forms a soft gel from which liquid (whey) exudes easily (Harwalkar & Kalab, 1986).

Parnell-Clunies *et al.* (1986) found that yoghurt from milk heated to 98 °C/0.5-1.87 min showed the highest water-holding capacity compared to UHT (140 °C/2-8 s) or vat (85 °C/10-40 min) heat treatments. Data from these studies indicated that denaturation of whey protein was not necessarily a precursor for improved water-holding capacity.

Firmness and syneresis of yoghurt are associated with the extent to which micelles coalesce during fermentation (Davies *et al.*, 1978). The appendages of micelles in heated milk appear to inhibit coalescence giving rise to a firmer curd with a lower tendency towards syneresis. Electron microscope studies showed that yoghurt from highly heated milks (95 °C/10 min or autoclaving at 121.7 °C/15 min) had filamentous appendages whereas that from raw milk had smooth contours with no appendages. The appendages were composed of β -lactoglobulin and were sensitive to sulphydryl blocking agents. The gel network in yoghurt from heat-treated milk

consists of thinner strands due to the reduced coalescence of casein micelles, which probably gives a firmer gel with greater water-holding capacity (Green, 1980).

The heat-induced association of α -lactalbumin with casein appeared to be important for fusion and hydration of the micelles during subsequent fermentation, which determined the rheological properties of yoghurt (Mottar *et al.*, 1989).

Stabilisers or gums are often added to yoghurt to provide a desirable consistency and prevent whey syneresis during storage. The stabiliser combines with water to form gels, increases the viscosity and reduces syneresis. In countries where the use of stabilisers is not permitted, there is heavy reliance on heat treatment regimes, adjustment of total solids and pH *etc.* to reduce syneresis. The denaturation of β -lactoglobulin and its interaction with casein micelles on heating milk for yoghurt manufacture helps in partly reducing this required increase in total solids and addition of stabilisers (Dannenberg & Kessler, 1988). The extension of holding time during heating beyond a certain point not only provides no improvement in gel consistency but possibly weakens it.

It is apparent that controlled heating of yoghurt milk and denaturation of whey protein are vital both for texture and reduction of whey syneresis in yoghurt.

Effect of homogenisation: Vaikus *et al.* (1970) observed that in acid gels from homogenised milk, where cream was homogenised and added back to milk, syneresis decreased only up to a certain pressure of homogenisation, *i.e.* 150 atm (15,151 kPa), along with an increase in viscosity. Syneresis increased with further increase in the homogenisation pressure. The increase in syneresis is attributed to the possible destabilisation of fat globules.

Effect of pH: The effect of pH on syneresis may be attributed to the variation in net electric charge of the casein micelles (Harwalkar & Kalab, 1986). Slow acidification of milk can promote undesirable effects like whey syneresis in yoghurt (Tamime & Deeth, 1980).

It is not clear whether lowering of pH causes syneresis or the problem is aggravated at pH values close to the iso-electric point of caseins.

Miscellaneous: Vibrations during transportation of the finished product, gravitational settling during storage and disruption of the microstructure of the protein matrix by accidental freezing during storage can all aggravate syneresis in yoghurt (Harwalkar & Kalab, 1983).

2.3 Emulsions

2.3.1 Introduction: An emulsion may be defined as "an intimate mixture of two immiscible liquids in which one liquid phase is dispersed throughout the other in the form of small, discrete droplets. In a good emulsion, these droplets remain dispersed indefinitely and the mixture remains completely homogeneous and gives the appearance of a uniform liquid *e.g.* homogenised milk" (Glicksman, 1969).

Emulsions are usually of two types: the oil-in-water type in which the oil is dispersed in small droplets throughout the water phase *e.g.* milk; and the water-in-oil kind in which the water is dispersed in small droplets throughout the oil phase *e.g.* butter. The dispersed droplets are the discontinuous internal phase, whereas the liquid surrounding the droplets is the continuous external phase. This review is restricted to the emulsions encountered in milk and cheese which are of the oil-in-water type.

An emulsifying agent or the stabilising agent in an emulsion assists in the formation of the emulsion, prevents or inhibits the creaming and coalescence of oil droplets, and contributes to the flow properties (*e.g.* pourability) and mouthfeel of the product (Marrs *et al.*, 1989).

Natural cheese is a nearly perfect emulsion, stabilized by natural surfactants - the cheese proteins (Shimp, 1985). Cheese contains an oil phase consisting of fats and oil-soluble substances, and a water phase consisting of a solution composed largely of water-soluble proteins and minerals. The two phases are naturally incompatible,

but are emulsified by surface-active proteins. The surface-active proteins are soluble in both the oil and water phases and tend to collect at the interfaces between the two phases (Shimp, 1985).

Emulsions are affected by heat treatment, addition of electrolytes (such as mineral acid, alkali, salt, alum *etc.*), centrifugation, mechanical agitation, and change in calcium content and pH (Glicksman, 1969; Shimp, 1985).

2.3.2 Emulsification of fat in milk and cheese

Milkfat has no affinity for water. However, emulsification of fat in the aqueous phase with the help of surface active agents influences the water-holding ability of the milk coagulum, which is supported by the effect of fat on the syneresis of renneted gels (described earlier). It is reported that the fat-casein interface in Cheddar cheese was the region of highest water content in the mature cheese where the fate of the membrane which originally surrounded the milk fat globule was less certain (Kimber *et al.*, 1974).

Homogenisation: Due to homogenisation, the average size of the fat globules in the milk is reduced from around 4 μm to as little as 1 μm (Mulder & Walstra, 1974), and the total fat globule surface area is increased by about six-fold (Morr & Richter, 1988). The creation of this extra fat-serum interface results in the adsorption of surface active material from the serum. The composition of the newly-created membrane makes a significant contribution to the physical properties of the emulsion, such as separation or coalescence and destabilisation (Darling & Butcher, 1978). Homogenisation of milk or cream results in the surface of fat globule being coated by casein micelles, and during the formation of this surface some casein micelles stabilise part of the surface of two adjacent fat globules (Brooker, 1987). During homogenisation, most of the caseins go to the oil-water interface in the form of proteinaceous colloidal particles (casein micelles) and this leads to a protein layer much greater than monolayer coverage (Dickinson *et al.*, 1989a).

Melsen & Walstra (1989) showed that creams made from anhydrous milk fat and skim milk were more stable than comparable natural creams. The effects of homogenisation in the former may explain the increase in emulsion stability.

Homogenisation of fat into reconstituted skim milk resulted in a soft rennet curd, and poor whey syneresis (Emmons *et al.*, 1980). The curd was similar to that obtained with the use of high heat skim milk powder (SMP) or homogenised whole milk. Reduced syneresis may be attributed to the adsorption of casein on the newly created surfaces of fat (Maxcy *et al.*, 1955).

Homogenisation of fat with whey protein concentrate, sodium caseinate and SMP resulted in fat globules with mean diameters 2.6, 1.4 and 1.3 μm , respectively; compared to 4.2 μm in unhomogenised washed cream (Aguilera & Kessler, 1988). Stability of the fat globules against coalescence increased as the mean fat globule diameter decreased.

It has been suggested that the fat globules interfere mechanically with syneresis (Lelievre & Creamer, 1978; Lawrence & Gilles, 1987), or act as filler particles in an aqueous matrix of swollen proteinaceous material (Luyten, 1988). However, the role of fat and materials adsorbed to fat globule surface on the water-holding ability of the protein matrix in cheese is not fully understood.

2.3.3 Influence of materials at the fat-serum interface on the emulsion stability

(i) Phospholipids

Phospholipids comprise approximately 1% of the total lipid in bovine milk. Phospholipids are able to form stable colloidal suspensions or emulsions in aqueous solution and have an important role in the formation and secretion of milkfat (Jensen & Clark, 1988).

Lecithins are a group of phospholipids, ubiquitous in the cell membranes of plants and animals, in which phosphoric acid is joined through ester linkages to a diglyceride and to an alcohol-containing group such as the amino alcohols, or glycerol, depending on the source of the lecithin (Marrs *et al.*, 1989). The lecithins

are soluble in fat but almost completely insoluble in water. When mixed with water, lecithins hydrate and disperse in the form of spherical globules or *liposomes*, which may be $< 1\mu\text{m}$ in diameter. The principal components of soybean lecithin, the major lecithin of commercial interest, are phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine. Lecithin also occurs naturally in milk. Lecithin was used to provide a coating of an inert layer free of milk proteins over the newly created surface of homogenised milk fat (Lelievre *et al.*, 1990b).

Melsen & Walstra (1989) observed that when fat globules in recombined milk were associated with appreciable amount of phospholipid, they were distinctly less stable. They were of the opinion that phospholipid will probably adsorb at the oil-water interface (thereby displacing proteins), considering the low interfacial tension they may cause. However, an appreciable amount of phospholipids would be needed at the surface layers of fat globules to displace the proteins. If enough serum proteins are present to cover newly-created surface, the phospholipid present in buttermilk does not adsorb onto the fat-serum interface during or after homogenisation, and when sufficient lecithin is added to the fat before homogenising, very unstable emulsions result (Melsen, 1989).

(ii) Caseins

Caseins, the important structural and emulsifying proteins in cheese, usually contain calcium phosphate groups carrying essentially all the protein charge at one end which is water-soluble or hydrophilic, while the other end is organic and non-polar in nature and is soluble in oil or lipophilic (Shimp, 1985). Compared to many other food proteins, the caseins are disordered and substantially hydrophobic (Dickinson *et al.*, 1988).

Sodium caseinate consists of the casein fraction of milk which has been precipitated by acid at pH 4.6, collected, redissolved by the addition of alkali to neutral pH, and then spray- or roller-dried (Dagleish & Law, 1988). Although sodium caseinate is composed mainly of a mixture of α_{s1} -casein and β -casein, it behaves rather differently at the oil-water interface than a simple mixture of these two individual caseins (Dickinson *et al.*, 1989a).

In oil-in-water emulsions stabilised by sodium caseinate (at concentrations of 1.8 to 5.0%, and protein to fat ratios of 0.022 to 0.07, respectively) there was no distinct preference for either α_{s1} -casein or β -casein to be adsorbed at the fat globule surface during homogenisation. On aging for 24 h β -casein replaced some but not all of the surface α_{s1} -casein. Complete replacement was never observed, indicating that some α_{s1} -casein may have been irreversibly adsorbed (Robson & Dalgleish, 1987).

In studying the competitive adsorption of α_{s1} -casein and β -casein in oil-in-water emulsions, Dickinson *et al.* (1988) detected that the ratio of individual casein components to one another in the proteinaceous membrane of homogenised milk is different to that found in skim milk. In particular, β -casein is generally found in larger proportions in the membrane than in the serum.

(iii) Whey proteins

Emmons *et al.* (1980) observed that when fat was homogenised into reconstituted dried whey, the fat globules became stabilised in the absence of casein, apparently by adsorbing whey proteins. Dispersion of fat into whey rather than skim milk in milk replacers yielded a stronger curd and more syneresis during cheesemaking.

Whey proteins are among the most water-soluble of all proteins. They tend to remain in the water phase of the cheese and not concentrate at water-fat interfaces. For this reason their emulsification power is poor. However, whey proteins are capable of complexing with casein under certain circumstances (Shimp, 1985).

The ability of one whey protein to replace another whey protein previously adsorbed at the oil-water interface is slow and limited in contrast to rapid and reversible exchange characteristics of caseins (Dickinson *et al.*, 1989b). Of the two whey proteins, β -lactoglobulin is much more difficult to displace. β -lactoglobulin, which has a more ordered globular structure and is able to form a tightly-packed viscoelastic structure at the oil-water interface, is more effective in relation to the long-term stability of the emulsion (Dickinson *et al.*, 1989a).

(iv) Competitive adsorption between caseins and whey proteins

When caseins and undenatured whey proteins are adsorbed to the fat-serum interface during homogenisation, the caseins are the more dominant group, but with no apparent preference for any individual protein. After subsequent pasteurisation, and on storage, whey proteins become tightly bound and are no longer readily removed by washing (Darling & Butcher, 1978).

While complete dominance of one protein over another does not occur on the fat globule membrane during the formation of an oil-in-water type emulsion, caseins generally tend to adsorb in preference to the whey proteins, and β -casein in preference to the other caseins (Dickinson *et al.*, 1989a). Whey protein does not displace β -casein from the emulsion droplet (Dickinson *et al.*, 1989b).

(v) Replacement of proteins at fat-serum interface by surfactants

Feijter *et al.* (1987) showed that in 50% oil-in-water emulsions, displacement of β -lactoglobulin and β -casein from the droplet surface by water- and oil-soluble surfactants could occur, either partly or completely, depending on the surfactant concentration and type. When water-soluble surfactants are used, binding occurs to protein in the bulk solution. However, binding as such cannot explain the displacement behaviour.

The presence of surfactants tends to lower the mass of protein adsorbed per unit surface area of fat globule membrane. Addition of sweet-cream buttermilk, monoglycerides or Tweens to milk or cream before homogenisation can thus replace the proteins at the interface considerably (Walstra & Jenness, 1984).

2.4 Incorporation of whey protein in cheese

2.4.1 Introduction: Incorporation of whey protein into cheese offers the advantages of increased yield, increased nutritional value of cheese and reduced amounts of whey solids for disposal. The water-holding capacity of whey protein; particularly heat-denatured whey protein, which has a higher water-holding capacity than casein (Maubois, 1987) is of particular significance in the present study. The following

review includes aspects related to various methods of incorporating whey protein into cheese with particular reference to the water-holding ability of cheese.

2.4.2 Incorporation of native whey protein in cheese by ultrafiltration (UF)

It is reported that the form in which the whey protein is incorporated into the UF cheese *i.e.* denatured or undenatured, may be important, both in terms of proteolysis and water-binding (Lawrence, 1989). Aspects of the incorporation of native whey protein into cheese, particularly by ultrafiltration, have been extensively reviewed by Lelievre & Lawrence (1988) and Lawrence (1989).

In the manufacture of cheese from UF concentrated milk, a reduced amount of whey is drained in comparison to the conventional process. This results in more whey protein being retained in the finished product, thus increasing the yield of cheese for a given volume of milk. Based on these principles, a continuous method of manufacture of Cheddar cheese with a claimed increase of 6-8% cheese yield, the "APV-SiroCurd process", has been developed (Jameson, 1987). Another method of incorporation of whey protein is to concentrate whey by ultrafiltration and add the retentate to the cheesemilk (Abrahamsen, 1979). In both the methods some denaturation of whey protein occurs during ultrafiltration (Lawrence, 1989), the degree of denaturation depending upon the extent of ultrafiltration and whether diafiltration is also being done (Lelievre & Lawrence, 1988).

Sutherland & Jameson (1981) concluded from laboratory-scale trials that Cheddar cheese comparable to conventional Cheddar could be manufactured from milk concentrated by ultrafiltration up to 4.8-fold and diafiltered at pH 6.2-6.4 to produce a retentate containing 3.3% lactose.

Abrahamsen (1979) reported an increase in yield and moisture content in St Paulin and Gouda cheeses made from milk fortified with different amounts of whey protein obtained by UF concentration. The soluble and amino nitrogen in cheeses with whey protein concentrate was lower during ripening, indicating a slower breakdown of the proteins. The quality of the cheeses was inferior.

Mozzarella cheese made from milk concentrated by ultrafiltration did not hold firm after grinding and seepage of serum occurred. The poor quality of the cheese was attributed to the presence of additional whey protein (Hansen, 1987). A Mozzarella-type cheese was made using retentates of 40% total solids (TS) from ultrafiltered and diafiltered milk (Covacevich, 1981). This milk was chemically acidified and salt was added to milk prior to or during diafiltration. The cheese had excellent stretch and melt properties. However, there was some exudation during ripening.

Considerable success has been attained in incorporating whey protein in soft or semi-soft cheeses using milk concentrated by ultrafiltration close to the level of total solids of the final product. Usually whey is not drained during manufacture which ensures retention of the whey protein in cheese. This process has been used in the manufacture of soft ripened cheeses (Furtado & Partridge, 1988) and in Cast Feta cheese. The presence of native whey protein in UF Cast Feta makes the texture of the cheese different from the traditional product (Lelievre & Lawrence, 1988). Increase in the yield of such cheeses up to 30% have been claimed (Hansen, 1977), but Lawrence (1989) was sceptical of the claimed yield increases, particularly in view of the possible losses that would occur from exudation during brining and storage.

Quarg, Ricotta and Cream cheeses may be manufactured using ultrafiltration technology, and the quality of such cheese is comparable to that of the traditional products. However, the economic advantages of the UF process in incorporating whey protein may be limited as considerable amount of whey protein is already retained in cheese made by the traditional processes where the heat applied is usually sufficient to denature the whey protein (Lelievre & Lawrence, 1988).

2.4.3 Incorporation of denatured whey protein in cheese by heat treatment of cheesemilk

Cheese made from pasteurised milk contains only a small fraction of whey protein, most of which is lost in the whey. Efforts to retain whey protein in cheese by treating milk at high temperatures have not been successful because of difficulties

in coagulating such milks with rennet. Hoodyonk *et al.* (1987) concluded that the poor rennetability of heated milk was related to the impaired aggregation properties of part of the rennet-converted casein micelles covered with denatured whey proteins and the diminished attraction between these proteins due to precipitation of calcium phosphate during heating. The retardation of rennet enzymatic activity in heated milks was attributed to the denaturation of β -lactoglobulin and its interaction with κ -casein. Complete denaturation of β -lactoglobulin caused a reduction of about 20% in the rate of κ -casein breakdown. Lowering of the pH was suggested as the most effective way to improve the renneting properties of heated milk.

Marshall (1986) reported that heating milk to 97°C/15 s did not inhibit enzyme action of rennet, but additional Ca^{2+} and initial pH of 6.4 were required for normal coagulation and curd-firming. Cheshire cheese made from this milk tended to be too moist with poor curd fusion, but raising the scalding temperatures and cheddaring the curds could possibly overcome this. An increase in yield was recorded. The process was recommended as being suitable for cheese with high moisture content and crumbly texture.

Banks *et al.*, (1987) manufactured Cheddar cheese from extensively heat treated milk (110°C/60 s). The rennet coagulability of the heated milk was improved by acidification of milk to pH 5.8 prior to starter addition and renneting. They claimed that improvement in cheese yield of the order of 10% (3.0 to 6.7% on a dry solid basis) could be achieved. A bitter off-flavour and a weak-bodied product resulted. The bitterness in cheese was attributed to the acidification process rather than to the incorporation of the whey protein. Banks (1988) further optimised the above process and eliminated the bitter flavour by using a reduced amount of rennet. However, the development of high quality Cheddar flavour was impaired. The yield increase resulted from a slight advantage in terms of fat retention and marked increase in protein retention in cheese. The increase in fat recovery was attributed to an increase in protein to fat ratio, and the increase in protein recovery to complex formation between β -lactoglobulin and κ -casein.

The adverse effects of severe heat treatment of milk (95°C for 1 min) on rennet coagulation may be reduced by the addition of low concentrations of CaCl_2 , acidification of the milk to pH 5.5 and reneutralisation to pH 6.0 (Singh *et al.*, 1988). It is suggested that the acidification of heated milks increases the Ca^{2+} and the subsequent neutralisation of the acidified milk partly restores the Ca^{2+} , *i.e.* acidified/reneutralised milk has a higher Ca^{2+} than normal milk. This accounts for the improvement in the coagulability of the milk. It is further suggested that this treatment of heated milks could be used to incorporate 50% of the whey protein into renneted gels and thereby increase the cheese yield.

In a process described by Cercetare (1983) for manufacture of fresh cheese from cow's milk 56-63% of the whey protein were retained in the cheese by coprecipitating casein and whey protein. It is claimed that the product has a better consistency and flavour in comparison to the conventional product.

2.4.4 Incorporation of whey protein in cheese by addition of heat-denatured whey protein to cheesemilk

The incorporation of heat-denatured whey protein into soft and semi-soft cheese in order to increase the water-holding capacity is a logical extension from the manufacture of yoghurt and traditional Cream cheese (Lawrence, 1989).

The *Centriwhey* process for recovering whey proteins and incorporating them into cheesemilk (Walker, 1970) involves the heating and acidification of whey to coagulate the proteins, and their concentration by centrifugation. Normal cheese whey is cooled to 8°C to stop acidification, neutralised to pH 6.25, heated at 96°C for 20 min, acidified to the iso-electric point, held for 1 min, cooled to 40°C, centrifuged in de-sludging separator and finally cooled for storage. The method has been used successfully in the manufacture of soft and semi-hard cheeses with the addition of up to 2.8 g dry matter per litre of milk. It is recommended that fat be added to maintain the desired protein to fat ratio in the cheese, and that slight modifications with the manufacturing method be done with the aim of making a slightly drier product. Another very similar process has been described by Genvrain (1968).

Advantages claimed for the Centriwhey process include the following: increased cheese yield due to the incorporation of albumin and globulin which are otherwise lost in the whey, greater uniformity of the weight of the cheeses, quicker ripening, suitability of the protein-free whey for lactose manufacture, and reduction in the biological oxygen demand of the whey for disposal as an effluent (Anon., 1969a).

Reported levels of incorporation of heat-denatured whey protein (on dry matter basis) varied from 3.0 to 6.0 g/kg cheesemilk (Kononova *et al.*, 1973; Krashenin *et al.*, 1974; Sakharov *et al.*, 1975). Addition of heat-denatured whey protein was reported to increase the retention of free and bound moisture in cheese considerably, due primarily to the greater hydrophilicity of these proteins compared to casein (Krashenin *et al.*, 1974). An improvement in the dispersion of the denatured whey protein in cheesemilk by homogenising at a pressure at 15,000 kPa resulted in reduced loss of whey protein and a slight increase in yield. Homogenisation of milk with added whey protein reduced hardness, and improved consistency and porosity of cheeses (Kononova *et al.*, 1973).

Brown & Ernstrom (1982) made Cheddar cheese by incorporating whey solids, obtained by ultrafiltering cheese whey to values from 9.8 to 20.3% solids and heat-denaturing at 75°C for 30 min. The acid production by starters was faster during cheese manufacture. The rennet coagulation was also faster apparently due to presence of residual enzyme from ultrafiltered whey. The cheese had a decreased fat content, decreased pH, increased moisture and increased yield. The cheese was comparable to the control cheese except for the 'acid' flavour defect. The lower pH of cheese was attributed to accelerated growth of starter organisms in the experimental cheese, possibly due to high free nitrogen and lactose obtained from the heated whey concentrate and due to the high moisture in cheese.

Pang (1989) reported similar trials involving manufacture of a Cheddar cheese base from milk into which denatured whey protein concentrate was incorporated. He was not able to obtain a consistent increase in yield. However, the cheese base was successfully used to manufacture processed cheese.

2.4.5 Role of whey proteins in proteolysis of cheese

Native whey proteins: Koning *et al.* (1981) stated that undenatured whey proteins in low fat semi-hard UF cheese form about 18.5% of the total protein. The whey proteins themselves were completely resistant to the proteolytic enzymes of rennet and starter during ripening. Proteolytic degradation of α_{s1} - and β -caseins was similar in cheeses with and without the undenatured whey proteins. In contrast, however, Harper *et al.* (1989) showed that the native whey proteins decreased the rate of α_{s1} -casein proteolysis in Cheddar cheese slurries, probably due to chymosin inhibition. β -casein proteolysis was also inhibited by native proteins, possibly due to suppression of both plasmin and chymosin activity. Further, in five-fold concentrated UF cheese, reductions were found in the activity of plasmin and in the hydrolysis of β -casein, α_{s1} - and α_{s1} -I-casein (Hansen, 1990).

Denatured whey proteins: The hydrolysis of denatured whey proteins during cheese ripening has been reported to give rise to atypical flavour and texture (Green *et al.*, 1981; Brown & Ernstrom, 1982; Banks & Muir, 1985). A study on the effect of whey proteins on the proteolysis of Cheddar cheese slurries revealed that denatured whey proteins had little effect on α_{s1} -casein degradation (Harper *et al.*, 1989). However, β -casein proteolysis was inhibited by the denatured whey proteins. The inhibitory effect of a high molar mass protein present in whey protein concentrate, which inhibited the action of chymosin and retarded proteolysis in cheese made with incorporation of native whey protein, was found to be destroyed by the heat-denaturation of whey protein (Lelievre *et al.*, 1990a).

Overall, it appears that the presence of large amounts of whey protein in cheese tends to change its textural properties and also its ripening pattern. The extent to which this occurs is dependent upon the amount of whey protein incorporated and the state of denaturation of the whey protein (Lawrence, 1989). The effect of an increase in concentration of native or denatured whey proteins in cheese on the proteolysis is not fully understood. It has not yet been established whether the rate of proteolysis of casein in UF cheese is affected by the proportion of whey proteins present or the extent to which they have been denatured (Lawrence, 1989). More work is necessary to confirm these reports (Lelievre & Lawrence, 1988).

2.5 Cream Cheese

2.5.1 Introduction

Cream cheese is a soft, unripened, non-rennet cheese with a rich, mildly acid flavour and a smooth, buttery consistency which permits slicing with a knife without breaking or crumbling (Slyke & Price, 1949; Kosikowski, 1977) . It should have good spreading qualities even at refrigerator temperature (Wilster, 1969). Cream cheese is used for cheesecake, salads, dips, and as a sandwich spread.

2.5.2 Composition

A typical composition of commercial unripened Cream cheese (Kosikowski, 1977) is as follows: 33.5% fat, 54.0% water, 9.8% protein, 0.75% salt, and 0.3% gums.

To comply with federal standards in USA, Cream cheese should contain at least 33% fat, and not more than 55% moisture. It is not legal to use sorbates or propionates in hot-pack Cream cheese, though these are allowed in cold pack natural ripened cheese.

Cream cheese is described as 'hot-packed' or 'cold-packed' depending on whether or not the final blend of curd, salt, gums and condiments are heat treated prior to packaging. The hot-pack method is the main method in use these days for reasons of a much improved keeping quality (Kosikowski, 1977; Honer, 1988).

A common problem for manufacturers of Cream cheese and Cream cheese spread is syneresis (exudation) after packaging (Modler *et al.*, 1985). The addition of a hydrocolloid or stabiliser prevents syneresis during cooling and storage (Wilster, 1969) and is more commonly used in the hot-packed Cream cheese (Slyke & Price, 1949). However, in some countries, *e.g.* Federal Republic of Germany, addition of colloids or stabilisers in Cream cheese is not permitted (Holdt, 1971).

2.5.3 Manufacturing technique

The basic operations in the manufacture of Cream cheese (Zakariasen & Combs, 1941; Kosikowski, 1977; Scott, 1981; Honer, 1988) are quite similar in spite of individual variations used by different manufacturers (Honer, 1988).

Typically a mix (standardised milk of about 11% fat and 8% solids-not-fat) is blended from milk and cream (other mixes containing cream, whole milk, condensed skim milk, SMP may be used), homogenised, pasteurised and acidified by lactic starter to pH of about 4.6 to form a coagulum (curd). In the short-set method of manufacture, $\approx 5\%$ starter is inoculated into the standardised processed milk at $\approx 31^\circ\text{C}$. A small amount of rennet may be added just after the addition of starter at the rate of 1 ml/1,000 kg standardised milk to facilitate setting. Acid production is rapid and coagulation is completed in 5-6 h. In the long-set method, starter is inoculated to standardised milk at $\approx 0.5\%$ at $\approx 22^\circ\text{C}$, and it takes 14-16 h for the curd to set.

The curd is either drained overnight in bags, or separated from whey through a centrifugal separator. The drained curd is then heated to $\approx 75^\circ\text{C}$ in a kettle/tubular heater/scraped-surface heat exchanger or similar device. Salt, stabilisers and condiments *etc.* are blended into the curd and the product is then packed into foil-lined boxes or containers.

Some manufacturers homogenise the blended product at 2,500 to 4,000 psi (17,225 to 27,560 kPa) to minimise oiling-off in the product. Homogenisation of the final blend ensures effective dispersion of the additives and their uniform distribution into the hot mix. Electron microscope studies have shown that viable lactic bacteria were killed in a whey protein-cream mixture base for making Cream cheese spread, when heat treated in the processing kettle (Kalab & Modler, 1985). The heat-treatment of the blend makes the product *commercially sterile* and increases its shelf-life to ≥ 60 days with refrigerated storage. Product deterioration is thus mainly due to oxidative reactions and a low level of residual activity of heat-resistant enzymes.

Process control

The fat content of the final product affects its texture. At lower levels of fat the cheese tends to be grainy and crumbly, while at higher levels it tends to be excessively smooth and sticky (Roundy & Price, 1941).

The pH at which the curd is cooked is important as it affects the whey drainage. The optimum pH is 4.6 when a cooking temperature of 55 °C is used. At higher pH values and higher cooking temperatures the resulting curd tends to be dry, crumbly and rubbery; while at lower pH values and lower cooking temperature the curd is smooth, soft, sticky and undrainable (Lundstedt, 1954; Wilster, 1969). Curd cooked at a pH of 4.6 results in cheese with a pH of 4.7 to 4.8.

The drainage of whey from curd is slowed with increase in fat content in standardised milk, increase in pasteurisation temperature and homogenisation pressure, decrease in cooking temperature, and addition of salt and neutralisers before cooking (Lundstedt, 1954). In general, faster the drainage the coarser the cheese; and slower the drainage the smoother the texture of the cheese.

2.5.4 Modified methods

(i) Cream cheese without whey drainage

The elimination of the curd-draining step in the manufacture of Cream cheese was introduced over 60 years ago by Dahlberg (1927). In this a high solids mixture of fresh cream (40 to 50% fat), skim milk powder (5% of the mixture) and gelatin (1%) or agar (0.5%) was pasteurised. Salt and commercial starter were added to the mixture. The mix was then homogenised, cooled to 21 °C and held for 10 to 15 h for the acid to develop, and then stored at refrigerated temperature. The effect of homogenisation in changing the fat from a dispersed to a continuous phase improved the body and texture of the cheese. Reducing the fat content resulted in some whey leakage. As this cheese was not hot-packed, the residual microflora would be expected to be active to some extent which would increase acidity and proteolyse caseins during refrigerated storage. More recently, somewhat similar procedures have been developed (Forman *et al.*, 1979; Davis, 1980).

Shchedushnov *et al.* (1978) developed a new Cream cheese by mixing homogenised cream and condensed whey, heating to 90-95 °C for 5-10 min and cooling. To this skim milk curd is added and the mix passed through a colloid mill, packaged and cold-stored.

Application of ultrafiltration: Protein-rich retentates from ultrafiltered milk have been used for manufacture of Cream cheese (Kosikowski, 1974; Maubois, 1980). The retentate is mixed with suitable proportions of cream, salt, gums and starter. The mix is then pasteurised, homogenised and hot-packed into containers. A similar method has been formulated with an exception in the use of glucono- δ -lactone for acidification instead of starters (Hansen, 1985).

(ii) Reduction in the rate of exudation from Cream cheese

Use of whey protein-rich protein base: Cream-type cheeses were prepared from blends of whey protein-rich protein bases and high-fat sour cream (Modler *et al.*, 1985). The blends were standardised with buttermilk, pasteurised, homogenised and hot-packed. The cheeses showed no evidence of syneresis (exudation) during storage presumably due to the presence of large amounts of whey protein.

Direct acidification method: Roundy (1960) described a rapid process of making Cream cheese by the direct acidification of a mixture of milk and cream with edible acid to pH 4.6-4.7 at 10-50 °C followed by centrifugal separation for removal of whey. The method is claimed to produce a Cream cheese that has little or no tendency to leak whey during storage. Corbin (1971) devised a process of direct acidification by using phosphoric acid and D-glucono- δ -lactone.

Blending of curds: Carswell & Hurlburt (1970) described a process of preparing Cream cheese with no non-dairy stabilisers, the cheese exhibiting minimal amount of whey separation during storage. This process involves preparing a Cream cheese mix (sour curd) to a predetermined acidity (say 0.85%) and adding a substantially unripened second Cream cheese mix (sweet curd) to reduce the acidity of the mixture to 0.7%. The mixture is then separated to Cream cheese and whey according to the conventional process.

2.6 Feta cheese

2.6.1 Introduction

Feta cheese is one of the family of white brined cheese that originated in the eastern Mediterranean countries (Caric, 1987). While Feta was originally manufactured from sheep's or goat's milk, it is nowadays also made from cow's milk (Lloyd & Ramshaw, 1979; Scott, 1981; Caric, 1987; Abd El-Salam, 1987). These days Feta cheese is made in several other countries.

Feta is a soft, high salt, low pH, rennet coagulated, non-pressed, brine-stored cheese. Specific characteristics of Feta vary widely depending on the milk used and the methods employed in its manufacture and ripening.

2.6.2 Flavour: The flavour of Feta may vary from mild aromatic to strongly lipolytic. Lipase is commonly used in manufacture, especially when pasteurised cow's milk is used. The cheese is frequently very salty from storage in brine, although excess salt is sometimes removed by soaking cheese portions in fresh water prior to consumption.

A wide range of fatty acids and their derivatives have been reported (Efthymiou, 1967; Ada, 1987) that contribute to the flavour and aroma of Feta cheese. Acetic acid is the most abundant acid present in Feta cheese. The presence of fatty acids and their derivatives reflect the lipolytic activity of added lipase or the lipase originally present in milk, and the activity of starter and non-starter microorganisms. Aspects related to lipolytic activity in cheese are not being reviewed here as it was not expected to influence exudation.

2.6.3 Texture: The texture of Feta cheese may vary from soft and crumbly, to smooth and sliceable (Kosikowski, 1977; Pernodet, 1987; Efthymiou & Mattick, 1964). Mechanical openness in the curd structure is typical of traditional Feta (Scott, 1981) but Cast Feta made by the UF process usually has a continuous porcelain-like texture devoid of mechanical openness.

2.6.4 Colour: Feta made from sheep's or goat's milk is very pale, almost white, whereas when made from cow's milk it has a yellow colour due to carotene that is considered undesirable by most consumers of the traditional product.

2.6.5 Composition: The composition of Feta cheese varies widely according to the milk type and composition, methods of manufacture, period of storage in brine, and other factors. The composition of best quality Feta cheese in Greece made from sheep's or goat's milk has been reported as moisture $\leq 52.5\%$ and fat $\geq 22\%$. Lower quality cheeses had higher moisture and lower fat contents (Anifantakis, 1986). This is in good agreement with the observations of Siapentas (1981) on Feta cheese made from cow's milk, the approximate composition from the best cheeses being 50% moisture, 25% fat, 15% protein and 3% salt.

2.6.6 Manufacturing techniques

In a typical procedure for the manufacture of Feta cheese (Anon., 1969b; Mehran & Kosikowski, 1972; Lloyd & Ramshaw, 1979; Scott, 1981; Anifantakis, 1986; Abd El-Salam, 1987), fresh whole milk is inoculated with $\approx 2\%$ starter at about 32°C and ripened for ≈ 2 h. Rennet and lipase are added, and after 45-90 min setting time the coagulum is cut into ≈ 2 cm cubes. The curd is held in the whey with intermittent stirring for up to 60 min, ladled into hoops and drained for ≈ 18 h (overnight) with successive turns in the first few hours. The young cheese may be either dry-salted by application of crystalline salt to the exterior of the cheese or brine-salted (23% brine) before final storage in $\approx 10\%$ brine at $5-10^\circ\text{C}$. Mansour & Alais (1972) showed that the Cryovac process of vacuum wrapping the Feta cheese after brining was an useful method for storage and ripening. The cheese is usually ripened for about 2 months before being ready for consumption. In earlier days pasteurisation of milk was not commonly practised. However, most manufacturers now pasteurise the milk for reasons of public health and uniform product quality. Homogenisation may form a part of the cheese manufacturing process. Bleaching agents may be added, particularly when cow's milk is used.

Feta cheese of good flavour, colour and body can be manufactured from a 'recombined milk' prepared from low heat skim milk powder (LHSMP) and

anhydrous milk fat (Gilles, 1974). Reconstituted skim milk (RSM) is prepared by mixing suitable proportions of LHSMP and water. Anhydrous milk fat (AMF) and LHSMP are used for preparation of reconstituted milk of 11 % solids-not-fat (SNF) and 3% fat. AMF is emulsified with RSM as a 20% cream by two-stage homogenisation at 200/50 kg/cm² (19,581/4,892 kPa). The uniform composition of this milk ensures that a cheese of consistent composition is obtained. CaCl₂ (0.04%) is added to reconstituted skim milk to get a firm curd. Commercial lipase is added to obtain a more acceptable product. The most acceptable cheese was made with the use of a starter comprising *Streptococcus lactis* and *Lactobacillus casei* or *Lactobacillus acidophilus*, the addition of commercial lipase, and the addition of CaCl₂ (0.04%) to favour the formation of a firm coagulum.

Feta cheese made by the application of ultrafiltration technique differs greatly in body and texture from the traditional variety due to incorporation of whey protein, and has not been included in this review.

2.6.7 Changes in brine-stored Feta cheese during Storage

(i) Loss and uptake of moisture, and change in weight of cheese

Because of the application of salt to the exterior of the cheese, either by dry or by brine-salting, some time is required for the salt to penetrate to the interior of the cheese and establish an equilibrium. This early imbalance in salt concentration between the outside and the interior of the cheese allows more acid development to occur in the interior of the cheese. This can lead to a rapid exudation of moisture from the young cheese and consequently a lower moisture content. However, the moisture loss that occurs in the early stages of salting and curing of Feta cheese is partly counterbalanced by an uptake of moisture from the brine after 10-30 days (Mansour & Alais, 1972; Alichanidis *et al.*, 1984; Vafopoulou *et al.*, 1989). The reabsorption of moisture has been attributed to the re-establishment of osmotic equilibrium (Mansour & Alais, 1972) and formation of new ionic groups due to peptide bond cleavage (Vafopoulou *et al.*, 1989). This phenomenon of initial moisture loss and subsequent rehydration has been observed particularly when the NaCl concentration in the brine was $\approx$ 10%. However, cheese stored in brine of

18% NaCl concentration lost moisture when stored at 5-15 °C and never regained weight (Mansour & Alais, 1972).

Omar & Buchheim (1983) showed a reduction in moisture content from 58.2% to 52.3% in case of Feta made from fresh milk in comparison to a reduction from 53.2 to 50.2% in cheese made from instant whole milk powder.

The loss of moisture from Feta-type cheese vacuum packed in Cryovac was only 2.4% in 120 d with a weight loss of 1.35%. There was slight exudation of whey between the cheese and the plastic film. Likewise, Domiati cheeses showed less weight loss when not stored in brine compared to when stored in 5% NaCl brine or 5% salt permeate (Abd El-Salam, 1981).

(ii) Change in lactose content and acidity of cheese: The conflicting reports on the changes of acidity in Feta-type cheeses may reflect the variety of conditions used in the manufacturing process.

Mansour & Alais (1972) reported a decrease of pH during storage until 30 d, after which pH started to rise again. According to Omar & Buchheim (1983) lactose in Feta-type cheese disappeared within 1 month. Thomas & Crow (1983) reported that 90% of the lactic acid present in commercial Feta was present as the D-isomer. This would indicate that the activity of non-starter lactic acid bacteria, salt-tolerant lactobacilli in particular, could be significant in completing the utilisation of any residual lactose present in the cheese, or in the racemisation of the L-isomer of lactic acid to the D-isomer.

In cheese stored traditionally in brine, some lactose and lactic acid may be expected to diffuse out of the young cheese into the brine, and thus limit the tendency for the acidity of cheese to increase during storage. In contrast, in Feta-type cheese stored in a Cryovac pack, acidity increased until 30 d and then remained stable. The lactic acid concentration in this cheese was three times higher than in brine-salted cheese (Mansour & Alais, 1972). This result may be

explained by the increased activity of microorganisms in the relatively low-salt cheese and the absence of any loss of acid into a brine.

The concentration of the salt in the cheeses, or the salt concentration of the brine in which the cheeses are stored, would be expected to have a marked effect on microbial activity during cheese storage.

(iii) Proteolytic changes

Continuous proteolysis during storage of brine-stored cheeses is indicated by the gradual decrease in total nitrogen (TN) and increase in the soluble nitrogen (Abd El-Salam, 1987). The decrease in total nitrogen is attributed to the conversion of proteins into the soluble fraction during proteolysis and the subsequent transfer of the soluble fraction into the brine. The proteins are hydrolysed by milk-clotting enzymes used during cheese manufacture and the protein breakdown products are further proteolysed to the soluble nitrogen by the cheese microflora.

In general, the proteolytic activity of rennet during ripening of cheese is markedly affected by the salt concentration (Fox & Walley, 1971). This could explain the resistance of para- κ -casein to hydrolysis throughout the storage period in Syrian white brine-stored Feta-type cheese from cow's milk (Mansour & Alais, 1972).

A number of fast- and slow-moving degradation products were apparent in the electrophoretogram of proteins from brine-stored cheeses. The breakdown products with mobilities higher than α_{s1} -casein were comparable to those formed by the action of chymosin on α_{s1} -casein, while the slow-moving fractions corresponded to the gamma-caseins produced from β -casein by the action of the indigenous milk proteinase, plasmin (Eigel, 1977; Abd El-Salam, 1987).

β -casein in Feta-type cheese from ewe's milk and cow's milk resists hydrolysis whereas α_{s1} -casein hydrolyses rapidly (Mansour & Alais, 1972; Alichanidis *et al.*, 1984). There was a loss of low molecular weight nitrogen (peptides, amino acids, ammonia) into brine during storage (Mansour & Alais, 1972). The rate of proteolysis that occurred in cheese stored at 10 °C in 15% brine was similar to that

observed in cheese stored at 20°C in 18% brine. Proteolysis of the cheese was almost completely inhibited at 5°C.

The rapid proteolysis detected in Cryovac wrapped Feta-type cheese in comparison to brine-stored cheese may have been due to the lower salt concentration in the former which had been brined for only 6 h in 12% NaCl (Mansoor & Alais, 1972).

(iv) Texture changes

Partial loss of Ca^{2+} bridges in the cheese matrix as a consequence of lowering of cheese pH and other soluble protein breakdown products into the brine (Mansour & Alais, 1972), and the continuous proteolysis of α_{s1} -casein lead to a change in the protein matrix and provide a smooth body to the ripened cheese (Abd El-Salam, 1987). When the α_{s1} -casein molecules are cleaved so that they lose their ability to act as a link in the protein network (Lin *et al.*, 1972), then the network disintegrates. This cleavage of α_{s1} -casein in brine-stored cheeses causes a loose structure, *i.e.* a crumbly texture.

2.7 Salt diffusion

2.7.1 Introduction: In cheeses which are brined, or salted by application of dry salt to the exterior surface, salt diffuses from the exterior to the interior over a period of time. In soft, brined cheeses, salt diffusion affects the growth of acid-producing lactic acid bacteria which in turn influences the cheese pH (Noomen, 1977). It is reasonable that salt diffusion, which affects microbial activity, cheese pH and consequently proteolysis, may also influence the exudation from cheese. It was therefore considered useful to review the aspects related to salt diffusion.

2.7.2 Theories on salt diffusion

Geurts *et al.* (1974b) described the uptake of salt and concurrent loss of moisture during brining of cheese as an impeded mutual diffusion process. Water is not displaced because of independent shrinkage of the matrix. A reduction in the volume of the cheese follows from the net transport of water and salt. Mansour

& Alais (1972) suggested that the osmotic equilibrium plays a major role in the exchange of the moisture and salt between the cheese and the brine.

Romano cheese is a hard, low moisture, heavily salted (8 to 14% salt-in-moisture concentration) cheese. The cheese is salted by immersion in brine as well as by surface application of dry salt. Therefore, a large salt-in-moisture concentration gradient occurs within a block of cheese and at different stages of ripening (Guinee & Fox, 1983). It is suggested that the transport of salt in Romano cheeses occurs by mutual diffusion process - water is lost by a combination of mutual diffusion and osmosis, the osmotic level being more pronounced as the surface to volume ratio of the cheese block increases, due to the higher salt uptake (Guinee & Fox, 1986a).

Geurts *et al.* (1980) postulated that the actual quantity of salt which passes through a flat cheese surface is proportional to the square root of the duration of the salting and the moisture content of the cheese. They stated that, if the salt uptake was known, it is possible to predict total salt uptake in spherical cheeses, the weight loss of the freshly salted cheese and the final moisture content of the brined cheese.

2.7.3 Factors affecting salt diffusion

Salt diffusion in cheese is affected by ion exchange phenomenon, electrostatic forces (as a consequence of charged groups on the protein matrix) and contraction of the solution (Geurts *et al.*, 1974b).

Cheese geometry: Cheese shape and size influence the rate at which salt (and salt-in-moisture concentration) becomes uniformly distributed throughout the cheese (Geurts *et al.*, 1980; Guinea & Fox, 1986c). The time taken for attaining equilibration of salt-in-moisture concentration in cheese was 8-12 d for Camembert (Kieferle & Seyrer-Reindl, 1953), about 40 d for Greek white Feta (Georgakis, 1973) and 80-90 d for spherical Romano (Guinea & Fox, 1986c).

Fat: High-fat cheese loses moisture less readily than does low-fat cheese under all manufacturing conditions (Geurts *et al.*, 1974b). Effective salt mobility is reduced by fat, hence the water flux will decrease when the fat content increases. The

sieving effect of the protein matrix, and the obstructions of the fat globules and protein strands through which salt can not penetrate (which increase the real distance travelled by a salt molecule on proceeding from one parallel to another), reduce the apparent diffusion rate relative to that in the pure water (Guinee & Fox, 1987).

Calcium in brine: Cheese takes up water and swells in the absence of calcium in brine solutions of low salt concentration (Geurts *et al.*, 1972). The use of CaCl_2 in brine at a level similar to that already present in cheese is therefore recommended.

Moisture content of cheese: The cheese takes up more salt when its moisture content is higher (Geurts *et al.*, 1980).

Duration of brining: The duration of brining greatly influences the salt uptake, the water content and the weight loss from cheese (Geurts *et al.*, 1980; Hardy, 1987).

Temperature of brining: An increase in the temperature of brining in Feta-type cheese increased moisture loss from the cheese and decreased the diffusion of salt into the cheese (Mansour & Alais, 1972). However, Geurts *et al.* (1974b) were of the opinion that salt uptake was not retarded or stopped during brining at a high temperatures although it may be affected at extremely low temperatures.

Storage temperature: At higher storage temperatures the time required for salt-in-moisture concentration to equilibrate in cheese after salting is expected to be less than the lower temperatures (Guinee & Fox, 1987).

Salt concentration gradient: The application of dry salt to the exterior surface of a cheese or the use of concentrated brines ($\approx 23\%$ salt) would result in a higher salt concentration gradient between exterior and interior than if lower strength brines were used. While the salt concentration gradient influences the rate of absorption of salt by a cheese during salting, it does not however affect the mobilities of the diffusing species except during brining in supersaturated salt solutions (Geurts *et al.*, 1974b; Guinea & Fox, 1987).

pH: Geurts *et al.* (1980) found that reducing the brine pH decreased the salt uptake and increased the moisture loss from Gouda cheese. This was attributed to the precipitation of casein in the outer layer of the cheese at pH values close to the iso-electric point, and consequently the formation of a hard low-moisture rind. When Camembert cheese was brined in brines at pH values from 1.5 to 8.3, the salt absorption was unaffected. The acid brines tended to reduce the moisture content of the cheese more than neutral or alkaline brines (Hardy & Weber, 1978).

2.7.4 Influence of salt on ripening of cheese

Salt has a major effect on the proteolytic activity of residual rennet and the varied activities of the microbial flora during the ripening of cheese. In one-month old Cheddar cheese containing 4% salt-in-moisture concentration, approximately 5% of the α_{s1} -casein and 50% of the β -casein remained unhydrolysed (Thomas & Pearce, 1981). In cheeses with 6% salt-in-moisture concentration, 30% of the α_{s1} -casein and 80% of the β -casein were unhydrolysed, while in cheeses with 8% salt-in-moisture concentration the corresponding figures were 60% and 95%. Proteolysis of β -casein by rennin was completely inhibited in the presence of 10% NaCl and was considerably reduced by 5% NaCl (Fox & Walley, 1971). The inhibitory effect of NaCl on the proteolysis of β -casein was independent of pH and incubation temperature. Rennin hydrolysates of β -casein were bitter in flavour whereas those of α_s -casein were not. The effectiveness of NaCl in controlling the development of bitter Cheddar cheese may be due to its relatively greater inhibitory effect on the proteolysis of β -casein than of α_{s1} -casein.

2.8 Changes in cheese during storage

2.8.1 Residual lactose, acidity and pH of cheese

The final stages of acid production in Cheddar cheese occur after the milled curd fingers have been salted. The rate at which this acid production occurs depends on the salt-in-moisture concentration. At high levels of salt-in-moisture concentration (6-7%) lactose persists in cheese for at least 6 months (Thomas & Pearce, 1981). At lower levels residual lactose is utilised with the formation of lactic acid. Consequently strong correlations are found between residual lactose

concentration and salt-in-moisture concentration, between cheese pH and salt-in-moisture concentration, and between residual lactose concentration and pH at 14 days after manufacture (Thomas & Pearce, 1981; Lawrence & Gilles, 1987).

The residual lactose, acidity and pH of cheese are interrelated, and may have a significant effect on the proteolysis and quality of all varieties of ripened cheeses.

2.8.2 Residual enzymes in cheese

To a very large extent the amount of residual rennet retained in cheese curd will determine the extent of proteolysis observed during ripening of cheese (Visser, 1977b; Koning *et al.*, 1981). The amount of residual calf rennet retained in the curd will be determined not only by the amount used initially to coagulate the milk, but also by the pH of the curd at draining, and other manufacturing conditions.

In Swiss cheese calf rennet is almost completely inactivated at the high cooking temperature of $\geq 50^{\circ}\text{C}$ used (Lawrence *et al.*, 1987), whereas in Cheddar ($\approx 38^{\circ}\text{C}$ cook) approximately 35% of the calf rennet activity is destroyed by the time the whey is drained (Holmes *et al.*, 1977). The distribution of calf rennet in cheese and whey is pH-dependent. In freshly-coagulated milk 31% of activity was retained in curd and 72% lost in the whey at pH 6.6; but 86% retained in the curd and 17% lost in the whey at pH 5.2 (Holmes *et al.*, 1977). The practical significance of this was demonstrated by Creamer *et al.* (1985) who showed that addition of calf rennet to milk of lower pH values resulted in Cheddar cheeses exhibiting more proteolysis, the low pH favouring the retention of chymosin and reducing denaturation of enzyme (Singh & Creamer, 1990). The age of cheese had no apparent effect on the activity of residual chymosin, suggesting that the chymosin is stable and active in cheese over a long period of ripening.

2.8.3 Calcium in cheese

The extent of acid production during cheese manufacture, especially before the curd is separated from the whey largely determines the calcium content in cheese (Lawrence & Gilles, 1987).

Cheddar cheese made from milk adjusted to a low pH value had lower concentrations of calcium and phosphate than cheese made from normal milk with a pH 6.6. Throughout maturation the texture of the cheeses made from the acidified milk was more crumbly, and less force was required to fracture the curd (Creamer *et al.*, 1985). The texture, however, seems to be related more to the differences in cheese pH than to the calcium content of the cheeses (Creamer *et al.*, 1988).

The extent to which the sub-microscopic structure of the casein micelles will be retained in the cheese in its original undisrupted form is largely determined by the loss of calcium phosphate. The quantity of micellar calcium phosphate lost is determined by the acidity developed before the whey is drained from the curd. In acid cheeses such as Feta, Cheshire, and the mould-ripened cheeses which have a relatively low mineral content, the casein sub-micellar units have been disrupted (Lawrence *et al.*, 1983).

In low pH cheeses the mineral level is low and does not provide much scope for variations in terms of calcium content in the final product. It needs to be established if this reduced level of calcium in low pH cheeses has any influence on the exudation of whey from cheese.

2.8.4 Proteolysis in cheese

This review summarises aspects of proteolysis, particularly related to soft and brine-salted (Gouda-type) cheese, which may be of special relevance to the present study. Proteolysis in cheese made with incorporation of whey protein and proteolysis in brine-stored Feta cheese have been described earlier in this Chapter. General aspects of proteolysis in cheese have recently been reviewed by Fox (1989).

The proteolytic enzymes potentially able to be involved in the ripening of cheese include rennet (chymosin), native milk protease (plasmin), and the enzymes of the starter and non-starter bacteria.

Chymosin and plasmin are responsible for specific proteolysis, while the bacterial and mould enzymes are responsible for non-specific proteolysis (Creamer, 1979).

In Gouda cheese rennet was shown to be responsible for the primary decomposition of α_{s1} -casein and part of β -casein, while starter bacteria degraded these proteins further during the long period of cheese ripening (Visser & Groot-Mostert, 1977).

The approximate proportions of α_{s1} -casein, α_{s2} -casein, β -casein and κ -casein in milk are 4:1:4:1. Presumably this ratio is carried over to freshly made cheese, except for κ -casein which is proteolysed during cheese manufacture. α_{s1} -casein is more easily broken down than β -casein by rennet in the ripening of most varieties of cheese (Mansour & Alais, 1972; Noomen, 1983; Fox, 1989). Hydrolysis of the most susceptible bonds in α_{s1} -casein ($\text{Phe}_{23}\text{-Phe}_{24}$ or $\text{Phe}_{24}\text{-Val}_{25}$) yields α_{s1} -I casein (Fox, 1989). The rate of breakdown of β -casein is reduced more than α_{s1} -casein due to an increase in salt concentration. This aspect has been described earlier in Chapter 2.6.7.

Starter enzymes are capable of degrading protein to small peptides and free amino acids (Noomen, 1983). Both α_{s1} - and β -caseins are broken down by starter enzymes. However, α_{s1} -casein is hydrolysed by the starter enzymes at a much slower rate than by chymosin. Free amino acids are generally not formed as a result of rennet action on caseins.

Visser (1977b) reported that rennet appeared to be responsible for the greater part of the soluble nitrogen production in Gouda cheese, with the starter bacteria and milk protease also making contributions though to a lesser extent. Milk protease on its own liberates amino acids and low molecular weight peptides but only in small amounts. The action of rennet clearly stimulates the formation of low molecular weight peptides and amino acids by the progressive degradation of the higher molecular weight products (of rennet action) by starter peptidases.

In Gouda-type cheeses the degradation of α_{s1} -casein is nearly complete in about a month's ripening; β -casein is more resistant, with about 50% of it still being intact after 6 months of ripening (Visser & Groot-Mostert, 1977). Rennet plays the major role in degrading α_{s1} -casein and decomposing β -casein in the first month of ripening and the enzymes of the starter bacteria contribute to further degrading

β -casein. Milk protease appeared to be responsible for the formation of the *minor caseins* from β -casein during the ripening of Gouda cheese.

The consequences of proteolysis in cheese include changes in cheese texture and the development of flavour. The texture of cheese is determined primarily by its pH and the ratio of casein to moisture (Lawrence *et al.*, 1987). The texture generally changes markedly in the first 1 to 2 weeks of ripening as rennet hydrolyses some of the α_{s1} -casein to the peptide α_{s1} -I-casein, causing a general weakening of the network (Jong, 1976). The relatively slow change in texture thereafter is determined mainly by the rate of proteolysis, which in turn is controlled largely by the proportion of residual rennet and plasmin in the cheese, salt-in-moisture concentration and storage temperature (Lawrence *et al.*, 1987). Cheese texture may be significantly changed by the use of coagulants other than calf rennet, addition of neutral proteases, and incorporation of whey protein.

2.8.5 Water activity (A_w) of cheese

The water activity of cheese is affected by the amount of free water and the soluble material present in cheese. In soft cheeses A_w is determined almost solely by the NaCl concentration in the aqueous phase in the cheese (Marcos & Esteban, 1982). The additional lowering of A_w in ripened cheeses below that corresponding to NaCl molality must be caused to a great extent by the aqueous concentration of other solutes such as low molecular weight non-protein nitrogen compounds released by proteolysis (Marcos *et al.*, 1981). A_w generally decreases with decreasing moisture in cheese. The A_w of soft cheeses with a moisture content > 40% can be predicted from the NaCl molality (M) in the total water of the product by the equation $A_w = 1 - 0.033 M$ (Marcos *et al.*, 1981).

The A_w of fresh curd is around 0.99 and is reduced by the addition of salt. During ripening of cheese there is a gradual fall in A_w due to evaporation, increased amounts low molecular compounds (peptides, amino acids, lactic acid *etc.*) resulting from proteolysis and possibly release of some bound salt (Kinsella & Fox, 1987). The amount of free water in cheese and the manner in which it is affected by the low molecular weight solutes, and the pH increase during ripening may influence the rate and extent of proteolysis (Ruegg & Blanc, 1977; Lawrence *et al.*, 1987).

2.8.6 Water-binding properties of proteins

The water sorption by milk proteins has been reviewed in detail by Kinsella & Fox (1987). The water associated with proteins has been categorised in terms of progressively increasing water activities, such as structural, monolayer, hydrophobic hydration, multilayer, unfreezable, capillary and hydrodynamic hydration water. Capillary water is defined as that water held physically in clefts, voids, or cavities by surface and capillary forces in the protein molecule at A_w 0.5 - 0.95, *e.g.* water entrapped in gels. It is similar to bulk water in physical properties, and is available as a solvent and for chemical reactions. At high $A_w > 0.9$, the amount of water bound ranges from 30 to 60 g water per 100 g protein.

Several types of interactions between water and food solids may occur at the molecular level. These include coulombic interactions between charged groups and bound ions, hydrogen bonding with polar groups, London-van der Waals forces, steric effects, solution effects, capillary condensation, plasticising of molecular structure and multiple effects between various components (Berg & Bruin, 1981). Water-protein interactions are affected by water content, amino acid composition, surface polarity or charge, conformation and topography of the protein, pH, ion species and temperature (Kinsella & Fox, 1987). Ionised amino acids bind 2 to 3 times more water than non-ionised groups.

Interaction of NaCl with paracasein was detected in the A_w range of 0.76 - 0.95 (Hardy & Steinberg, 1984). The amount of interacting salt increased with added salt, decreasing A_w and moisture content. The binding of salt to the protein may have reduced the net water sorption capacity of the protein by displacing water, inducing some conformational changes, limited solubilisation or more likely by reducing the effective concentration of salt *per se* thereby reducing the amount of water bound by salt (Kinsella & Fox, 1987).

Water vapour sorption by whole casein and micellar casein was measured in the water activity range of 0.2 to 0.98 (Ruegg & Blanc, 1976). At high water activities, whole casein revealed minimum hydration near its isoelectric point (approximately pH 4.6). Water sorption by micellar casein increased on either side of a pH close

to that of original milk serum. It is suggested that acidification during lactic acid fermentation decreases the water activity in milk products significantly.

Pure casein or paracasein presumably binds (by chemi-sorption and ice-structuring) about 0.55 g water/g protein (Geurts *et al.*, 1974a). In milk products the bound water is much less. In cheese, it is 0.10 to 0.15 g/g, which is hardly affected by pH and salt content. In milk, the amount of bound water may be slightly higher than cheese, but the bound water is not significantly affected by renneting or acidification.

During proteolysis in cheese greater water-binding properties are exhibited by the newly formed amino and carboxyl groups (Fox, 1989). As each peptide is cleaved during ripening of Cheddar cheese, two new ionic groups are generated and each will compete for the available water in the system (Creamer & Olson, 1982). Thus the water previously available for solvation of the protein chains will become tied up with new ionic groups making the cheese harder and less easily deformed.

The following relationship between water-holding ability of the protein gel matrix in cheese, and exudation, appears probable. The water-holding ability of the gel matrix is influenced by the composition of cheese and other forces described earlier in this section. For a given set of conditions and composition of cheese, the gel matrix will have the ability to hold a certain maximum amount of water. When the moisture present in cheese is lower than the water-holding ability of the gel matrix, as may be the case in hard varieties of cheese, exudation is unlikely, even when the matrix is affected by changes that occur during storage. On the other extreme, when the available moisture in cheese is in excess of the water-holding ability of the gel matrix, *e.g.* Cottage cheese, exudation invariably occurs. However, when the moisture content in the cheese is at equilibrium with the water-holding ability of the gel matrix, changes in cheese during storage (such as proteolysis) that affect the composition and structure of the protein matrix may have a marked influence on the exudation. When the net effect of such changes during the storage of cheese results in decreasing the water-holding ability of the gel matrix, it leads to exudation.

CHAPTER 3

SCOPE AND OBJECTIVES OF THE PRESENT INVESTIGATION

The scope of the present investigation was primarily to determine the factors affecting exudation during storage of cheeses of low pH and high moisture, and to elucidate the underlying mechanism. The following were the broad objectives:

- To study the effect of selected manufacturing variables and processing conditions on the exudation of whey from Cream and Feta cheese.
- To correlate the biochemical changes during storage of Cream and Feta cheese with exudation.
- To gain knowledge on aspects related to recombined milk cheese.
- To investigate the role of some milk components on the exudation of whey from Cream and Feta cheese.
- To determine the effect of incorporation of heat-denatured whey proteins on the yield and exudation of whey from Feta cheese.
- To examine the effect of the type of material adsorbed to the fat globule surface on exudation of whey from Feta cheese.

CHAPTER 4

ANALYTICAL METHODS AND SENSORY EVALUATION

4.1 Introduction: Standard chemical methods of analyses of milk, cream, whey, exudate, curd and cheese have been summarised in Appendix 4.1. Specific methods used in this study, and modifications of the standard procedures, are described in the first section of this Chapter. Procedures for sensory evaluation of Cream and Feta cheese are described in the second section of this Chapter.

SECTION ONE

4.2 Specific methods

4.2.1 Sample preparation

Cream cheese: An entire cup of Cream cheese stored at 5°C was mixed and a sample was drawn.

Feta cheese: A whole block (500-600 g) of Feta cheese was grated and a sample of the grated mixture was drawn. When required, grated Feta samples were packed tightly into sample bottles (so that very little air space was left) and frozen at - 20°C for analysis at a later date. The frozen samples were thawed at 4°C for 24 h before analysing the sample.

Exudate from Feta: The exudate that was released from Feta and accumulated in the plastic pouch was centrifuged at 3,000 rpm for 5 min, filtered through Whatman filter paper (No 41) and used for analysis.

4.2.2 Measurement of amount of exudate

(a) Exudate from Cream cheese: A typical example of exudation from Cream cheese is illustrated in Fig. 4.1. The following procedure was used to isolate and measure the amount of exudate from Cream cheese.

- (i) Individual cups and lids to be filled with Cream cheese were coded and their weights recorded separately.
- (ii) The cups were filled up to the brim with Cream cheese and the lids were applied. The cups were then turned upside-down.



Fig. 4.1 A sample of Cream cheese showing exudate on the surface. The cups with Cream cheese were stored upside-down. The exudate settled at the bottom. The cups were inverted prior to measurement of exudation.

- (iii) Any air entrapped in the body of the Cream cheese was removed by gentle tapping of the cups.
- (iv) The filled cups were stored in the upside-down position at the specified temperatures. Storage of the cups in the upside-down position facilitated easier measurement of the exudate at a later stage, because the exudate being heavier than the Cream cheese tends to settle at the bottom of the cup. Storing the cups upside-down also prevented mixing of the condensate with the exudate.
- (v) Prior to measurement of the amount of exudate, the cups were drawn and stored at 5°C (not applicable to those already stored at 5°C) for an hour. This was done to provide a firm body to the Cream cheese by the cooling effect. Firming-up of the Cream cheese helps in the easy separation of the liquid phase (exudate) from the solid phase.
- (vi) The cup was weighed and then reverted back to the normal position. The lid was taken off and the exudate on the top was poured out by holding the cup at an angle of about 45°C to the horizontal for a minute. A Whatman filter paper (No 41) was used to absorb some exudate sticking to the cup or the lid. The weight of the cup with the lid and the Cream cheese was taken.

Calculation

Amount of exudate (g/kg moisture in cheese) =

$$\frac{(\text{Weight of exudate in g} \times 100 \times 1000)}{(\text{weight of cheese in g} \times \text{moisture percent in Cream cheese})}$$

Amount of exudate (g/kg cheese) =

$$\frac{(\text{Weight of exudate in g} \times 1000)}{\text{weight of cheese in g}}$$

(b) Exudate from Feta cheese: A typical example of exudation from Feta cheese is illustrated in Fig. 4.2. In a somewhat similar study, Pederson *et al.* (1971) measured exudation in 60-day-old Blue cheeses by providing temperature-stressed treatment to the cheeses. In the present study, however, the actual amount of exudate that leaked during storage of cheese was measured. The following procedure was used to determine the amount of exudate from Feta cheese.



Fig. 4.2 Feta cheese samples at various stages after manufacture.
Left to right: A block of Feta cheese after brining; Brined Feta cheese vacuum packed in plastic pouch (and stored in this state); Exudate released from Feta cheese in the vacuum packed pouch during storage (≈ 6 months); A pack containing Feta cheese is cut open to measure the amount of exudate.

- (i) The initial weights of the blocks of Feta cheese were recorded immediately after brining.
- (ii) At the time of measurement of the amount of exudate, one block of Feta was removed from the plastic pouch and the moisture on its surface was blotted using paper towels.
- (iii) The block of cheese was further rested on an absorbent paper towel for a minute to ensure removal of all exudate sticking to its external surface of the cheese and then weighed.
- (iv) The difference between the weight of cheese after brining and the weight of cheese after removal of exudate provided the net weight of the exudate.

Calculation

Exudation has been expressed in terms of g exudate/kg cheese, g exudate/kg moisture in cheese, final moisture in non-fat substance and % reduction in moisture in non-fat substance (MNFS). Details of the calculations are provided in Appendix 4.2.

4.2.3 Electrophoresis of cheese: Standard analytical methods for poly-acrylamide gel electrophoresis (PAGE) were used (Appendix 4.1).

Sample preparation: The procedure involved in the sample preparations for urea-PAGE or Sodium dodecyl sulphate (SDS)-PAGE are similar except for the following:

- Sample buffers are different as follows.
 Urea-PAGE sample buffer: 0.092 g EDTA, 1.08 g Tris base, 0.55 g boric acid, 36.0 g urea, 0.75 ml of 2-mercaptoethanol, 1 ml of 0.1% bromophenol blue solution made up to 100 ml and adjusted to pH 8.4 with 1 N HCl.
 SDS-PAGE sample buffer: 20.0 ml of 10% (w/v) SDS solution, 10 ml glycerol, 5 ml of 2-mercaptoethanol, 12.5 ml '0.5 M pH 6.8' Tris-HCl buffer, 2.5 ml of 0.05% (w/v) bromophenol blue made up to 100 ml with distilled water.
- For urea-PAGE usually few drops of a reducing agent, mercaptoethanol or dithioerythritol (DTE), are added to the sample extract prior to electrophoresis. For SDS-PAGE the sample extract is imparted heat treatment at 95°C for 10 min in the presence of the reducing agent so that the sulphide bonds of the protein are reduced and complete protein denaturation occurs.

The samples are diluted to an extent such that the concentration of protein is 1 to 2 mg per ml of the sample extract.

Sample preparation of cheese: Cream or Feta cheese was dissolved in sample buffer in the proportion of 1:50, held in a water-bath at 40°C for 2 h and then centrifuged at 10,000 rpm for 10 min. Samples were drawn from the fat-free portion and

diluted with the sample buffer in the ratio of 1:1. The dilutions were altered, when necessary, to achieve the desired concentration.

Sample preparation of exudate: Exudate from Cream cheese was diluted with the sample buffer to a suitable level and used for electrophoresis. In exudate from Feta cheese, presence of high concentration of NaCl acted as impurities, interfered with the mobility of the proteins during electrophoresis and subsequently made it difficult to identify and quantify the protein bands. This difficulty was overcome by dialysing the exudate in water to remove the excess NaCl. The extent of dilution of the high molecular weight proteins/peptides in the exudate sample during the dialysis was determined from the recorded weights of the contents in the dialysis tubing before and after dialysis. The dialysed exudate was further diluted with the sample buffer to obtain 1-2 mg protein/ml of sample extract. It may be noted that only the soluble and low molecular weight material in the exudate is lost during dialysis (Chapter 10) and, therefore, the determination of proteins/peptides of relatively high molecular weight by SDS-PAGE would be unaffected.

4.2.4 Proteins adsorbed to fat globule surface

The objective was to identify and quantify the proteins adsorbed to the surface of fat globules in cheese. This involved the separation of the fat content from cheese, extraction of protein adsorbed to the fat globule surface and finally SDS-PAGE of the fat-free extract. It would be nearly impossible to demarcate the protein adsorbed to FGM from the rest of the protein in the cheese. Any washing procedure involved is likely to take away some of the proteins adsorbed to the fat globule surface. Further, the washing step could also change the proportions of adsorbed protein and result in new configurations. Accordingly, it was intended to obtain a relative estimate of the nature of the proteins firmly adsorbed to FGM. Preliminary trials were performed to determine a suitable washing solution, the number of washings and the quantities of washing solutions to be used for extracting the fat globules with least possible damage to the fat globules. The procedure used is described below:

- (i) About 5 g cheese was dissolved in 45 g of 0.2 M sodium citrate solution and held in a water bath at 55°C for one hour. The contents in the flask were swirled at regular intervals.
- (ii) The contents were centrifuged at 7,000 rpm for 15 min at 4°C. Fat layer on top was scooped out (the contents were filtered at refrigerated temperatures, if necessary).
- (iii) The fat was dissolved in 35 ml of 0.2 M sodium citrate and held in the water bath at 55°C for another hour.
- (iv) The contents were again centrifuged as done earlier, the fat layer on top was collected and dissolved in 6 ml SDS sample buffer.

- (v) A few grains of DTE were added, the contents heated at 95 °C/10 min, cooled and once again centrifuged.
- (vi) The fat-free liquid (filtration was done at refrigerated temperature, if required) was analysed by SDS-PAGE as described in the standard method.

4.2.5 Whey protein nitrogen index (WPNI): WPNI is a measure of the undenatured whey proteins. Preliminary trials showed that it was difficult to ascertain the WPNI of curd and Cream cheese because they did not dissolve fully in the NaCl solution. Addition of NaOH to the mixture of NaCl and cheese/curd did not solubilise the cheese/curd. Estimation of WPNI in milk and whey was, however, satisfactory.

The accuracy of the test method to determine WPNI was verified by comparing with the results of the undenatured whey proteins determined by using SDS-PAGE as the reference method. The samples used were skim milk powder of known WPNI, whey from Cream cheese curd cooked at 60 °C and whey from Cream cheese curd cooked at 75 °C. The undenatured whey proteins present in the samples were filtered from the solutions of the samples saturated with NaCl (as described in the standard method for WPNI in Appendix 4.1). In the experimental method, the filtrates were used to determine the WPNI by the dye-binding method. In the reference method, the three filtrates were dialysed with water to get rid of NaCl and then analysed by SDS-PAGE. The ratios of undenatured whey proteins in the salt extracts of the three samples estimated by experimental and reference methods were compared and found to be very close (20.1:15.0:7.5 & 20.1:15.1:7.4). The dye-binding method could be thus effectively used to obtain a quick estimate of the amounts of the undenatured whey proteins in whey and milk.

Procedure: All the steps were identical to the reference method (outlined in Appendix 4.1) for dried milks except for the use of 23.0 g of milk or whey, or 8.0 g of Cream cheese or curd with 15.0 g water, instead of taking 3.0 g milk powder and 20.0 g of water.

4.2.6 Hardness of Cream cheese: A penetrometer attached with a cone-shaped probe/plunger was used to measure the hardness of Cream cheese. Cream cheese

was tempered to a temperature at which the hardness was to be estimated. The cheese was thoroughly mixed and its surface was smoothed flat using a spatula. The cheese was placed in the penetrometer and the probe was moved to touch the cheese surface. The probe was allowed to penetrate the Cream cheese for a fixed time interval. The depth of penetration was used as an indicator of the hardness of the product. Hardness was expressed in terms of hundredths of a centimetre.

4.2.7 Curd-fines lost in whey: The standard method of centrifuging whey to obtain an estimate of the amount of fines could not be used here because of the presence of too much fat in the product. Association of proteins with fat resulted in some curd-fines (mixture of protein and fat) floating on the supernatant liquid after centrifugation. Use of a projection microscope to measure the fines did not succeed because of too much variation in the sizes of curd fines. A simple method was therefore developed to isolate the insoluble material in the whey. The method is based on the assumption that the curd-fines were insoluble and that the total insoluble dried matter provides an indication of the fines lost in whey.

Procedure: Weighed quantities of whey were filtered through pre-weighed Whatman filter paper No 41. Three washings were given to the residue on the filter paper with distilled water. Following washing, the filter paper along with the residue on it was carefully transferred into a pre-weighed petri dish, dried in an oven at 105 °C for 16 h and weighed. The curd-fines were calculated as a percentage of the quantity of dried residue in the whey (w/w).

4.2.8 Test for emulsion stability of 'manufactured cream' made from fresh frozen milkfat for recombining and reconstituted skim milk: Presence of free fat and formation of a cream plug during storage of the manufactured cream and recombined milk over a certain period of time were the attributes considered in measuring the emulsion stability.

Procedure: Cream samples were filled in 100 ml graduated cylinders up to the 100 ml-mark and stored at 20 °C. After 18 h any *free fat* or *cream plug* were directly read from the markings on the cylinder and expressed as a percentage (v/v). Presence of free fat, if any, was also recorded.

Further, the cream samples were mixed with reconstituted skim milk (RSM) in the proportion of 1:4 so as to have a composition similar to that of cheesemilk. The recombined milks (cheesemilks) were filled in 500 ml beakers and stored at 20 °C. The extent of free fat and cream layer formed were recorded visually after 18 h. In context to the present study, cheesemilk prepared from mixture of manufactured cream and RSM was expected to provide more useful information. During cheese manufacture the milk needs to retain emulsion stability up to whey drainage (≈ 4 h). The emulsion stability of manufactured cream was considered satisfactory if the cheesemilk did not have much phase separation over 18 h.

4.2.9 Gel strength: Formagraph (A/S N. Foss electric, Type 11700) was used for measurement of curd gel strength. The instrument plots time-graphs as a measure of firmness of the coagulating milk. The measurement is based upon tiny forces picked up during linear oscillations of pendulums suspended in the coagulating milk. The time-graph provides an indication of the time at which the gel formation in the milk starts after the addition of rennet. As the gel firmness increases, the forces resisting the linear oscillations of the pendulum are recorded on the output chart as a bell-shaped projection. The increase in the width of the bell-shaped curve is related to the increase in the firmness of the gel. However, this increase is considered accurate until 15-20 min after the addition of rennet. Thereafter, the results become doubtful due to the continuous shattering effect of the oscillations.

Procedure: RSM (10% total solids) was prepared by dissolving low heat skim milk powder with water at 40 °C. 0.1% HCl was added to RSM to lower the pH of milk to a level similar to the pH of milk (≈ 6.5) at the time of rennet addition during cheese manufacture. Calculated quantities of emulsifying agents and diluted calf-rennet were added in proportions similar to that added at cheese manufacture. The samples were analysed in the Formagraph at 32 °C up to 50 min.

4.2.10 Differential Scanning Calorimetry: The method is based on the principle of measurement of the energy changes in a sample on heating (or cooling). The measured heat flow rate is proportional to the instantaneous specific heat of the sample. When phase change, or transitions involving an energy change, occur

during the temperature scan, the contribution to the heat flow is also measured. A computer controlled differential scanning calorimeter (Perkin-Elmer, DSC - 2C, Norwalk, Connecticut, USA) with a refrigerated block that allows a temperature range of - 60°C to 700°C was used.

Procedure: An empty sealed container was placed in the reference pan. Accurately weighed samples of Feta cheese or exudate (≈ 10 mg) were packed in hermetically sealed standard volatile sample pans, placed in a slot adjacent to the reference pan in the calorimeter, allowed to equilibrate at 295 K and then cooled to 215 K at the rate of 10 K/min. The sample was held at 215 K for 3 min and then heated at the rate of 5 K/min. The energy transfer (Joules/g) during cooling and heating the sample between 220 and 285 K was measured and plotted in a thermogram. At around the melting or freezing point of the sample there is an onset of a peak in the thermogram. The peak represents the phase conversion of the water in the sample (refer to Chapter 6 for details). The melting point was estimated by extrapolating the onset of the peak from the DSC thermograms. The melting and freezing points are only approximate estimates because of lack of a linear portion in the peak for extrapolation. The lack of linearity may be attributed to the experimental samples being complex mixtures.

4.2.11 Microbiological tests: Cheesemilk and cheese after brining were tested for the presence of coliforms. Total counts and lactobacilli counts in cheese at various stages of storage were determined as described in NZDDM (1984).

SECTION TWO

4.3 Sensory evaluation

4.3.1 Introduction: The objective of the sensory evaluation was to ensure that the cheeses made with the wide range of manufacturing variations were generally of acceptable quality.

4.3.2 Feta cheese: Feta cheese was assessed for flavour and textural characteristics eight weeks after manufacture. The cheese was evaluated by a minimum of seven

judges from a trained and experienced panel of nine. The evaluation was carried out at the NZDRI cheese grading room at 20°C. Prior to evaluation the cheeses were tempered to 20°C. The blocks of cheese were removed from the vacuum sealed plastic pouches and blotted with a paper towel to remove the moisture sticking to its surface. Exudate present in the plastic pouches was discarded.

Sample presentation was random and the origins of the samples were not revealed to the panel. The panellists were informed that no efforts had been made to reduce the yellow colour of the cheese, nor to reproduce the characteristic rancid flavour in Feta cheese, as lipase was never used. The panellists were advised not to downgrade the cheese on these accounts.

The cheeses were evaluated for flavour attributes of acidity, saltiness, oxidised and bitterness; and for textural characteristics of mouthfeel and structure. The evaluation was done on a freshly-cut (sliced-off) cheese. The flavour was evaluated by smell and tasting. The body was assessed by rubbing a portion of the sample between the forefinger and the thumb, by mouthfeel, and by slicing a layer of the cheese with a knife. The evaluation was based on a five point scale (1 - 5). Close distinctions between samples were recorded by using decimal points in the specified scale. Based on analysis of variance the data were statistically analysed using the SAS package (1985) in a micro-vax computer. The questionnaire used is presented in Appendix 4.3.

4.3.3 Cream cheese: Sensory evaluation of Cream cheese was identical to that of Feta cheese except for the following changes:

- Usually the cheese was evaluated 2 weeks after manufacture. Prior to evaluation, the cheese stored at 5°C was tempered to 20°C in about 2 h. The cheese was presented in the cup it was stored for evaluation.
- One of the ways of differentiating the smoothness, spreading properties and the presence of coarse particles in the cheese was by spreading a portion of cheese with a knife or spatula as a thin layer on a paper.

The questionnaire used is presented in Appendix 4.4.

CHAPTER 5

EXUDATION OF WHEY FROM CREAM CHEESE DURING STORAGE

5.1 Introduction

Cream cheese (hot-packed) was chosen to study the exudation of whey because:

- it is an unripened cheese,
- use of rennet is not required,
- it is less complicated in comparison to other cheeses,
- exudation is commonly encountered, and
- it was expected to provide results in a short time (10-15 weeks).

Exudation of whey from Cream cheese during storage is a common defect (Modler *et al.*, 1985). A process modification to prevent this defect in Cream cheese has been described (Carswell and Hurlburt, 1970). There are some passing remarks in published reports on ways of reducing the extent of exudation (Dahlberg, 1927; Modler *et al.*, 1985). No scientific study has been undertaken to determine the causes, and the extent to which the manufacturing variables influence exudation. Therefore, the objective of this study was to investigate the effects of manufacturing variables on the exudation of whey from Cream cheese and to establish practical procedures to reduce the extent of exudation. Composition and quality aspects of Cream cheese were also studied. The hot-pack method for making Cream cheese was chosen because it provides a long shelf-life and is a widely used commercial process.

This Chapter is divided into two sections. The first section describes the effect of process variables on exudation from cheeses with constant moisture. The second section describes the effect of process variables on cheeses with constant moisture in non-fat substance (MNFS), the chemical changes occurring in cheese during storage, and the possible mechanism of exudation.

SECTION ONE

5.2 EFFECT OF SELECTED MANUFACTURING VARIABLES ON EXUDATION FROM CHEESES OF CONSTANT MOISTURE

5.2.1 **Introduction:** Variation in manufacturing conditions during manufacture of Cream cheese results in variable drainage of whey from curd (Lundstedt, 1954), and consequently variable moisture content in the finished cheese. As the bulk of the exudate is comprised of moisture, it is reasonable to assume that exudation would be influenced by the moisture content in cheese. Exudation from cheeses made with variations in the manufacturing conditions would thus be influenced by not only the manufacturing variables, but also the resultant variation in moisture contents. However, the objective of the present investigation was to clearly identify the effect of the manufacturing variables. This was achieved by adjusting the product to a constant moisture during heat-processing of curd.

A constant moisture of 54% was targeted to comply with the US federal standards which prescribe a maximum of 55% moisture in the finished product. Lower moisture in cheese reduces yield and is commercially unacceptable.

5.2.2 **Experimental approach:** A large number of manufacturing variables could have been chosen for the study but it would have made the experiment excessively complex and time-consuming. The variables chosen for study had to be readily controlled by the cheesemaker. In choosing the high and low levels of the variables two aspects were considered. Firstly, they should permit exudation from Cream cheese during storage. Secondly, they should not significantly alter the quality and properties of the cheese.

Based on findings from trials of a very preliminary nature, a set of manufacturing variables and their respective levels were chosen.

The findings of this experiment are interpreted in terms of composition, quality and cheese exudation.

5.2.3 Experimental plan: Manufacturing variables and the levels of variation chosen for study are listed in Table 5.1.

Table 5.1 Selected manufacturing variables and their respective levels of variation for studying the effects on exudation of whey from Cream cheese during storage

Treatments	Levels	Reference in text
Protein to fat ratio in milk	0.30 0.22	A High Low
Homogenisation pressure (psi) ¹ (two stage) (single stage)	2,000/500 600	B High Low
Pasteurisation temperature (°C) ²	82.0 72.0	C High Low
Curd pH at cooking	4.95 - 5.0 4.65 - 4.7	D High Low
Cooking temperature (°C)	75.0 60.0	E High Low
Storage temperature (°C)	5.0 20.0 30.0	G Refrigerated Ambient Elevated
Storage time (weeks)		S
- at 5°C	2, 4, 6, 8, 12, 16	
- at 20°C	2, 3, 4, 6, 9	
- at 30°C	1, 2, 3, 4, 6	

¹ 1 psi = 6.89 kPa

² The terms 'low' and 'high' have been used for describing the levels of pasteurisation temperature for convenience only. 'Low pasteurisation temperature' refers to the minimum prescribed heat treatment for pasteurisation and the 'high pasteurisation temperature' refers to a heat treatment higher than the minimum prescribed limit.

Basis of selection of the levels of manufacturing variables

The chosen protein to fat (P/F) ratios for standardised milk were based on preliminary studies. The fat content of standardised milks with P/F ratios of 0.22 and 0.30 ranged from 9.0 to 12.0 % fat. A fat percentage of 10.5% is required in milk for making Cream cheese with about 33% milkfat.

Homogenisation of milk is usually done in two stages: the first at high pressure, typically 2,000 psi (13,780 kPa); and the second at low pressure, typically 500 psi (3,445 kPa). The higher level of homogenisation pressure was arbitrarily chosen as that of normal two-stage homogenisation (2000/500 psi). Single stage homogenisation at 600 psi was chosen as the lower level of homogenisation pressure because any further lowering was likely to result in a substantial amount of fat rising to the top during the incubation period. Preliminary studies showed that this would lead to high fat losses in the whey and a less smooth product.

The minimum prescribed legal requirement for pasteurisation (72°C/15 s) was chosen as the lower level. The higher pasteurisation temperature (82°C) was limited by the temperature that could be attained with the equipment available.

The lower level of curd pH at cooking (4.7) was chosen from a consideration of practical handling. At $\text{pH} \leq 4.6$ the curd particles were too fine and clogged the pores in the sieves. The upper limit of curd pH at cooking was chosen as 5.0 because a pH higher than this was likely to lead to less acid development in curd and incomplete flavour in the final product.

The cooking temperatures (60°C, 75°C) were chosen with a view to keep the process close to the commercial method of manufacture which commonly involves centrifugal separation of curd and whey at temperatures approximating 75°C.

The three storage temperatures (5°C, 20°C, & 30°C) were selected with the objective of gaining information about: refrigerated temperature (5°C) at which the product would normally be stored, ambient temperature (20°C) at which the product would be expected to be handled by the consumers, and elevated

temperature of storage (30 °C) that might provide a quick indication of the trends in exudation.

Statistical design of the experiment: A split-plot 2^4 factorial experiment was performed. The combinations of high and low levels of four manufacturing variables with which each trial was carried out were: P/F ratio, homogenisation pressure, pasteurisation temperature and curd pH at cooking. Each trial was split at the following three stages for study of the effect of other variables: cooking temperature of curd, storage temperature of Cream cheese and storage time of Cream cheese. A total of 16 trials were conducted. The experiment was performed in two blocks of eight trials each, where the blocked effect was the time and the defining contrast was $I = ABCD$ (see Table 5.1 for nomenclature). Trials in each block were randomised.

The results were analysed by tests for analysis of variance using the statistical computer package SAS (1985). However, for analyses on exudation data, this statistical method was not appropriate and the Chi-squared test was used. The details of statistical analyses are described in Chapter 5.2.7.(c) and Appendix 5.5. SAS was used for calculating the least square mean (LSM) values of the variables.

5.2.4 Experimental: Preliminary trials were carried out to establish the general manufacturing procedure, analytical methods, sampling frequency, and the method of measurement of the amount of exudate.

Manufacturing process: The method of manufacture was similar to the commercial 'long-set' and 'hot-pack' Cream cheese. The major deviation from the commercially practised method of manufacture of Cream cheese (Honer, 1988; Kosikowski, 1977) was that the product was not homogenised after heat processing. This was because the product showed a satisfactory consistency even without homogenisation and in some batches the product did not pass through the homogeniser because of high viscosity. The other deviation was the use of potassium sorbate in the processing mix. This was done to prevent mould growth in cheese during storage. Preliminary trials had shown that in some samples,

particularly those stored at high temperatures, moulds grew and the exudate became slimy. The slimy state of the exudate interfered with the accurate measurement of the quantity of exudate.

The cheese was manufactured in the pilot-plant (Dairy Product Development Centre) of New Zealand Dairy Research Institute (NZDRI). Brief descriptions of the equipment and accessories used in milk processing and cheese manufacture have been provided in Appendix 5.1. A flow diagram of the manufacturing process of Cream cheese is outlined in Fig. 5.1. Some of the manufacturing steps have been shown pictorially in Fig. 5.2. The manufacturing process for Cream cheese is described in Appendix 5.2.

Moisture adjustment of curd: Preliminary trials showed that, due to evaporation during heat-processing of curd, Cream cheese mixes calculated to have a moisture content of 55% produced finished product with about 54% moisture. Adjustment of the moisture content to the target value was achieved by either adding water, when the curd had less than required moisture, or by heat-processing curd with the lid open for varying periods, up to a maximum of 10 min, to allow evaporation when the curd had more than the required moisture content. An example of the calculation for the amount of water required to be added or removed for adjustment of moisture in cheese has been provided in the Appendix 5.3.

5.2.5 Analytical methods: Raw whole milk, cream and standardised milk were analysed for fat, protein and total solids by the Milko-Scan. Curd was analysed for pH, fat (Babcock) and moisture (microwave analyser). Cream cheese was tested for pH, fat (Babcock), moisture and salt (potentiometric method). Details of all methods are reported in Chapter 4 and Appendix 4.1.

5.2.6 Sensory evaluation: The product was evaluated by a panel after two weeks of storage for body (firmness) and textural (softness and smoothness) characteristics using a 5-point scale. Details of the evaluation procedure are provided in Chapter 4.

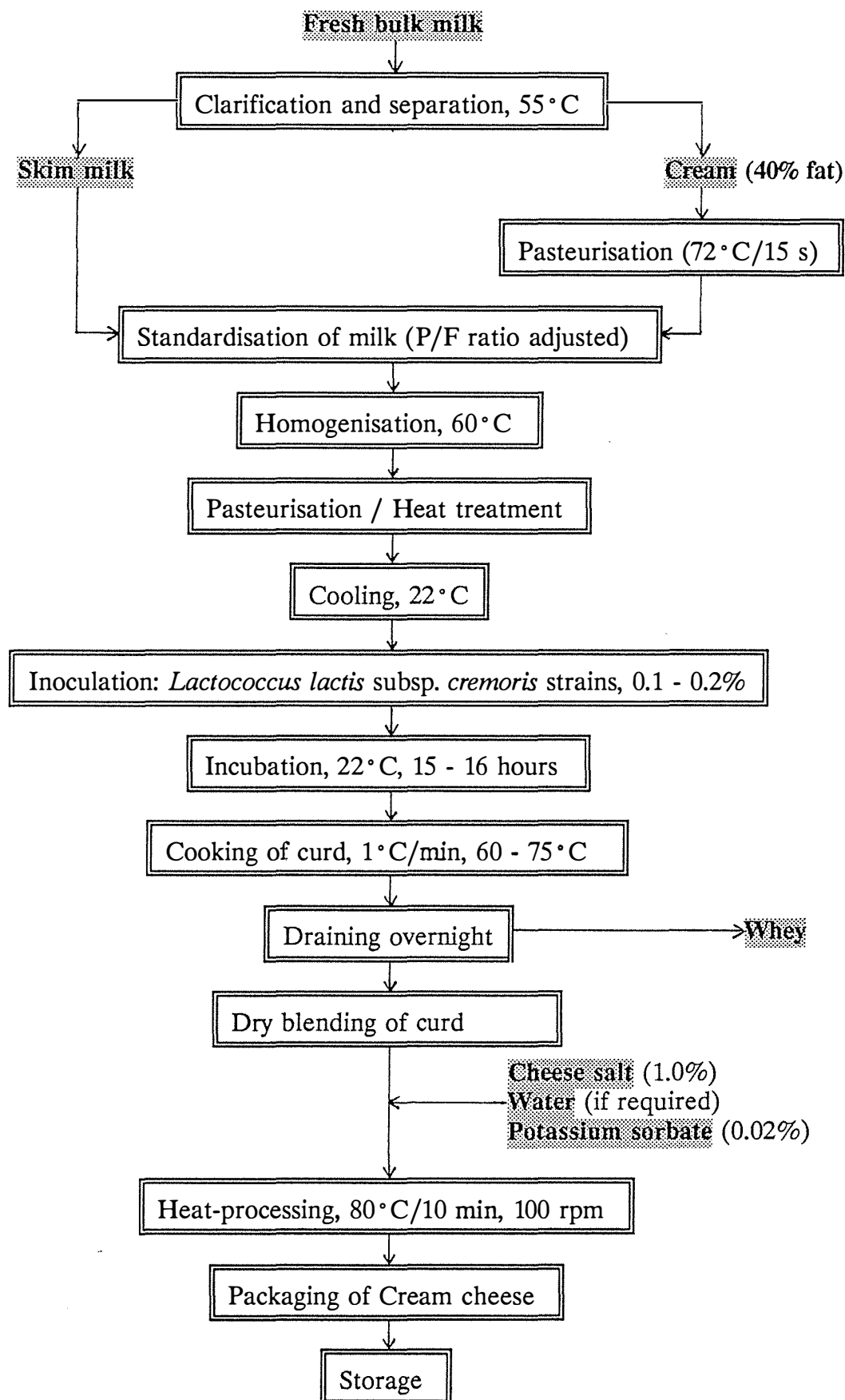


Fig 5.1 Manufacturing process of Cream cheese



Fig. 5.2 Selected stages in manufacture of Cream cheese.

Top row (left):	Cheese vat used for incubation of standardised milk and cooking;
Bottom row (left):	Sieves used for overnight draining of curd;
Top row (right):	Kettle used for heat-processing of Cream cheese;
Bottom row (right):	Following heat-processing the Cream cheese was packed in cups, inverted and stored at the specified temperature.

5.2.7 Results and discussion

For the sake of convenience, the results are described in 3 parts:

- (a) Composition of milk, curd and cheese.
- (b) Manufacturing aspects and cheese quality.
- (c) Effect of manufacturing variables on exudation.

(a) Composition of milk, curd and cheese

Composition of milk: The desired variations in the P/F ratio of milk were achieved (Table 5.2). Other details of composition of standardised milk are provided in Appendix 5.4.

Table 5.2 P/F ratio of standardised milk

Source of variation & the levels	P/F ratio	
	LSM	F
Protein/Fat ratio		522.0***
High	0.302	
Low	0.218	
Homogenisation pressure		2.87
High	0.263	
Low	0.257	
Pasteurisation temperature		1.18
High	0.258	
Low	0.262	
Curd pH at cooking		0.90
High	0.262	
Low	0.258	
Cooking temperature		0.00
High	0.260	
Low	0.260	
Standard deviation	0.007	

LSM = Least square mean; F = F ratio;

*** $p < 0.001$; F values without any asterisks denote 'not significant'.

Composition of curd (Table 5.3): The composition of the curd was affected by the manufacturing variables. The variation in the composition may be attributed primarily to the effects of the manufacturing variables on the syneresis of whey and draining of curd. In general, increased amounts of moisture were retained in curd made with lower P/F ratio, higher homogenisation pressure, higher pasteurisation temperature and lower curd pH at cooking. These results are consistent with the findings that the drainage of whey from curd is slowed by high pasteurisation temperature, high homogenisation pressure and high acidity (Lundstedt, 1954).

Variation of curd pH was achieved as planned. The data on curd pH at cooking and cheese pH are pooled together in Table 5.5.

Table 5.3 Effect of manufacturing variables on the composition of curd

Variables	Fat (%)		Moisture(%)		MNFS (%)		FDM (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
A		10.49*		0.01		66.5***		53.9***
High	33.53		54.75		82.38		74.12	
Low	35.44		54.65		84.57		78.17	
B		33.6**		30.0**		46.9**		1.23
High	32.78		56.74		84.40		75.84	
Low	36.19		52.71		82.55		76.45	
C		18.5**		16.8**		30.5**		0.87
High	33.22		56.20		84.20		75.89	
Low	35.75		53.19		82.73		76.40	
D		17.6**		22.9**		39.1**		0.04
High	35.72		52.95		82.37		75.92	
Low	33.25		56.30		84.32		76.10	
E		0.11		6.87*		6.44*		5.58*
High	34.44		54.51		83.14		75.73	
Low	34.53		54.91		83.81		76.56	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature; D = Curd pH at cooking; E = Cooking temperature; LSM = Least-square mean; F = F ratio; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; F values without asterisks denote 'not significant'.

Composition of Cream cheese: The objective of attaining moisture of about 54% in all Cream cheeses was mostly achieved (Table 5.4). The average moisture content of the individual trials was 53.9% with a standard deviation of 1.31.

Table 5.4 Composition of Cream cheese with relation to the manufacturing variables

Variables	Fat (%)		Moisture (%)		Salt (%)		pH	
	LSM	F	LSM	F	LSM	F	LSM	F
A		14.83*		0.78		0.15		0.22
High	33.06		53.87		1.13		4.96	
Low	34.66		54.23		1.13		4.94	
B		2.51		0.59		2.05		0.25
High	33.53		54.21		1.14		4.96	
Low	34.19		53.89		1.12		4.94	
C		4.16		6.76*		2.97		0.77
High	33.44		54.58		1.14		4.93	
Low	34.28		53.53		1.12		4.97	
D		0.28		2.08		8.84*		52.5***
High	33.97		53.76		1.12		5.11	
Low	33.75		54.34		1.15		4.80	
E		0.14		1.06		4.4		0.9
High	33.81		53.98		1.12		4.95	
Low	33.91		54.12		1.14		4.96	
Variables & levels	MNFS (%)		FDM (%)		S/M (%)			
	LSM	F	LSM	F	LSM	F		
A		34.67**		43.70**		0.38		
High	80.49		71.70		2.10			
Low	82.99		75.72		2.08			
B		0.68		2.21		0.21		
High	81.57		73.26		2.10			
Low	81.92		74.16		2.09			
C		1.62		0.07		0.05		
High	82.01		73.63		2.10			
Low	81.47		73.78		2.10			
D		1.88		0.59		1.02		
High	81.45		73.47		2.08			
Low	82.03		73.94		2.11			
E		2.45		1.08		2.0		
High	81.58		73.50		2.08			
Low	81.90		73.92		2.10			

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature; D = Curd pH at cooking; E = Cooking temperature; LSM = Least-square mean; F = F ratio;
 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;
 F values without asterisks denote 'not significant'.

Constant moisture was attained in all cheeses except for slight difference between two levels of 'pasteurisation temperature' treatment. Significant variations in FDM and MNFS for the two levels of P/F ratio were due to the imposed variations in the standardised milk.

Table 5.5 shows the pH of curd and Cream cheese (1 d and 16 wk old). Variation in pH of Cream cheeses is correlated to the variation of 'curd pH at cooking'. The pH of Cream cheese was about 0.1 units higher than the pH at which curd was cooked. This difference in pH is consistent with the earlier findings (Lundstedt, 1954). Cream cheese stored at 5°C did not show a change in pH during storage.

Table 5.5 Effect of manufacturing variables on the pH of curd and Cream cheese (1 day and 16 weeks old)

Source of variation & the levels	pH of curd		pH of Cream cheese at 5°C			
	-----		-----			
			1 day		16 weeks	
	LSM	F	LSM	F	LSM	F
Protein/Fat ratio		25.8**		0.22		2.92
High	4.98		4.96		4.98	
Low	4.90		4.94		4.94	
Homogenisation pressure		1.35		0.25		0.79
High	4.95		4.96		4.97	
Low	4.93		4.94		4.95	
Pasteurisation temperature		0.84		0.77		1.05
High	4.94		4.93		4.95	
Low	4.95		4.97		4.97	
Curd pH at cooking		791***		52.49***		135.3***
High	5.16		5.11		5.11	
Low	4.72		4.80		4.81	
Cooking temperature		1.68		0.9		1.95
High	4.94		4.95		4.97	
Low	4.95		4.96		4.96	
Storage time (weeks)						0.36
2					4.95	
6					4.95	
12					4.97	
16					4.97	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature;

D = Curd pH at cooking; E = Cooking temperature;

LSM = Least-square mean; F = F ratio; ** p < 0.01; *** p < 0.001;

F values without asterisks denote 'not significant'.

(b) Manufacturing aspects and cheese quality

Cheese manufacture: Cheeses made with the following combinations resulted in a fluid-like, atypical and unsatisfactory product.

1. High P/F ratio + low homogenisation pressure + low pasteurisation temperature + low pH at cooking + low cooking temperature.
2. High P/F ratio + high homogenisation pressure + low pasteurisation temperature + low pH at cooking + low cooking temperature.

The above products were unsatisfactory because of the separation of aqueous phase from the fat phase. Cream cheese with the first combination had the phase separation at all storage temperatures, while Cream cheese with the second combination did so at storage temperatures of 20°C and 30°C. It was difficult to estimate the amount of exudate in these products as the fat phase separated from the aqueous phase. The statistical analyses had to be carried out with values for these combinations missing.

In general, cheeses made with the higher pasteurisation temperature or higher cooking temperature were more viscous. The viscous nature of the product was indicated by its flow characteristics after heat-processing of curd. The curd made from milk homogenised at higher pressure or pasteurised at higher temperature was difficult to drain. When the curd was cooked to the higher temperature, it had a thinner consistency and more curd-fines appeared to be lost in whey. Cream cheeses made with the higher cooking temperature always had a thicker consistency.

It was not always possible to adjust the moisture in the final product to below 55%, as planned, particularly when very high moisture was retained in the curd.

Sensory evaluation: Except for the cheeses with an atypical fluid consistency, all were graded 'acceptable' by the panel (Table 5.6). Cheeses of lower pH were rated less smooth, which may be attributed to the fact that low pH cheeses tend to have a 'short' body and are crumbly. The higher cooking temperature produced a firmer cheese which is in accord with the earlier observation of a thick and viscous product during cheese manufacture.

Table 5.6 Effect of manufacturing variables on the mean scores of sensory parameters of cheeses

Source of variation & the levels	Body		Texture	
	LSM	F	LSM	F
Protein to fat ratio		0.50		0.31
High	2.36		3.29	
Low	2.55		3.36	
Homogenisation pressure		0.93		4.29
High	2.58		3.44	
Low	2.33		3.20	
Pasteurisation temperature		5.41		1.27
High	2.77		3.39	
Low	2.14		3.26	
Curd pH at cooking		6.27		40.24**
High	2.12		2.95	
Low	2.79		3.69	
Cooking temperature		14.99**		0.01
High	2.72		3.34	
Low	2.19		3.31	

LSM = Least-square mean; F = F ratio;

F values without asterisks denote 'not significant'; ** $p < 0.01$.

(c) Effect of manufacturing variables on exudation

In this Chapter exudation is expressed in terms of g exudate per kg moisture in the cheese. Tests for correlations were also made for exudation expressed as: a percentage of the total weight of cheese, percent MNFS of cheese, and percentage reduction in MNFS. However, in all cases the correlations were similar or of lower statistical significance.

The least-square mean values (calculated using analysis of variance) of the amount of exudate for the selected levels of manufacturing variables are reported in Table 5.7. Frequency distribution (incidences of exudation for the selected ranges) and Chi-squared values for the manufacturing variables are shown in Table 5.8. Interpretation of the effect of manufacturing variables on exudation has been made using results from both these tables.

Table 5.7 Effect of manufacturing variables on the exudation of whey from Cream cheese during storage (g exudate per kg moisture in cheese)

Sources of variation and the levels of variation	L.S.M. values of amount of exudate			
	Storage temperature of Cream cheese			
	5°C	20°C	30°C	Combined ¹
Protein to fat ratio				
High	5.47	16.45	20.22	16.28
Low	4.39	19.23	18.18	13.15
Homogenisation pressure				
High	1.98	10.01	11.65	8.62
Low	7.88	25.66	26.75	20.82
Pasteurisation temperature				
High	0.96	7.58	6.79	5.49
Low	8.90	28.09	31.62	23.95
Curd pH at cooking				
High	2.04	10.14	7.10	6.28
Low	7.82	25.53	31.30	23.16
Cooking temperature				
High	0.32	1.59	1.70	1.28
Low	9.54	34.08	36.71	28.16
Storage time				
1 week	n.d.	7.99	n.d.	n.d.
2 weeks	1.16	8.59	10.88	7.72
3 weeks	n.d.	12.99	19.03	n.d.
4 weeks	1.67	13.60	24.24	14.27
6 weeks	2.45	19.37	33.86	22.17
8 weeks	4.18	n.d.	n.d.	n.d.
9 weeks	n.d.	34.65	n.d.	n.d.
12 weeks	6.06	n.d.	n.d.	n.d.
16 weeks	14.07	n.d.	n.d.	n.d.

¹ Includes data on exudation at storage periods (2,4,6 weeks) common to all the three storage temperatures;
L.S.M. = Least Square Mean; n.d. = not determined.

Table 5.8 Effect of manufacturing variables on the exudation of whey from Cream cheese during storage (g exudate per kg moisture in cheese): based on X^2 test of significance

Source of variation & levels	Stored at 5°C				Stored at 20°C				Stored at 30°C				Combined ^a			
	x	y	z	X^2	x	y	z	X^2	x	y	z	X^2	x	y	z	X^2
A				10.74**				10.26**				9.85**				13.75**
High	82	6	8		60	5	15		55	11	14		110	14	20	
Low	68	22	6		42	16	22		36	24	20		82	34	28	
B				10.14**				4.33**				7.65*				12.10**
High	83	11	2		55	12	13		52	18	10		105	26	13	
Low	67	17	12		47	9	24		39	17	24		87	22	35	
C				19.3***				8.78*				5.06				14.4***
High	87	8	1		60	7	13		52	16	12		111	15	18	
Low	63	20	13		42	14	24		39	19	22		81	33	30	
D				14.2***				4.69				10.76**				9.44**
High	73	21	2		49	15	16		44	25	11		95	32	17	
Low	77	7	12		53	6	21		47	10	23		97	16	31	
E				32.4***				49.0***				45.5***				63.4***
High	91	5	0		72	5	3		65	13	2		127	13	4	
Low	59	23	14		30	16	34		26	22	32		65	35	44	
Storage time				2.93				0.43				5.16				2.03

^a Includes data on exudation at storage periods (2, 4 & 6 wk) common to three storage temperatures; A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature; D = pH at cooking; E = Cooking temperature; x = No of incidences of 'no exudation'; y = No of incidences of 'slight/moderate (1-20 g/kg cheese moisture)' exudation; z = No of incidences of 'excess' exudation (> 20 g/kg cheese moisture); X^2 = Chi-square; * p < 0.05; ** p < 0.01; *** p < 0.001; X^2 values without asterisks denote 'not significant'.

Statistical method: The data on exudation did not have a normal distribution. Attempts to induce normality by various mathematical transformations did not succeed. Therefore, ANOVA could not be used for the test of significance.

Chi-square test was used to determine the significant effect of treatments above the split *i.e.* for P/F ratio, homogenisation pressure, pasteurisation temperature, curd pH at cooking. The limiting factor for the Chi-squared test method is that it is based on a frequency distribution¹ of observations, *i.e.*, the number of incidences (or occurrences) of exudation (none/slight/excess), rather than the actual amount of exudate. Thus, the Chi-square test does not adequately take into account the exact increase in the amount of exudate.

From a customer's viewpoint the findings of Chi-square test are important as it indicates whether or not there was exudation. However, for gaining an insight into the mechanism of exudation ANOVA is more useful because it takes into account the actual data at the two levels and indicates the effect of manufacturing variable. The details on why ANOVA could not be used for the test of significance, and how Chi-square test has been used to test the significance of variation has been provided with an example in Appendix 5.5.

Effect of P/F ratio (0.23 & 0.30) on exudation: The cheeses made from milk with the high P/F ratio showed a lower incidence of exudation than the low P/F ratio at all storage temperatures (Table 5.8). However, when the high P/F ratio cheeses showed exudation the amount of exudate was generally greater than the low pH cheeses (Table 5.7). From a practical viewpoint a lower incidence of exudation is preferable even if the extent of exudation is greater. This is because the customer readily notices the presence of exudate. The amount is of secondary importance.

All the cheeses had similar total solids. The increased protein, which has better water-holding ability than fat in the cheeses made from milk with higher P/F ratio,

¹ The criteria used in choosing the ranges for the frequency distribution of exudation (None/Slight/Excess) are outlined in Appendix 5.5.

may be responsible for the lower incidence of exudation in these cheeses. The influence of fat on exudation is not clear from these experiments.

Effect of homogenisation pressure (2,000/500 psi & 600 psi) on exudation: Increase in homogenisation pressure resulted in a decrease in the amount of exudate and incidence of exudation at all storage temperatures (Table 5.7 & Table 5.8). The effect of the increase in homogenisation pressure may be explained by the increase in fat globule surface area due to decrease in size and the increase in the number of fat globules. Homogenisation is known to reduce syneresis during cheese manufacture (Emmons *et al.*, 1980; Storry *et al.*, 1983; Vaikus *et al.*, 1970). The effect is possibly due to fat mechanically blocking casein-casein interaction (Lelievre & Creamer, 1978). It is possible that the mechanism involved in reduced syneresis due to homogenisation may also apply to exudation during storage of cheese.

Effect of pasteurisation temperature (72°C & 82°C) on exudation: With an increase in pasteurisation temperature, a decrease in the amount of exudate and incidence of exudation was observed at all storage temperatures (Table 5.7 & Table 5.8). This was in spite of a slightly higher moisture content in the cheese made from milk pasteurised at a higher temperature. The effect of higher temperature may be explained as similar to that of reduced syneresis in yoghurt from high heat treated milk. Syneresis in yoghurt is eliminated by heating milk to higher temperatures (95°C/10 min) due to binding of β -lactoglobulin to κ -casein and prevention of fusion of casein micelles (Brooker, 1987).

This effect of high heat denaturation of whey protein is a widely used practice to retard syneresis in yoghurt (Tamime & Deeth, 1980; Brooker, 1987; Dannenberg & Kessler, 1988). A linear increase in denatured whey protein from 10 to 40% of the total protein has been reported when the pasteurisation temperature of milk was increased from 72 to 85°C (Garrido *et al.*, 1983). Modler *et al.* (1985) observed better water retention and reduction in the amount of exudate in a hot-packed Cream cheese spread, and were of the opinion that this might have been due to the hydrating properties of whey protein.

Evidence for the effect of heat-denaturation of whey protein in Cream cheese in reducing the extent of exudation has been provided in Section Two.

Effect of curd pH at cooking (4.7 & 5.0) on exudation: The amount of exudate from cheese was higher with the lower curd pH at cooking (Table 5.7). This may be explained by the fact that the lower pH is closer to the iso-electric point of the casein protein at which the protein-protein interaction is maximal, the molecules are compact and hydration is minimal (Kinsella & Fox, 1987). However, the total incidence of exudation was less for lower curd pH at cooking (Table 5.8). This implies that the amount of exudate from cheese cooked at low pH was much greater in quantity than cheese cooked at a higher pH. This supports the view that exudation is commonly observed in low pH cheeses (Lawrence, 1989). The reason for a greater number of high pH cheeses showing exudation than the low pH cheeses could be due to the effect of interaction with other process variables.

Effect of cooking temperature (60°C & 75°C) on exudation: Cooking temperature had a highly significant effect on exudation at all storage temperatures (Table 5.7 & Table 5.8). Temperature rise during cooking was 1°C per min. Thus, cheese made with a high cooking temperature had an additional heating time of 15 min to reach 75°C from 60°C. Another 5 - 10 minutes was taken for cooling. Therefore the net holding time for cheese made at higher cooking temperature was considerable. This would have denatured a substantial amount of whey protein. Details of the possible effect of high heat treatment has been described earlier under the effect of 'pasteurisation temperature'.

Effect of storage time and storage temperature (5°C, 20°C & 30°C) on exudation: The effect of storage time and temperature on the exudation is shown in Fig. 5.3. The average amounts of exudate, the frequency distribution of incidences of exudation at the three storage temperatures, and the X^2 value is shown in Table 5.9. Until the sixth week of storage at 30°C the increase in amount of exudate had been proportional to the storage time. A similar pattern, though at a slightly reduced rate, was observed for cheeses stored at 20°C. However, for cheeses stored at 5°C, which is of more practical significance, the increase in the

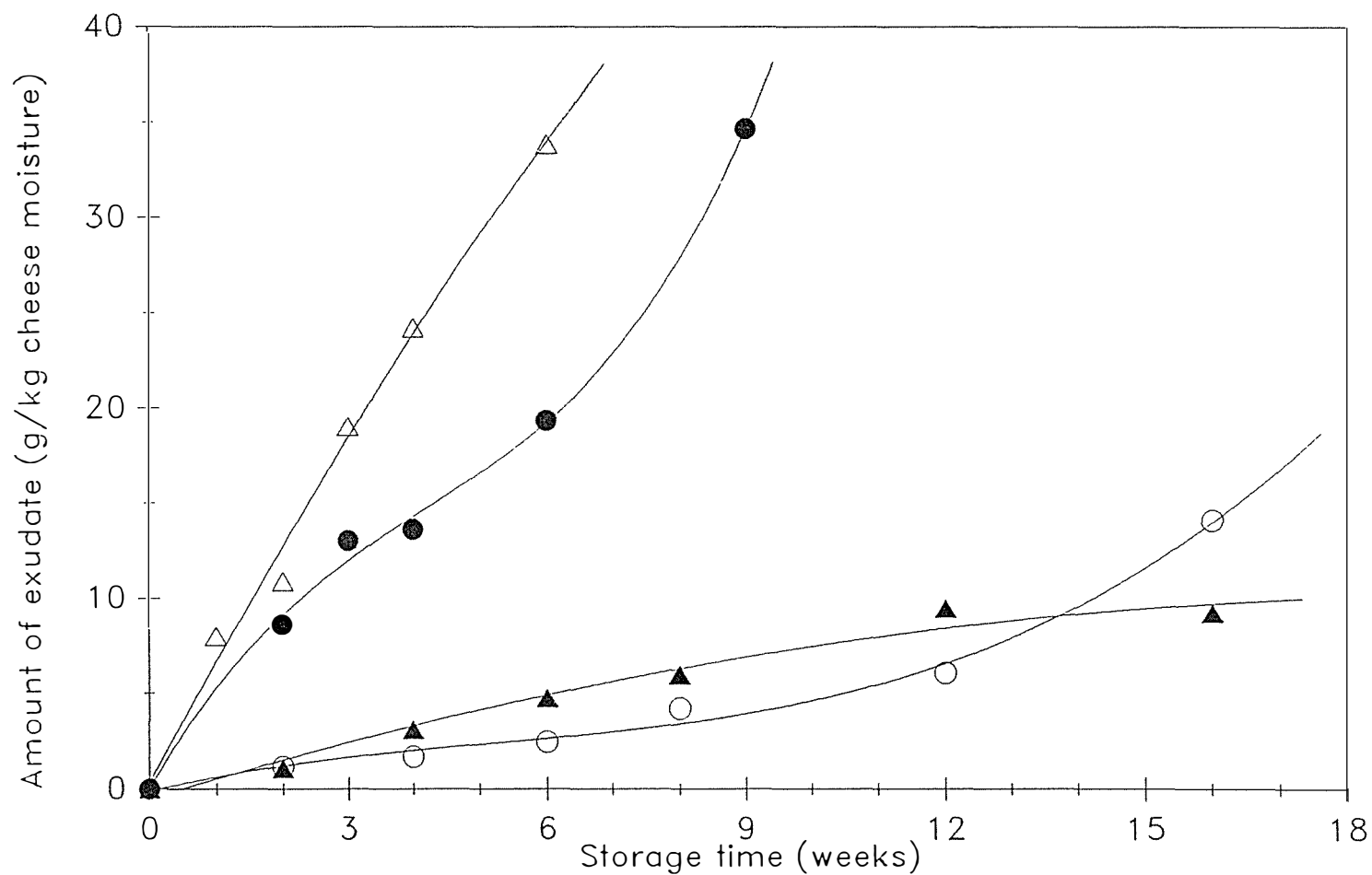


Fig 5.3 Exudation of whey from Cream cheese during storage
 ○ Stored at 5°C (constant moisture); ● Stored at 20°C (constant moisture);
 △ Stored at 30°C (constant moisture); ▲ Stored at 5°C (constant MNFS).

extent of exudation was much slower until about 9 weeks but started to rise thereafter. The reduced amount of exudate from Cream cheese at the storage temperature of 5°C is similar to ultrafiltered Cast Feta cheese where very little moisture is lost during storage at 7°C compared to 15°C (Lawrence, 1989). Increase in the amount of exudate with increase in storage temperature could be due to the general decrease in water sorption of milk proteins with increasing temperatures (Kinsella & Fox, 1987). Walstra *et al.* (1985) cited shrinkage of casein particles with rise in temperature as the likely cause for increased syneresis in acid coagulated gels of milk products.

It is hard to predict the pattern of exudation after 16 weeks, particularly for those cheese which have shown exudation. Presumably further exudation would be impeded by the decrease in the amount of potential exudate remaining in the cheese. However, within the selected periods of study - which is of practical relevance - the exudation increased with increase in storage temperature.

The Chi-square test on frequency distribution shows that the incidence of exudation was not affected with increase in storage time. This is a reflection of the fact that most cheeses had exudation in the first two weeks of storage and the subsequent additional incidences of exudation were negligible.

Table 5.9 Effect of storage temperature on the exudation

Storage temperature (°C)	Least-square mean of exudation ^a (g exudate/kg cheese moisture)	No of incidences of exudation			X ²
		Nil	Slight	Excess	
5.0	2.91	79	13	4	
20.0	17.73	61	14	2	21.91 ***
30.0	23.52	52	21	23	

^a Data includes exudation from Cream cheeses at 2, 4 and 6 weeks of storage at the three storage temperatures;

X² = Chi-square; *** = p < 0.001.

Most of the microorganisms and enzymes would be inactivated during the high heat-processing ($\approx 74^{\circ}\text{C}/30\text{ min}/25\text{-}40\text{ psi}$ steam pressure) of curd with other ingredients (Kosikowski, 1977; Kalab & Modler, 1985; Modler *et al.*, 1985; Honer, 1988). It is unlikely that there would be any proteolysis during the selected periods of storage. There is no evidence to link exudation in hot-packed Cream cheese with proteolysis or chemical changes during the selected periods of storage. The plausible explanation for the variation in amount of exudate in Cream cheese (or even absence of exudation up to considerable periods of storage) appears to have been influenced by the process treatments, casein gel structure, fat emulsion stability and extent of whey protein denaturation. The effect of storage time on syneresis could be attributed to the fact that the gel systems in foodstuffs have cross-linkages that are not permanent (Oakenfull, 1984) and proteins are known to have reduced water-holding capacity with increased temperature and time (Kinsella & Fox, 1987). This is supported by the observation that all Cream cheeses stored at 5°C , irrespective of the process treatment, had clearly visible and profuse exudation after 18 months of storage, though it has not been ascertained how far the bio-chemical changes to the cheese contributed to this effect. Nevertheless, it was encouraging to note the absence of exudation in some Cream cheeses stored up to a year at 5°C .

SECTION TWO

5.3 EFFECT OF MANUFACTURING VARIABLES ON EXUDATION FROM CHEESES OF CONSTANT MNFS

5.3.1 Introduction: From the previous factorial experiment the role of fat and the effect of curd pH at cooking on exudation was not clear. The experiment suffered due to unsatisfactory product in two trials. The mechanism of how the process variables affected exudation was only speculative. In the present experiment, it was aimed to provide evidence to support the proposed theories and confirm the trends observed in the previous experiment. The scope of the experiment was:

- (i) To determine the role of fat on exudation.
- (ii) To determine the effectiveness of heat-denatured whey protein in retarding the extent of exudation.

- (iii) To determine the residual lactose present in Cream cheese at various periods of storage.
- (iv) To determine the extent of proteolysis in Cream cheese during storage.
- (v) To estimate the extent of loss of curd-fines through whey in relation to process treatments.
- (vi) To correlate hardness of Cream cheese with process variables, presence of denatured whey proteins and exudation of whey.
- (vii) To determine the effect of homogenisation pressure on fat globule size and the protein adsorbed to the fat globule surface.

5.3.2 Experimental approach: The process variables and the limiting values for the variables were the same as in the previous experiment (Table 5.1). The major difference was to have a constant MNFS in Cream cheeses instead of a constant moisture. This was because, if the non-fat-substance in the cheese is considered to have the ability to hold a certain maximum amount of moisture, an increase in the amount of moisture in the non-fat-substance (MNFS) above this level would result in a proportionate increase in the amount of moisture (*i.e.* exudate). It was hoped that a constant MNFS would negate any effect exerted by the non-fat-substance *per se* on the exudation.

Only one storage temperature (5°C) was chosen for study because the results at other storage temperatures were unlikely to be of much practical use.

The combinations of manufacturing variables that did not provide a satisfactory product in the previous experiment were eliminated from the present study to avoid any possible missing data. The experiment was only fractional-factorial. The emphasis was in providing supportive and conclusive evidence for the findings of previous experiment.

Statistical design: A half replicate [$2^{(4-1)}$] of the full factorial [$2^{(4)}$] experiment was performed. The trials were conducted with the following variables (see Table 5.1 for nomenclature): abcd, aBcD, aBCd, AbcD, AbCd, ABcd, ABCD, abCD. The upper case refers to the higher level and the lower case refers to the lower level for the respective manufacturing variables. Analysis of variance (ANOVA) technique was employed for the entire set of data using the statistical package SAS (1985). Chi-squared test of significance was performed with the data on the incidence of exudation.

Because of insufficient error terms in the fractional factorial design, the effects of manufacturing variables (particularly above the split) may not show the actual significance. The results for the different levels of the variables are to be treated on a relative basis except for the variable 'cooking temperature' which has the appropriate error terms and is statistically accurate.

5.3.3 Experimental: The manufacturing conditions for Cream cheese were the same as described in the previous experiment (page no. 65 & Appendix 5.2). The only deviation was to aim for a constant MNFS (83.5%) in curd. This MNFS value was chosen on the basis of the average value of the MNFS of curd obtained in the previous experiment. Adjustment of moisture was done by adding a calculated amount of the whey or by evaporation during processing of curd as explained in the previous experiment. An example of the calculations for adjustment of MNFS level in Cream cheese has been provided in Appendix 5.6.

5.3.4 Analytical methods: The Milko-Scan was used to measure fat, protein and total solid contents of the skim milk, cream and standardised milk. Lactose content of the standardised milk was also estimated by the Milko-Scan. Because of the high fat content ($\approx 11\%$ fat), the standardised milk was diluted with an equal quantity of skim milk prior to analysis. Raw standardised milk and processed (homogenised and pasteurised) milk were analysed for calcium (complexometric method), WPNI (dye-binding method) and fat globule size (spectroturbidimetric method).

Whey was analysed for fat, protein, lactose and total solids in the Milko-Scan. The whey was also analysed for calcium, curd-fines and WPNI (dye-binding). Curd was analysed for fat (Babcock method) and moisture (microwave analyser). Cream cheese was tested for pH, fat (Babcock method), moisture, salt (potentiometric method), calcium (complexometric) and hardness (Penetrometer). Urea-PAGE on Cream cheeses was done at 0, 2, 4, 8 and 16 weeks after manufacture. Residual lactose in Cream cheese was estimated at 2 and 16 weeks of manufacture. The protein adsorbed to the surface of the fat globule was extracted from raw

standardised milk, homogenised milk and Cream cheese. The type and concentration of proteins were estimated by SDS-PAGE.

Details of all these methods are outlined in Chapter 4 and Appendix 4.1.

5.3.5 Sensory evaluation: The product was evaluated by a panel after two weeks of storage for body (firmness) and textural (softness and smoothness) characteristics using a 5-point scale. Details are provided in Chapter 4.

5.3.6 Results and Discussion

The results are discussed under the following headings:

- (a) Composition of milk, whey, curd, cheese and exudate.
- (b) Manufacturing aspects and cheese quality.
- (c) Physical and biochemical aspects of cheese.
- (d) Effect of manufacturing variables on exudation.

(a) Compositions of milk, whey, curd and cheese

Composition of standardised milk: Milks were standardised to the desired high and low P/F ratios (Table 5.10). Details of composition of standardised milk have been provided in Appendix 5.7.

Composition of whey (Appendix 5.8): Most variations with the composition of whey are not of any practical concern. Protein content in whey from cheeses made with higher pasteurisation temperature or higher cooking temperature were lower. This could be due to the retention of heat-denatured whey protein in the curd. Whey obtained during cheese manufacture at the lower level of curd pH at cooking had significantly higher calcium. This was due to more of the soluble calcium being lost in whey at a lower pH (Casiraghi *et al.*, 1987).

Composition of curd (Appendix 5.9): The manufacturing variables influenced the extent of syneresis and whey drainage during cheese manufacture, and consequently the moisture retained in the curd. Variation of moisture, in turn, affected the fat

content in the curd. Results were similar to those described in the previous experiment.

Table 5.10 Effect of manufacturing variables on the protein to fat (P/F) ratio of raw standardised milk

Source of variation & the levels	P/F ratio	
	LSM	F
Protein/Fat ratio		344.8***
High	0.301	
Low	0.225	
Homogenisation pressure		0.54
High	0.261	
Low	0.264	
Pasteurisation temperature		0.96
High	0.261	
Low	0.265	
Curd pH at cooking		2.15
High	0.260	
Low	0.266	
Cooking temperature		0
High	0.263	
Low	0.263	

LSM = Least-square mean; *** $p < 0.001$;
F values without any asterisks denote 'not significant'.

Composition of Cream cheese (Table 5.11): Constant MNFS was attained in most cheeses except for the variable P/F ratio. Variation in the curd pH at cooking had the desired significant variation on the pH of Cream cheeses. The calcium content in cheese reflected the extent of acid production before the curd was separated from whey which is consistent with the findings of Lawrence & Gilles (1987).

Composition of Exudate obtained from Cream cheese: Often it was not possible to analyse the exudate because of the small quantity. A typical composition of exudate was: 0.32% fat, 3.51% protein, 1.82% NaCl, 10.90% total solids, 4.72% lactose, 24.3 mMol/kg calcium.

Table 5.11 Composition of Cream cheese with respect to the manufacturing variables

Source of variation	Fat (%)		Moisture (%)		NaCl (%)		Ca ²⁺ (mM/kg)		pH	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A		11.55*		3.52		1.0		4.04		0.04
High	30.67		55.60		1.1		24.8		5.04	
Low	34.22		53.94		1.1		23.3		5.02	
B		6.41		6.41		2.78		0.11		1.87
High	31.12		55.89		1.1		23.9		4.99	
Low	33.76		53.65		1.1		24.2		5.07	
C		0.84		0.79		2.78		1.00		0.53
High	31.97		55.16		1.3		24.4		5.01	
Low	32.92		54.38		1.1		23.7		5.05	
D		4.72		5.59		9.01		13.57*		20.50*
High	33.58		53.72		1.1		25.4		5.16	
Low	31.31		55.82		1.1		22.7		4.89	
E		0.15		0.82		3.95		1.50		17.48*
High	32.55		54.53		1.1		24.2		5.01	
Low	32.34		55.01		1.1		23.9		5.04	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature;
D = Curd pH at cooking; E = Cooking temperature;

LSM = Least-square mean; F = F ratio;

* $p < 0.05$; F values without asterisks denote 'not significant'.

Table 5.11 continued ...

Table 5.11 continued

Variables & levels	MNFS (%)		FDM (%)		S/M (%)	
	LSM	F	LSM	F	LSM	F
A		50.72**		29.10**		3.82
High	80.07		69.07		2.0	
Low	81.99		74.18		2.1	
B		0.99		5.40		7.23
High	81.16		70.52		2.0	
Low	80.89		72.72		2.1	
C		0.23		0.93		0.33
High	80.97		71.16		2.1	
Low	81.09		72.08		2.1	
D		1.03		3.26		3.82
High	80.89		72.48		2.1	
Low	81.17		70.77		2.0	
E		5.60		0.64		1.95
High	80.87		71.44		2.1	
Low	81.20		71.80		2.1	

A = P/F ratio; B = Homogenisation pressure;

C = Pasteurisation temperature; D = Curd pH at cooking;

E = Cooking temperature; LSM = Least-square mean; F = F ratio;

** p < 0.01; F values without asterisks denote 'not significant'.

(b) Manufacturing aspects and cheese quality

Cheese manufacture: Manufacture of cheese did not pose any problems. A greater quantity of curd-fines were lost in the whey when the cheese was made with a higher cooking temperature (Appendix 5.8) which confirmed the visual observation. The effect could be due to thermal (high heat treatment) and mechanical (additional agitation) factors. However, this is less likely to be a concern in commercial scale manufacture where curd is mechanically separated.

Evaluation of Cream cheese: In general the cheeses were acceptable (Appendix 5.10). The body of the product was rated as significantly firmer with higher cooking temperature.

(c) Physical and biochemical aspects of Cream cheese

Fat globule size: The mean diameter of fat globules in raw standardised milk and processed (homogenised and pasteurised milks) are provided in Table 5.12. The mean diameter of fat globules in milk homogenised at a higher pressure was significantly smaller when compared to that with lower homogenisation pressure. The values of mean diameter compare favourably with the reported values of about 1 μm for homogenised milks (Walstra, 1975).

The mean diameter of fat globules could not be estimated in some Cream cheeses because the turbidity spectra did not match with the standard theoretical spectra of known fat globule sizes. Interestingly, it was observed that the size of fat globules for all Cream cheeses homogenised at higher homogenisation pressure could be detected, and the values ranged from 0.303 - 0.574 μm . The fat globule size of all Cream cheeses made with homogenisation at a lower pressure could not be estimated. This could have been due to the fat globules being very large, or non-homogeneous. While dissolving Cream cheese in alkaline EDTA (ethylene diamine tetra-acetic acid) solution during sample preparation for estimation of fat globule size, free fat could be seen floating on the surface. This indicates that the fat globules had undergone clumping and clustering during processing. Because of

some free-fat separation during sample preparation, it was difficult to draw a representative sample. Therefore, the estimated fat globule sizes in Cream cheeses are not likely to be very accurate. However, it can be safely interpreted that the fat globules in Cream cheese made from milk homogenised at lower pressure were larger than those from milks homogenised at higher pressure. The total area occupied by the surface of the fat globules in Cream cheeses made from milk homogenised at lower pressure may therefore be expected to be less than cheese made with higher homogenisation pressure.

Table 5.12 Effect of manufacturing variables on the mean diameter of fat globules in raw standardised milk and processed (homogenised and pasteurised) milk

Levels	Mean diameter of fat globules (μm)			
	Raw milk		Processed milk	
	LSM	F	LSM	F
A		5.19		3.43
High	4.5		0.6	
Low	3.9		0.8	
B		3.53		41.87**
High	3.9		0.4	
Low	4.4		1.0	
C		0.04		0.07
High	4.2		0.7	
Low	4.1		0.7	
D		0.01		0.23
High	4.2		0.7	
Low	4.2		0.7	
E		0.00		0.00
High	4.2		0.7	
Low	4.2		0.7	

A = P/F ratio; B = Homogenisation pressure;
 C = Pasteurisation temperature; D = pH at cooking;
 E = Cooking temperature;
 LSM = Least-square mean; F = F ratio; ** $p < 0.01$;
 F values without asterisks denote 'not significant'.

Whey Protein Nitrogen Index (WPNI): The severity of heat treatment is directly related to the denatured whey protein and inversely related to the undenatured whey protein (or WPNI) in milk or whey. In this experiment as WPNI values of curd and Cream cheese could not be accurately estimated, the results are being inferred from the WPNI values of raw standardised milk, processed (homogenised and pasteurised) milk and whey. It is expected that with higher heat treatment during pasteurisation, more whey protein would be denatured in the milk and retained in the curd. An indication of the retention of the denatured whey protein in the curd would be supported by a corresponding lower WPNI in whey. Likewise, increased heat-denaturation of whey protein at a higher cooking temperature and their retention in the curd would be indicated by a lower WPNI of whey.

The WPNI for the standardised milk, processed milk and whey are shown in Table 5.13. The low WPNI of whey (alternatively, more denatured whey protein retained in curd) for higher pasteurisation temperature is consistent with the findings reported by Garrido *et al.* (1983) that increase in pasteurisation temperature from 73°C to 85°C resulted in linear increase in denatured whey protein from 10 to 40%. A similar effect was also observed with increase in cooking temperature. A low WPNI for the higher homogenisation pressure may be traced to the initial difference in raw standardised milks. It is unlikely that the effect could have been due to the treatment itself.

It is apparent that variation in heat treatment due to the process variables 'pasteurisation temperature' and 'cooking temperature' was effective in obtaining a variation in the denaturation of whey protein. It follows from this that Cream cheeses made with higher levels of pasteurisation temperature and cooking temperature had increased amounts of heat-denatured whey protein.

Protein adsorbed to surface of fat globules in milk: Protein adsorbed to fat globules in raw standardised milk and processed milks homogenised at high and low pressures were extracted and analysed by SDS-PAGE. The visibility of protein bands in the gels from samples of homogenised milks was not very clear. In some instances even the densitometer failed to plot the bands clearly. This was due to

Table 5.13 Effect of manufacturing variables on the WPNI
[mg undenatured whey protein/g milk (or whey)]

Treatment & Levels	WPNI (mg/g)					
	Raw standardised milk		Processed (pasteurised & homogenised milk)		Whey	
	LSM	F	LSM	F	LSM	F
A		0.26		0.01		0.28
High	3.12		2.14		1.55	
Low	3.09		2.15		1.46	
B		6.77		11.79*		3.56
High	3.03		1.96		1.34	
Low	3.18		2.33		1.66	
C		1.09		33.44*		6.44
High	3.07		1.84		1.29	
Low	3.13		2.45		1.71	
D		3.86		0.83		0.57
High	3.16		2.10		1.56	
Low	3.04		2.19		1.44	
E		0.00		0.00		41.54**
High	3.10		2.14		1.27	
Low	3.10		2.14		1.73	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature;
D = pH at cooking; E = Cooking temperature; LSM = Least-square mean;
F = F ratio; * $p < 0.05$; ** $p < 0.01$;
F values without asterisks denote 'not significant'.

insufficient recovery of 'homogenised fat' by centrifugation during the extraction procedure.

Both raw and homogenised milks had all the major milk proteins. The effect of homogenisation on the concentration of casein and whey protein adsorbed to the fat globule surface, and the fat globule size, have been reported in Table 5.14. With increase in homogenisation pressure more casein was adsorbed to the fat globule surface. This observation is consistent with the reported literature that during homogenisation the newly created fat globules are coated by the casein protein (Vliet & Dentener-Kikkert, 1982; Brooker, 1987).

Table 5.14 Effect of homogenisation of milk on the concentration of proteins (casein and whey protein) adsorbed to fat globule surface, and the mean diameter of fat globules

Particulars	Fat globule size (μm)	Results from densitometer plots		
		Area of casein protein	Area of whey protein	Ratio of casein to whey protein
Raw standardised milk	4.0	0.13	0.095	1.37
Processed milk (Homogenised at 600 psi)	0.9	0.13	0.07	1.86
Processed milk (Homogenised in two stages at 2000/600 psi)	0.5	0.20	0.05	4.0

Note: Areas of the proteins have arbitrary units;
1 psi = 6.89 kPa.

Protein adsorbed to surface of fat globules in Cream cheeses: SDS-PAGE showed the presence of major milk proteins in the proteins adsorbed to the fat globule surface in cheese. These were similar to the proteins extracted from fat globule surface of homogenised milks. However, unlike the protein bands from fat globule surface of homogenised milks, these were clearly visible and were present in much

higher concentration. The ratio of casein and whey protein as estimated with the densitometer plots are reported in Table 5.15. The casein to whey protein ratio was significantly higher with higher P/F level. This may be due to availability of more protein in proportion to fat. Further, the ratio of casein to whey protein was significantly lower for pasteurisation treatment of milk at a higher level. Availability of increased amounts of denatured whey proteins in the milk at the higher pasteurisation, which followed immediately after homogenisation, might have facilitated the adsorption of increasing amounts of whey proteins onto the fat globule surface.

A slightly increased casein to whey protein ratio was observed in fat extracted from cheese made with higher homogenisation pressure. This is due to the increased adsorption of casein proteins to fat globules in the milk at the higher homogenisation pressure.

Table 5.15 Effect of manufacturing variables on the protein adsorbed to fat globules (casein to whey protein ratio) extracted from Cream cheese

Source of variation & the levels	Casein to whey protein ratio ¹	
	Least square mean	F ratio
Protein/Fat ratio		14.14*
High	2.164	
Low	1.621	
Homogenisation pressure		3.37
High	2.025	
Low	1.760	
Pasteurisation temperature		14.93*
High	1.614	
Low	2.171	
Curd pH at cooking		0.17
High	1.863	
Low	1.923	
Cooking temperature		0.44
High	1.800	
Low	1.985	

¹ Densitometer plots from gels of SDS-PAGE; * $p < 0.05$; F values without any asterisks denote 'not significant'.

Hardness of Cream cheese: Hardness of cheese was estimated by a penetrometer (Table 5.16). Cream cheese made with a higher cooking temperature was significantly harder than that with a lower cooking temperature. This is in accord with the cheese made at higher cooking temperature being rated as firmer by the panel of judges (described earlier).

Slightly firmer body was noticed in Cream cheeses made with the following process variables: lower P/F ratio, higher homogenisation pressure, higher pasteurisation temperature, lower curd pH at cooking. Some of these effects may have been due to the presence of more fat (solidified state of the fat contributing to hardness), or more denatured whey protein (better water-binding ability).

Cream cheeses at 5°C were firmer in comparison to 20°C. This may be attributed to more of the fat fraction in Cream cheese being in a solid state at the lower temperature (MacGibbon & McLennan, 1987). Cream cheeses after 16 weeks were firmer than those after two weeks of storage. The increase in firmness was detected in all the cheeses, irrespective of whether or not there was exudation. This increase in firmness during storage may be similar to a process of setting in butter where hardness of the butter increases rapidly immediately after manufacture and then at a decreasing rate, approaching a final value asymptotically (Taylor *et al.*, 1973). It is also possible that the cross-linking in the protein network of the Cream cheese increases during aging and increase the firmness.

No relationship between hardness of Cream cheese and exudation could be established.

In a separate experiment it was found that addition of stabilisers *e.g.* Locust bean gum at the rate of 0.025%, helped considerably in firming the Cream cheese body. The stabilised product showed no exudation over eight months of storage at 5°C.

Residual lactose in Cream cheese: Lactose content and the corresponding pH in Cream cheeses of varying age are tabulated in Table 5.17. Lactose was detected in Cream cheese at 16 weeks after manufacture. Repeatability of lactose estimates

in Cream cheese at 16 weeks was not satisfactory. This was due to the difficulty in drawing a representative sample from the thawed Cream cheese. It is unlikely that there has been any breakdown of lactose during storage, because the pH of Cream cheese did not change during this period. In a similar process involving manufacture of Cream cheese spread, electron microscope studies on the microstructure showed that viable lactic bacteria were killed by the heat treatment resulting in highly vacuolised dead cells (Kalab & Modler, 1985).

Table 5.16 Effect of manufacturing variables on the hardness of Cream cheese. Hardness is expressed in terms of distance of penetration (one hundredths of a centimetre) of the cone, with a total weight of 50 g, in a fixed time.

Source of variation & levels	Hardness of product at 5°C				Hardness of product at 20°C			
	Storage period				Storage period			
	Two weeks		Sixteen weeks		Two weeks		Sixteen weeks	
	LSM	F	LSM	F	LSM	F	LSM	F
A		0.16		1.92		0.59		0.51
High	242.4		207.95		190.9		156.31	
Low	213.5		121.45		160.7		142.51	
B		0.13		0.88		0.44		2.35
High	214.9		135.41		162.7		134.58	
Low	240.9		193.98		188.9		164.23	
C		1.10		3.57		0.34		4.66
High	190.5		105.78		164.4		128.52	
Low	265.4		233.61		187.2		170.30	
D		1.45		1.78		1.18		2.67
High	270.9		206.32		197.2		165.20	
Low	185.0		123.07		154.4		133.62	
E		10.44*		2.58		22.76*		99.1**
High	118.6		125.81		144.1		124.11	
Low	337.3		203.58		207.6		174.71	
S.D.	135.4		96.8		26.6		10.2	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature; D = Curd pH at cooking; E = Cooking temperature; LSM = Least-square mean; S.D. = Standard deviation of raw data; F = F ratio; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; F values without asterisks denote 'not significant'.

Table 5.17 Lactose level and corresponding pH of Cream cheeses of varying age

Age of cheese	Lactose content ¹		pH
	(mMol/kg cheese)	(%)	
One day	74.60	2.68	5.08
	74.07	2.67	
	73.97	2.66	
	68.41	2.46	
Sixteen weeks	70.50	2.54	5.04
	71.02	2.56	
	49.89	1.80	
	57.51	2.07	

¹ Results of replicates.

Proteolysis in Cream cheese: Urea-PAGE on Cream cheeses of varying age showed that the casein fractions did not undergo any significant change during the 16 week storage period (Fig 5.4). The areas of caseins determined from densitometer plots are provided in Table 5.18. There was no evidence of any proteolysis in Cream cheese during storage. The ratios of α_{s1} -casein to β -casein should have been close to 1 for the standard casein and the Cream cheeses. This was not so because the bands in the gel were thicker at the centre and gradually narrowed towards the sides for some unknown reason. However, no new bands had appeared in the gel, and visual observation of the protein bands showed no signs of proteolysis.

Proteolysis in exudate of Cream cheese: Urea-PAGE showed that the exudate had a slight increase in the casein fractions with increase in storage time (Table 5.19). This may be due to a general trend, or to a lack of accuracy in quantitative electrophoretic studies (Creamer, 1991). However, no new casein bands or sub-bands had appeared, which further confirmed the absence of proteolysis in Cream cheese.

HPLC studies showed that the amount of peptides detected in the molecular weight range 500 - 10,000 daltons were very low. A small amount of amino acids was detected. Most of the peaks were in the region of casein proteins. It was difficult

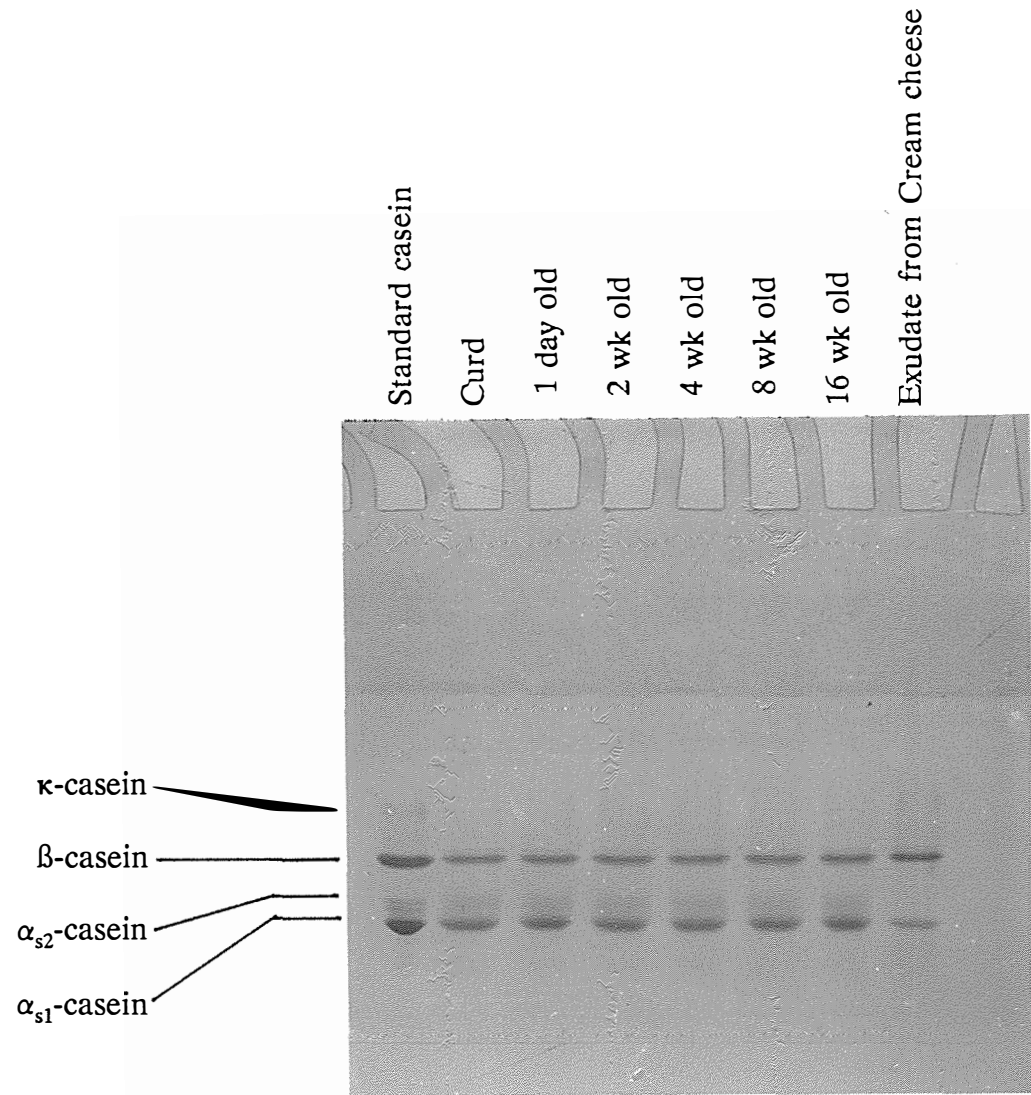


Fig. 5.4 Urea-PAGE of Cream cheese during storage at 5°C.

to identify the peak for whey proteins as they might have overlapped with that of casein.

Conclusion: Proteolysis did not occur during storage of Cream cheese up to 16 weeks. Thus, occurrence of exudation was not caused by any proteolytic changes in Cream cheese. It has been shown earlier that there was no change of pH of Cream cheese during storage. It follows from these findings that exudation is more likely to be influenced by manufacturing conditions rather than the changes during storage.

Table 5.18 Effect of storage time on the casein fractions in Cream cheese (urea-PAGE results)

Particulars	Area occupied by Casein bands ¹				
	α_{s1} - casein	α_{s2} - casein	β - casein	κ - casein	Ratio of α_{s1} - to β -casein
Sample - I					
Standard Casein	1.09	0.1	0.46	0.03	2.36
Curd	0.49	0.12	0.32	0.05	1.53
Cream cheese					
1 day	0.39	0.11	0.23	n.d.	1.69
2 weeks	0.43	0.08	0.23	0.04	1.87
4 weeks	0.39	0.11	0.23	0.04	1.70
8 weeks	0.46	0.09	0.25	0.04	1.84
16 weeks	0.42	0.07	0.23	0.02	1.83
Sample - II					
Standard Casein	0.86	0.23	0.55	0.14	1.56
Curd	0.39	0.15	0.26	0.03	1.50
Cream cheese					
1 day	0.46	0.18	0.29	0.03	1.59
2 weeks	0.41	0.20	0.30	0.03	1.37
4 weeks	0.41	0.15	0.26	0.04	1.58
8 weeks	0.40	0.12	0.28	0.07	1.43
16 weeks	0.48	0.15	0.28	0.05	1.71

¹ Estimated from the densitometer plots. The units are arbitrary.

n.d. = Not detected by the densitometer plots.

Table 5.19 Effect of storage time on the casein fractions in exudate from Cream cheese (urea-PAGE results)

Age of Cream cheese from which exudate was obtained	Area occupied by Casein bands ¹				
	α_{s1} -casein	α_{s2} -casein	β -casein	κ -casein	Ratio of α_{s1} - to β -casein
8 weeks	0.19	n.d.	0.23	n.d.	0.83
18 weeks	0.21	0.02	0.29	0.15	0.72

¹ Estimated from the densitometer plots. The units are arbitrary.

n.d. = Not detected by the densitometer plots.

(d) **Effect of manufacturing variables on exudation:** The effects of the manufacturing variables on the exudation (mean values estimated using ANOVA and test of significance using X^2 test) are listed in Table 5.20. Most of the effects were similar to the previous experiment. Since the statistical design was only fractional-factorial, the effects detected were less significant.

Effect of P/F ratio (0.23 & 0.30) on exudation: Similar MNFS values for the two levels of P/F ratio could not be attained apparently due to lack of adequate control during cheese manufacture and analyses. Estimation of the moisture content in the microwave analyser, though quick, provides only a rough estimate. This might have been the biggest source of error.

Cheese made from milk standardised to the lower level of P/F ratio had higher MNFS and the amount of exudate from this was greater (Table 5.20). The increase in the amount of exudate with increased MNFS is consistent with the presumed concept that an increase in the moisture content of the non-fat substance, beyond the limit of what it can hold, would be proportional to the amount of exudate released from cheese.

The moisture content of cheese made from milk with the lower level of P/F ratio was slightly lower, and the fat content was significantly higher (Table 5.11). In spite of a slightly lower moisture and a higher fat content, the amount of exudate

Table 5.20 Effect of manufacturing variables on the exudation of whey from Cream cheeses of constant MNFS

Sources of variation & levels	Least-square means of exudation (g/kg cheese moisture)	No of incidences of exudation			χ^2
		Nil	Slight ^a	Excess ^b	
A					5.95*
High	0.87	43	5	0	
Low	10.31	36	7	5	
B					13.65**
High	2.43	37	11	0	
Low	8.75	42	1	5	
C					20.66***
High	0	48	0	0	
Low	11.17	31	12	5	
D					5.32#
High	1.56	42	6	0	
Low	9.61	37	6	5	
E					20.66***
High	0	48	0	0	
Low	11.17	31	12	5	
Storage time					0.05ns
2 weeks	1.07	14	2	0	
4 weeks	3.09	13	2	1	
6 weeks	4.74	13	2	1	
8 weeks	5.96	13	2	1	
12 weeks	9.46	13	2	1	
16 weeks	9.21	13	2	1	

^a slight = 1-20 g exudate/kg cheese moisture;

^b excess = > 20 g exudate/kg cheese moisture;

A = P/F ratio; B = Homogenisation pressure;

C = Pasteurisation temperature; D = Curd pH at cooking;

E = Cooking temperature;

χ^2 = Chi-square; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;

= $p < 0.10$; ns = not significant.

from these cheeses was significantly higher. This clearly showed that fat was not effective in reducing the extent of exudation. It is reported that in yoghurt the mechanical properties of the milk gel is changed and the water-binding capacity of the gel is improved by increasing the levels of milk solids (Brooker, 1987). However, present findings on Cream cheese indicate that it is primarily the protein that accounts for the water-binding capacity of the gel structure, and that fat is not effective in binding the moisture.

Effect of homogenisation pressure (2000/500 psi & 600 psi) on exudation: The average amount of exudate was significantly less in Cream cheeses made from milk homogenised at a higher pressure (Table 5.20). This happened in spite of a slightly higher moisture in cheese. It was shown earlier that increase in homogenisation pressure resulted in a decrease in the mean diameter of the fat globules. Walstra (1975) reported that size-reduction of fat globules in milk occurs due to homogenisation with about a six-fold rise in fat globule surface area. It was found that with an increase in homogenisation pressure more casein are adsorbed to the fat globule surface. This is expected to make the casein gel structure of casein matrix more rigid due to a net increase in the number of linkages of molecules between fat and protein. The gel matrix is also likely to be less permeable to water due to an increase in the fat globule surface area. These effects of homogenisation would have contributed to the reduction in the amount of exudate from Cream cheese made with higher homogenisation pressure.

While the amount of exudate was higher from cheese made with low pressure homogenisation, the incidence of exudation was lower. This was due to a large amount of exudate released from the cheeses in which exudation occurred. The practical implication of this is significant. If it is possible to reduce the amount of exudate in the cheese by interaction with other manufacturing conditions, it would be possible to use the low homogenisation pressure during cheese manufacture.

Effect of pasteurisation temperature (72°C & 82°C) and cooking temperature (60°C & 75°C) on exudation: Cream cheese made from milk pasteurised at a higher temperature did not have any exudation (Table 5.20). Similarly, cooking

curd at a higher temperature also prevented exudation from Cream cheese. The analyses on WPNI showed that more whey protein was denatured due to the higher heat treatments. The effect of denatured whey protein in binding more moisture and providing a firm body to the product is likely to have prevented exudation. These results are comparable with the studies on yoghurt where the effect of heating milk results in greater water-binding ability due to interaction of denatured whey protein (β -lactoglobulin) with casein micelles (Dannenberg and Kessler, 1988). The high water-holding capacity and greater firmness due to high heat treatment was explained as due to the formation of a compact, heavily branched protein network of the gel (Parnell-Clunies *et al.*, 1987).

Effect of curd pH at cooking (4.7 & 5.0) on exudation: The average amount of exudate and the incidence of exudation were both greater at the lower curd pH at cooking (Table 5.20). This might have been caused by the fact that the lower curd pH at draining, and consequently the cheese pH, were both closer to the isoelectric point of casein at which the hydrating properties of casein is expected to be the minimum.

Effect of storage time (Table 5.20): The Chi-squared test showed that the increase in the incidence of exudation was not affected by the increase in storage time. Pooled data from all the trials on Cream cheeses showed that of all the cheeses that had exudation, 90% exuded within the first two weeks of manufacture. This observation may have a practical relevance. Usually it would take over a week for the product to reach the costumers. This time provides an opportunity for the manufacturer to examine whether or not the cheeses exude and to decide about releasing the product to the market.

The least-square mean values of the amount of exudates are consistent with the earlier findings of a gradual increase in the amounts with increase in storage time (Fig. 5.3, page no. 78).

5.4 OVERALL SUMMARY AND CONCLUSION TO CHAPTER 5

Composition of curd was primarily influenced by the effect of the selected manufacturing variables on the amount of whey drained during the manufacture of cheese. Pasteurisation of milk at a higher temperature, homogenisation of milk at a higher pressure and cooking curd at a lower pH resulted in slow drainage of whey (during cheese manufacture) and increased moisture in curd. Accurate process control is required, particularly during heat-processing of curd, to adjust the moisture or MNFS to the desired level in Cream cheese. A greater amount of curd-fines are lost at a higher cooking temperature. The curd pH at cooking primarily determined the pH of Cream cheese.

There was no evidence of proteolysis up to 16 weeks in Cream cheese stored at 5°C. Residual lactose was detected in Cream cheese at the end of 16 weeks of storage. There was very little change in pH of Cream cheese during this storage period.

The amount of exudate increased with increase in the MNFS in Cream cheese. Within the selected levels of variation of P/F ratio, change in fat content of Cream cheese did not affect exudation. Homogenisation resulted in a decrease in the mean diameter of fat globules in the milk. Homogenisation of milk at a higher pressure resulted in adsorption of increased amounts of casein on the fat globule surface, both in the milk and Cream cheese. Coating of fat globules with casein resulted in fat globules behaving as part of the casein matrix. The increase in the fat globule surface area and casein-coating over the fat globule surface explain the reduced amount of exudate in Cream cheese made from milk homogenised at a higher pressure. Increased heat treatment through pasteurisation and cooking of curd resulted in increased denaturation of whey protein which was effective in reducing the amount of exudate. In most cases cooking of curd at the higher temperature prevented exudation from Cream cheese. The curd pH at cooking had a smaller effect on exudation in comparison to other variables. Increase in the amount of exudate from cheese at lower pH is possibly due to the closeness of the pH of cheese to the iso-electric point of casein, the major protein in Cream cheese.

Increase in the amount of exudate was observed with increase in storage temperature and increase in storage time. Use of a stabiliser, locust bean gum at the rate of 0.025%, was effective in preventing exudation.

The effect of the selected manufacturing variables in reducing the extent of exudation from Cream cheese was in the following decreasing order of importance: cooking temperature > pasteurisation temperature > homogenisation pressure $\geq$ P/F ratio in milk > curd pH at cooking.

Cream cheeses which do not develop exudation in the first two weeks are unlikely to exude at later stages of storage. No relationship could be established between the hardness of Cream cheese and the exudation. It is concluded that exudation is not caused by any gross change in the composition of hot-packed Cream cheese.

CHAPTER 6

EXUDATION OF WHEY FROM FETA CHEESE DURING STORAGE

6.1 Introduction

Feta cheese was chosen as a model cheese for the study on exudation because:

- it is a ripened cheese,
- exudation occurs during storage of all Feta cheeses,
- it is an important variety of cheese and has commercial significance,
- it represents brine salted cheese,
- it was desirable to improve the understanding about recombined¹ milk cheeses, and
- it was expected to provide results in a reasonably quick time (<6 months).

Very little is reported on factors affecting exudation from Feta cheese during storage and the mechanism of exudation. However, some information is reported on the change in weight of Feta type cheeses during storage (Mansour & Alais, 1972; Abd El-Salam *et al.*, 1981; Alichanidis *et al.*, 1984; Abd El-Salam, 1987; Vafopoulou *et al.*, 1989) and variation in moisture content during storage (Omar & Buchheim, 1983). Most of these studies are related to storage of cheese in brine. Mansour and Alais (1972) investigated some aspects on Feta type cheese vacuum-packed in Cryovac wrapped plastic bags. They reported that the loss of weight in this cheese was about 1.35% (2.4% moisture) when stored at 10°C for 120 days, which was less compared to the weight loss from brine-stored cheese. The present investigation is restricted to vacuum-packed Feta cheese made from recombined milk.

¹ Feta cheese is normally made from fresh milk. In this study Feta cheese was made using recombined milks. The technology of recombined milk cheese was important for New Zealand cheese industry in view of its potential for export. The other advantage of using recombined cheese is described later in this Chapter.

The scope of this study was to determine the effect of selected manufacturing variables on exudation and determine the possible causes. This Chapter is divided to following sections:

- (a) Preliminary studies.
- (b) Chemical, biochemical and microbiological changes in Feta cheese and exudate during storage at 10°C.
- (c) Effect of selected manufacturing variables on exudation from Feta cheese.

SECTION ONE

6.2 PRELIMINARY STUDIES

The objective of the preliminary studies was to develop a scientific method for study of exudation of whey from Feta cheese during storage. The scope of this experiment was:

- (i) To standardise the manufacturing process for Feta cheese using low heat skim milk powder (LHSMP) and fresh frozen milkfat for recombining (FFMR).
- (ii) To study the effect of vacuum packing cheese in plastic pouches and determine the suitability of the vacuum-packed cheese for the measurement of the amount of exudate.
- (iii) To determine the effect of storage temperature of Feta cheese on exudation.
- (iv) To determine the effect of factors related to handling of cheese on the exudation, *e.g.* size of cheese blocks, temperature variation, turning the blocks, vacuum packaging.

6.2.1 Experimental

Major deviations in the manufacture of Feta cheese from the conventional process were:

- Cheese after brining was packed in vacuum sealed plastic bags instead of storing in brine. The procedure was similar to that described by Mansour

& Alais (1972). This form of packaging is more suitable as a consumer pack and also convenient to measure the amount of exudate during storage. It was hoped that the findings from this study could be easily applied to other cheeses because of the packaging resemblance to most other varieties of cheeses.

- Recombined cow's milk was used instead of fresh sheep's milk. The process is similar to that described by Gilles (1974).

Feta cheese made during these studies was used to acquaint the panel of judges with the product characteristics, and develop the sensory profile. Variations were applied as described in the results and discussion.

Cheese manufacture

Raw Materials: LHSMP and FFMR were used as the source of milk solids. In the text Cream prepared using FFMR and reconstituted skim milk is referred as *manufactured cream*. Recombined milk was chosen for manufacture of Feta cheese to avoid the effect of seasonal variation in the composition of fresh milk, and to gain knowledge on aspects related to recombined milk cheese.

Manufacturing process: The equipment and accessories used for manufacture of Feta cheese are outlined in Appendix 6.1. The flow-diagram for the manufacturing process is outlined in Fig. 6.1. Detailed description of the process is provided in Appendix 6.2. Some of the manufacturing stages are shown pictorially in Fig. 6.2.

6.2.2 Results and discussion

This is described in two parts - the first part is on aspects related to cheese manufacture and the second part is on aspects related to exudation.

(a) Aspects related to cheese manufacture

(i) Bacteriological quality: The recombined milk, cheese before brining and cheese after brining were analysed for bacteriological quality periodically. Coliforms were

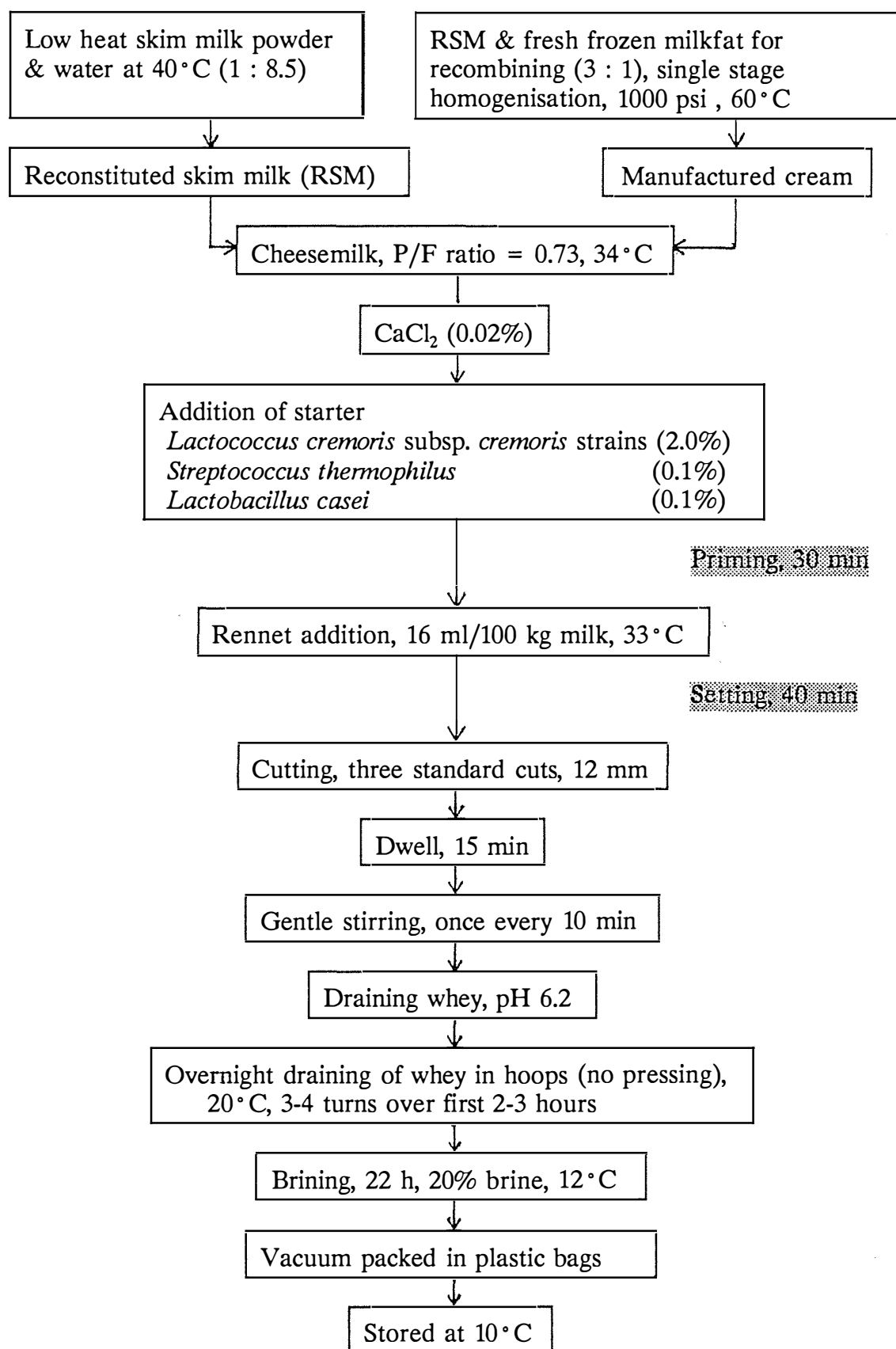


Fig. 6.1

FLOW DIAGRAM FOR MANUFACTURE OF FETA CHEESE



Fig. 6.2 **Selected stages in Feta cheese manufacture.**

Top row (left):	Reconstitution of low heat skim milk powder in water;
Top row (right):	Cheesemilk in vat;
Bottom row (left):	Hoops used in cheese manufacture;
Bottom row (right):	One-half of a block of Feta cheese, removed from a hoop after overnight draining (cheese before brining).

usually absent in milk and cheese, which indicated that satisfactory hygienic conditions were maintained during manufacture of cheese. Details of total microbiological counts have been described later (Chapter 6.3.2).

(ii) Sensory evaluation: Feta cheeses were generally found acceptable by the sensory panel.

(iii) Type of SMP: Low heat, medium heat and high heat SMP were used for making Feta cheese. With the use of high heat SMP the curd was extremely soft and difficult to handle, too many fines were lost in whey, and the moisture in cheese before brining was about 60%. The problem was less severe with medium heat SMP. There was no such problems with the use of low heat SMP and moisture content in cheese before brining was around the desired value of 52%.

(iv) Cutting of curd: With two cuts, too many curd-fines were lost into whey and the moisture content of cheese before brining was slightly high (54.0%). With three standard cuts, the moisture dropped to about 52.0% and fewer fines were lost to whey.

(v) Curd size: When the curd was cut with 9 mm knives the moisture in cheese was lower, *i.e.* 48 - 50%. This was overcome by using 12 mm knives.

(vi) Brining time: Typical data on the effect of brining time and block size on S/M in cheese, and consequently on exudation is presented in Table 6.1. The effects are similar to findings on Gouda cheese (Geurts *et al.*, 1980) in that the duration of brining affected the salt uptake, the water content and weight loss. The period of brining was chosen as 22 h for block sizes of (100 X 100 X 50) mm.

Amount of exudate decreased with increase in brining time probably because of reduced moisture content in cheese.

Table 6.1 Effect of brining time on the salt content and exudation of Feta cheese

Brining time h	Approximate block size mm	Moisture %	Salt %	S/M %	Exudate amount after 7 weeks g/kg cheese
48	200 X 100 X 50	49.0	5.56	11.3	44.9
28	200 X 100 X 50	52.9	4.65	8.8	49.5
24	200 X 100 X 50	56.1	3.88	6.9	74.8
22	100 X 100 X 50	51.0	4.34	8.5	n.d.

n.d. = not determined

(b) Aspects related to exudation

(i) Effect of variation in size of cheese block on exudation: The exudation was expected to be influenced by the geometric constraints of the curd, like surface area and distance over which the exudate has to flow. Variation of size had a slight effect on the amount of exudate during the early stages of storage, but this was not detected with increase in storage time (Table 6.2).

Table 6.2 Effect of variation in size of cheese block on the exudation of whey from Feta cheese

Weight of block (g)	Amounts of exudate (g/kg cheese) at 10°C			
	7 days	35 days	77 days	105 days
1550	9.7	20.6	34.2	41.3
874	11.4	22.9	34.3	41.2

Note: Data presented above is a typical example. A number of such trials were carried out and the trend in the results were similar.

(ii) Effect of block to block variation of Feta on exudation No definite trend in the extent of exudation was detected due to block to block variation of cheese from the same batch (Table 6.3). It appears that there is a slight variation in the amount of exudate during the initial storage, but this becomes less evident with increase in time. This however provided the useful information that different cheese blocks of similar size, shape and weight from the same batch could be used for measuring the amounts of exudate at different storage intervals.

Table 6.3 Effect of block to block variation in cheese manufactured from the same vat on the exudation

Storage at 10°C			Storage at 15°C		
Weight of block (g)	Amount of exudate (g/kg)		Weight of block (g)	Amount of exudate (g/kg)	
	10 days	30 days		10 days	30 days
735	15.0	25.9	796	26.4	46.5
766	13.1	23.5	809	27.2	44.5
817	14.7	24.5	818	28.1	45.2
827	13.3	23.0	851	27.0	42.3

(iii) Effect of mechanical handling of cheese blocks on exudation: The effects of some of the factors expected to be encountered during the course of routine handling of cheese on the exudation were investigated. To minimise the experimental error due to block to block variation, blocks of cheese of approximate size (200 X 100 X 50) mm were divided into two equal halves and used for comparative study.

Effect of vacuum packaging (Table 6.4): Amount of exudate was less from vacuum-packed cheese. This effect may be explained as follows. The exudate may be assumed to be held by a capillary action in the protein matrix in cheese. Vacuum packaging results in the plastic bag firmly sticking on to the cheese surface

and thereby blocking the openings at the ends of the capillaries. This restricts the outward flow of exudate. The concept of plastic layer sticking to the opening of capillaries and restricting the release of exudate was experimentally verified by providing space between the cheese and the plastic bag. Free space was artificially created around cheese by wrapping it with cheese cloth or cleaning pads before vacuum packing. Large amounts of exudate were released (Table 6.5). In addition to the advantage of reducing the extent of exudation from cheese, vacuum packaging of cheese was considered a necessity for reasons of controlled microbial activity and increased shelf-life of the cheese.

Unpacking and repacking of cheese in vacuum each day significantly affected the amount of exudate released (Table 6.5). This implied that one block of cheese could be used only once for determining the amount of exudate.

Table 6.4 Effect of vacuum packaging on the exudation of whey from Feta cheese

Treatment	Amount of exudate (g/kg cheese)			
	Storage at 10°C		Storage at 15°C	
	10 days	30 days	10 days	30 days
No vacuum	31.8	33.2	61.6	74.2
Vacuum packed	18.0	30.4	42.9	58.6

Turning and piling (Table 6.5): Turning and piling cheese blocks (one on top of another) over a period of two weeks did not affect the exudation.

Thermal shock: Thermal shock provided to the cheese by shifting the product from 10°C to 20°C for 30 min every day resulted in an increase in the amount of exudate (Table 6.5). The product is expected to have thermal shocks during transfer at different stages after manufacture, such as during transportation from

factory to retail outlet and then to the consumer. These results are consistent with the observation that exudation occurs during shipment of Blue cheeses, particularly when temperature fluctuations occur (Pedersen *et al.*, 1971).

Table 6.5 Effect of miscellaneous factors on the exudation of whey from Feta cheese

Treatment	Amount of exudate (g/kg cheese) after 1 wk of storage at 10°C
Control	11.15
Cheese cloth wrapped between vacuum-packed cheese and plastic pouch	71.42
Control	24.16
Cleaning pad wrapped between vacuum-packed cheese and plastic pouch	139.57
Control	10.37
Vacuum-packed cheese unpacked and vacuum packed on 3rd and 5th days	17.0
Control	14.68
Turning/piling every day	14.58
Control	20.51
Pack left at 20°C for 30 min every day	23.77

(iv) Effect of fat content in cheese on exudation: A decrease in the amount of exudate was observed in cheese with increased fat content (Table 6.6).

Table 6.6 Effect of fat content in cheese on exudation

Age of cheese (weeks)	Amount of exudate (g/kg cheese) at 10°C	
	Control (23%)	High fat (29%)
1	8.7	6.8
7	30.2	22.6
15	47.7	35.0

(v) Effect of storage temperature on exudation: Increase in storage temperature increased the amount of exudate obtained from cheese during storage (Table 6.7). This effect may be the result of a variation in solid fraction of fat due to variation

in storage temperature (MacGibbon & McLennan, 1987), or decrease in water sorption by milk proteins with increasing temperatures (Kinsella & Fox, 1987). The mechanism of increase in the amount of exudate with increased storage temperature may be somewhat similar to the increase in syneresis in renneted gels with increase in temperature during cheese manufacture (Dijk, 1982; Walstra & Vliet, 1986). Increase in storage temperatures in this range has an increasing effect on proteolysis (Lawrence *et al.*, 1987). It is likely that exudation and proteolysis are interrelated.

Table 6.7 Effect of storage temperature and storage time on exudation of whey in Feta cheese (Composition of cheese: 23% fat, 50.7% moisture, 4.1% NaCl, pH 4.7)

Age of cheese (weeks)	Amount of exudate (g per kg cheese)		
	Storage temperature		
	5 °C	10 °C	20 °C
1	4.7	8.7	25.9
7	12.8	30.2	63.6
15	21.5	47.7	137.0

(vi) Suitability of measurement of the amount of exudate: It was easy to isolate the cheese from the exudate and record the weights. Details on measurement of exudation are provided in Chapter 4.

(vii) Conclusion: Some of the findings of the above study have important commercial and scientific implications. It appears that the exudate is readily available for exudation from cheese. The thermodynamic effect helps accelerate the process of exudation; while the kinetic effect on exudation is impeded by barriers, such as blockage of the ends of capillaries of the protein matrix and the presence of fat in the protein matrix. From a commercial viewpoint the cheesemaker may find it an advantage to be able to pile cheese blocks one over another. However, it is important to ensure proper vacuum packaging and impart minimal thermal shocks to the product during transportation and distribution.

From a scientific viewpoint, it is worthwhile noting that the number of times of vacuum packaging of the product, particularly every time a plug of cheese is drawn for sampling, could be contributing to the exudation from cheese.

SECTION TWO

6.3 CHEMICAL, BIOCHEMICAL AND MICROBIOLOGICAL CHANGES IN FETA CHEESE AND EXUDATE DURING STORAGE AT 10°C

The aim of this study was to investigate some of the chemical, biochemical and microbiological changes in Feta cheese during storage at 10°C.

6.3.1 Experimental: Cheese was manufactured as described in Chapter 6.2.1. Cheese samples were drawn at the required storage interval. Usually one whole block of cheese was grated and a representative sample was drawn for analysis. Exudate from cheese was centrifuged at 3,000 rpm at 4°C for 5 min and filtered through Whatman filter paper (No. 41) prior to analysis. Analyses were performed as specified in the results and discussion. Details on analytical methods are provided in Chapter 4.

6.3.2 Results and discussion

(a) Proteolysis

Urea-PAGE of Feta cheese: The proteolysis of casein proteins in Feta cheese at various stages of storage is shown in Fig. 6.3. Breakdown of α_{s1} -casein and β -casein during storage of cheese is shown in Fig. 6.4.

The α_{s1} -casein had undergone substantial degradation by the end of four weeks. Subsequent breakdown appeared to be much slower in comparison. After twelve weeks very little of the α_{s1} -casein remained and further breakdown was very slow. In contrast, the β -casein was resistant to proteolysis with very little degradation in the first seven weeks. This was followed by a slow and gradual breakdown up to 26 weeks.

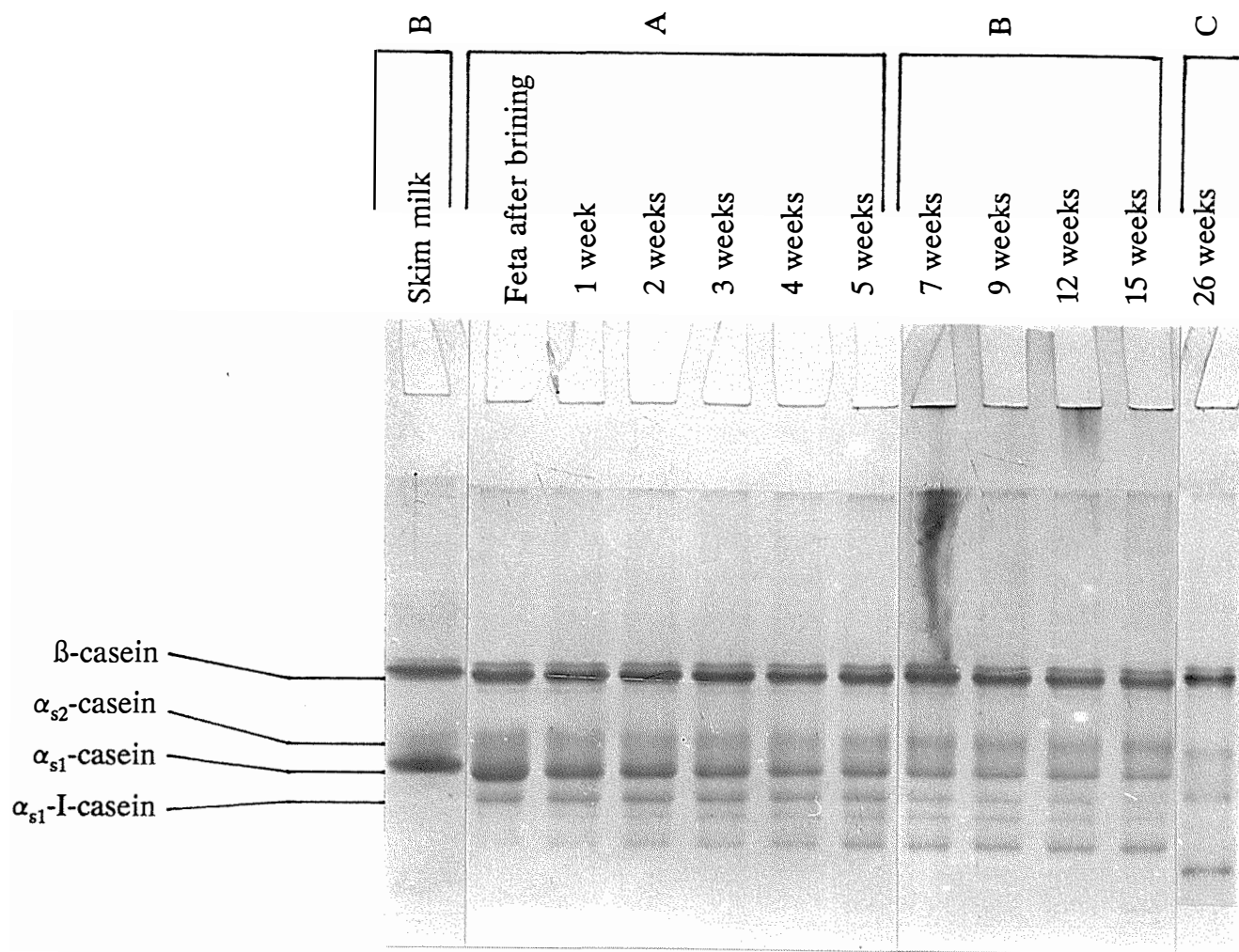


Fig. 6.3 Casein proteolysis in Feta cheese during storage at 10°C (Urea-PAGE). A, B and C are different gels.

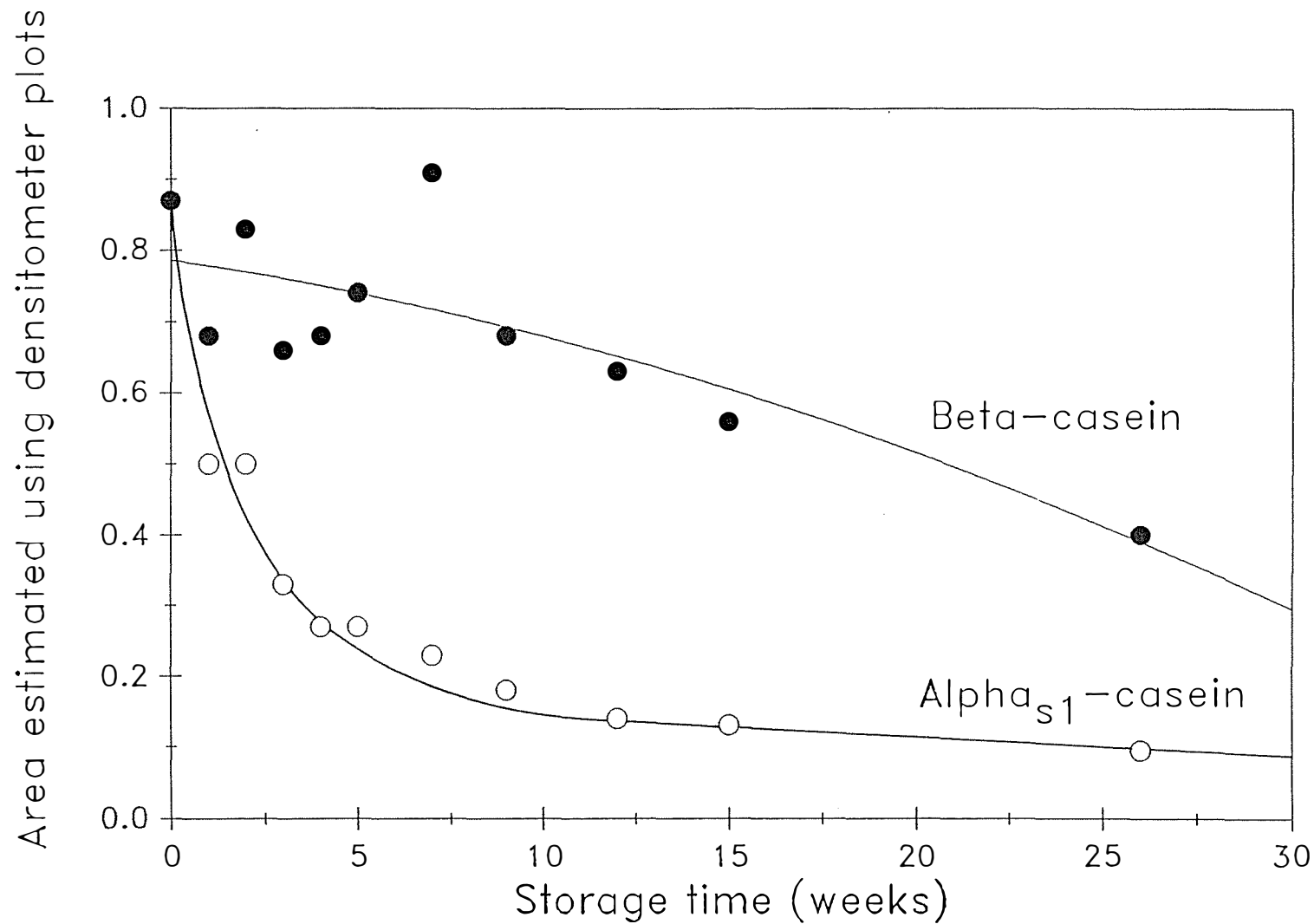


Fig. 6.4 Casein protein degradation during storage of Feta cheese (Urea-PAGE)

Nearly half of the β -casein was intact in cheese after 26 weeks (Table 6.8). These results are similar to proteolysis reported for Gouda cheese (Visser & Groot-Mostert, 1977). Alichanidis *et al.* (1984) reported that β -casein in Feta cheese from ewe's milk resists hydrolysis, with α_{s1} -casein hydrolysing rapidly.

Proteolysis in Feta cheese was thus detected in the early stages after manufacture and continued at least up to 26 weeks of storage. α_{s1} -casein was most susceptible to proteolysis. β -casein was more resistant to proteolysis though it was slowly degraded at later stages of storage.

Proteins in exudate and their proteolysis

SDS-PAGE on exudates from Feta cheese during storage: Fig. 6.5 shows the proteins present in exudates at various storage intervals. The exudate contained mostly whey proteins and virtually no casein proteins. Densitometer plots were used to measure the area of α -lactalbumin and β -lactoglobulin bands in the gel (Table 6.8). The areas are relative estimates with arbitrary units. A slight increase in the whey protein content was detected with increase in storage time. This was probably due to the release of whey proteins held in the protein matrix into the aqueous fraction. This may be explained by the gradual weakening of the casein gel matrix due to proteolysis during storage. Both α -lactalbumin and β -lactoglobulin did not degrade during this period, and no new bands appeared. It was concluded that the whey protein fraction was not affected by proteolysis during storage.

HPLC on exudate: Profiles of low molecular weight peptides from exudates at different storage intervals have been provided in Fig. 6.6. The whey protein fraction (10,000 - 50,000 daltons) appears to have had little change all along this period. The peptides below molecular weight 5,000 daltons were presumably casein fragments. There was a gradual increase in the peptides in this lower molecular weight range, and the breakdown products seem to contain di- and tri-peptides, and amino acids. The protein fractions eluting prior to whey proteins (molecular weight > 50,000 daltons) are probably serum albumins and immunoglobulins. There is indication of ongoing casein proteolysis during storage.

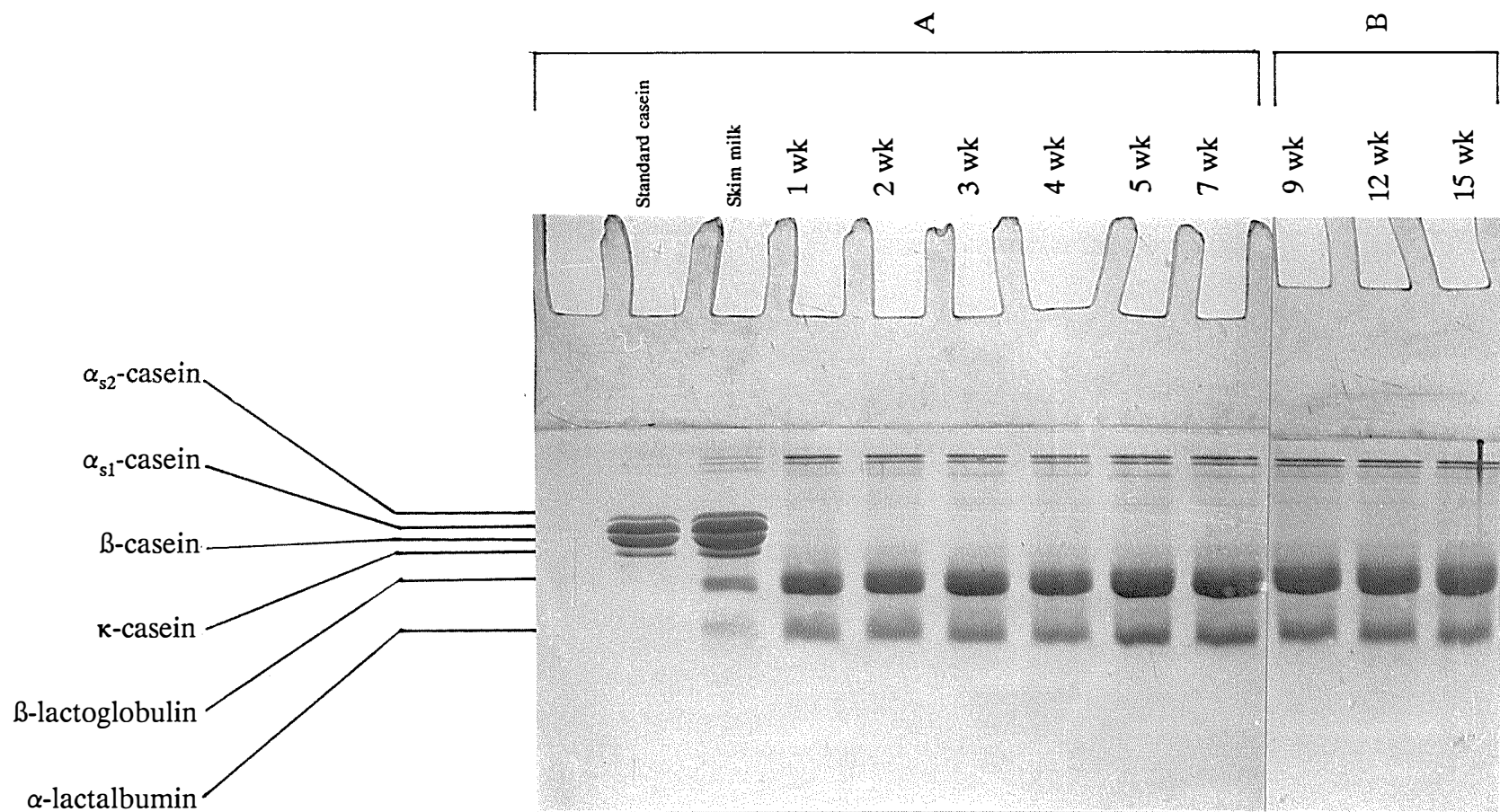


Fig. 6.5 SDS-PAGE on exudates from Feta cheeses of different age. A and B are different gels.

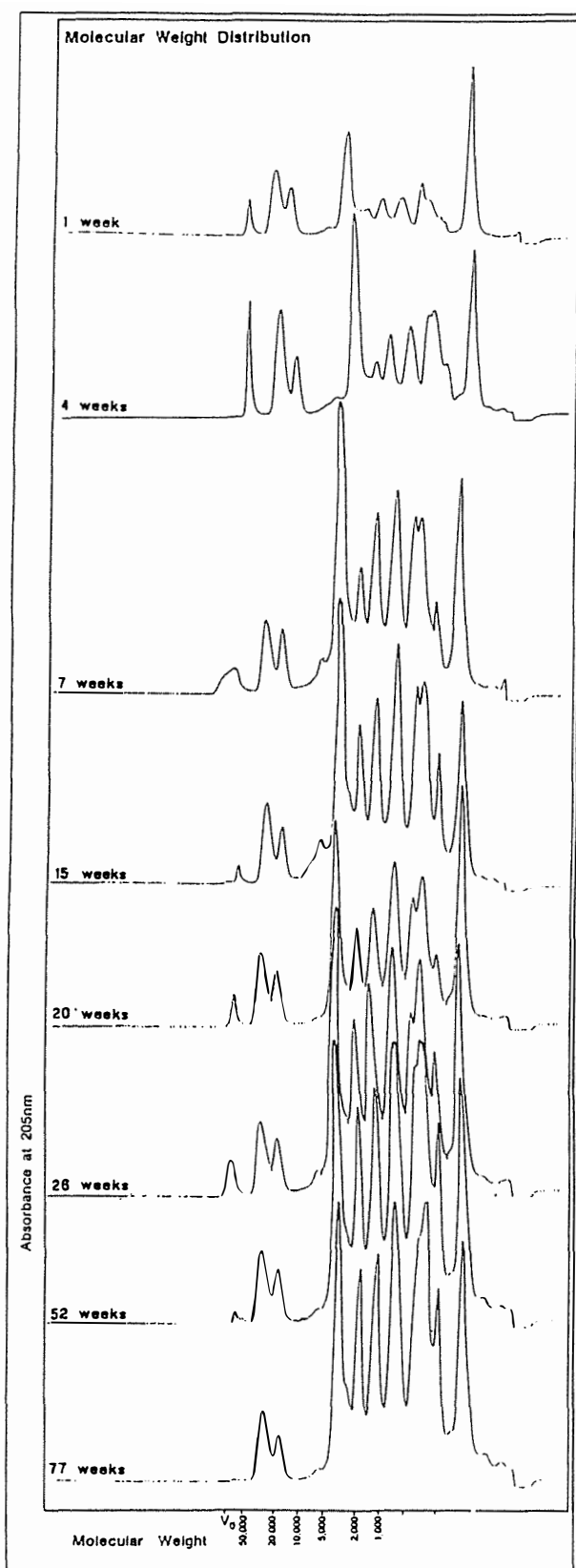


Fig. 6.6. Pattern of distribution of peptides in exudates from Feta cheeses of different ages (HPLC technique).

Table 6.8 Concentration of major proteins in Feta cheese and exudate during storage at 10°C

Area (arbitrary units) obtained using densitometer				
Storage period	Feta cheese		Exudate from Feta	
(weeks)	α_{s1} -casein	β -casein	α -la ¹	β -lg ²
0	0.87	0.87	n.a.	n.a.
1	0.50	0.68	0.55	1.31
2	0.50	0.83	0.67	1.33
3	0.33	0.66	0.56	1.32
4	0.27	0.68	0.60	1.31
5	0.27	0.74	0.76	1.64
7	0.23	0.91	0.71	1.59
9	0.18	0.68	0.73	1.56
12	0.14	0.63	0.69	1.53
15	0.13	0.56	0.71	1.49
26	0.09	0.40	n.d.	n.d.

¹ = α -lactalbumin; ² = β -lactoglobulin;
n.d. = not determined; n.a. = not applicable.

(b) Changes in pH and residual lactose of Feta cheese and exudate during storage

A substantial amount of residual lactose was detected in Feta cheese and exudate after 26 weeks of manufacture (Table 6.9). The pH of Feta cheese decreased very slowly during this storage period. These results are in contrast to the reported findings that lactose in Feta type cheeses disappeared within one month of storage (Omar & Buchheim, 1983) and the acidity of Feta cheese stored in Cryovac wrapped cheese stopped increasing after 30 days (Mansour and Alais, 1972). However, this is consistent with the evidence that lactose persisted for at least six months in Cheddar cheese with 6-7% S/M (Thomas and Pearce, 1981). The decrease in lactose content was very slow during storage. This is consistent with the findings that at high S/M levels (about 6%) in Cheddar cheese, starter metabolism virtually stopped (Turner and Thomas, 1980). In a study on 'Noordhollandse Meshanger' cheese, Noomen (1977) reported that the pH of cheese was regulated by the growth of very slow-producing lactic acid bacteria, whose activity depended upon the salt to moisture ratio in cheese. Here it is very unlikely that any of the lactic organisms would have survived the low pH and high

salt concentration for such a long time except for the lactobacilli strain (*Lactobacillus casei*) and the non-starter lactic acid bacteria (NSLAB). Anon. (1969b) determined that after two months of manufacture Feta cheese made with *Lactobacillus casei* strain had much higher total counts than Feta cheese made without it. This showed the ability of *Lactobacillus casei* to survive in conditions of high acid and low pH.

Table 6.9 Residual lactose in Feta cheese and exudate at different storage intervals

Storage time	Feta cheese		Exudate of Feta	
	Lactose (mM/kg)	pH	Lactose (mM/kg)	pH
Before brining	48.0	4.84	n.a.	n.a.
After brining	38.0	4.67	n.a.	n.a.
4 weeks	35.7	4.64	n.d.	4.64
9 weeks	34.4	4.57	67.5	n.d.
15 weeks	24.5	4.56	57.6	4.54
20 weeks	28.1	4.50	n.d.	n.d.
26 weeks	27.0	4.48	47.6	4.44
52 weeks	n.d.	n.d.	46.1	n.d.
77 weeks	n.d.	n.d.	n.d.	4.20

n.a. = not applicable; n.d. = not determined

It was hypothesised that metabolism of residual lactose to lactic acid could be contributing to the slow decrease in pH during storage. An experiment was carried out to check if the drop in pH of cheese during storage was proportional to the presumed conversion of lactose to lactic acid. Feta cheese after brining was grated and aliquots of this (20 g) were mixed with increasing amounts of lactic acid of known concentration at 20°C. The corresponding changes in pH values were measured. To negate the effect of water present in lactic acid, equal quantities of milli-Q² water were added to aliquots of cheese, and the corresponding change in pH were measured. The net difference in pH values obtained were plotted against the added lactic acid (Fig. 6.7). The curve in the normal pH range for Feta cheese (4.7-4.3) was nearly a straight line. The buffering capacity of curd, largely

² Water is purified by reverse osmosis and Milli-Q treatment (Millipore Corporation, Bedford, MA).

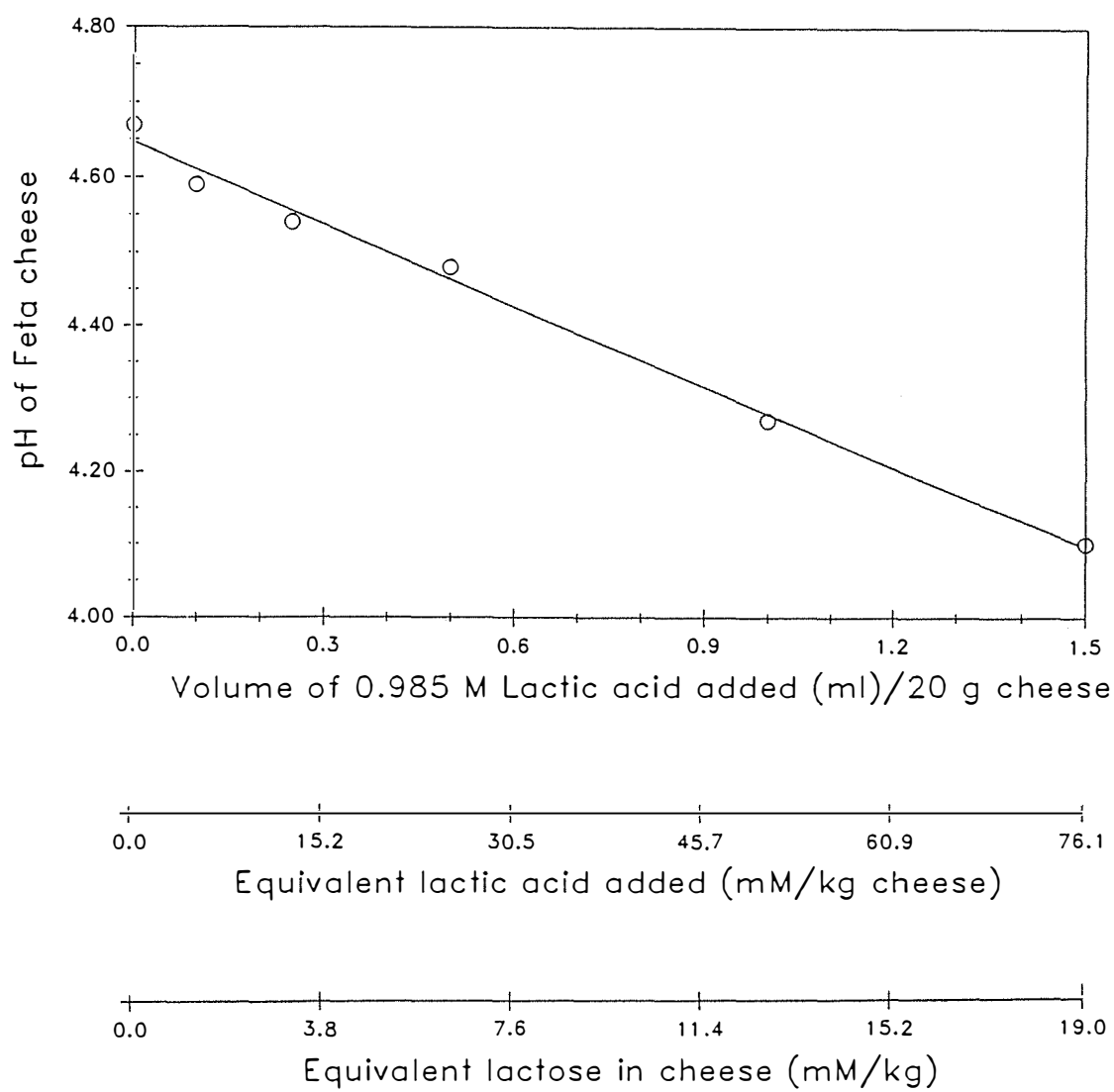


Fig.6.7 Change in pH of Feta cheese (after brining) with addition of lactic acid.

determined by the protein and the phosphates present (Lawrence and Gilles, 1987), was very low due to a reduced pH and reduced mineral level in the cheese. Using this curve it was estimated that the lactic acid formed as a result of the decrease in quantity of lactose in cheese over six months of storage would have lowered the cheese pH slightly more than the observed decrease. This 'reduced decrease' in pH may be attributed to the formation of proteolytic breakdown products like ammonia (Fox, 1989), which neutralises some of the effects of lactic acid.

(c) Formation of lactates in Feta cheese and exudate during storage (Table 6.10):

Detection of lactates in stored cheese and exudate indicated the metabolism of lactose to lactic acid by the microbial flora. Starter bacteria produce only L-(+)-lactate. Conversion of L-(+)- to D-(-)- lactates represents racemisation and indicates the presence of non-starter lactic acid bacteria (NSLAB). 90% of the lactates present in Feta cheese obtained from a commercial source were in D-form, suggesting growth of adventitious bacteria (Thomas & Crow, 1983). However, in this experiment both Feta cheese and exudate contained negligible amounts of D-(-)-lactate while most of the lactates were present as L-(+)-Lactate. This suggested that NSLAB were not active in cheese. Even if NSLAB were present in cheese, they were probably inactive due to the high salt concentration and low pH of cheese.

Table 6.10 Quantity of lactates present in Feta cheese and exudate at varying storage intervals (mM/kg)

Storage time	Lactates in Feta cheese			Lactates in exudate		
	L-(+)	D-(-)	Total	L-(+)	D-(-)	Total
BB ¹	114.9	1.7	116.6	n.a.	n.a.	n.a.
3 days	104.7	1.9	106.6	n.d.	n.d.	n.d.
4 weeks	105.7	2.3	108.0	n.d.	n.d.	n.d.
9 weeks	107.0	2.5	109.5	207.5	2.6	210.0
26 weeks	n.d.	n.d.	n.d.	191.9	9.2	201.1
52 weeks	n.d.	n.d.	n.d.	234.2	7.8	242.0

¹ BB = Before brining; n.d. = not determined; n.a. = not applicable.

(d) **Citrates and acetates in Feta cheese and exudate** (Table 6.11): The quantity of citrates present in Feta cheese and exudate are comparable to the 0.2% w/w (6.8 mM/kg) citrates normally present in Cheddar cheese curd (Thomas, 1987). The quantity of citrates did not decrease and the acetates did not increase with increase in storage time. This supports the earlier proposed view that NSLAB were not active. The level of acetates present in Feta cheese were in contrast to reports that acetic acid is abundantly available in pickled Feta type cheeses (Efthymiou & Mattick, 1964; Efthymiou, 1967; Ada, 1987).

Table 6.11 Quantities of acetates and citrates present in Feta cheese and exudate during various storage intervals (mM/kg)

Storage time	Feta cheese		Exudate	
	Citrate	Acetate	Citrate	Acetate
8 weeks	n.d.	n.d.	5.1	2.6
15 weeks	7.8	1.6	6.0	1.3
20 weeks	8.5	2.7	4.1	2.5
26 weeks	7.3	1.3	5.9	7.6
52 weeks	n.d.	n.d.	5.4	3.0

n.d. = not determined

(e) **Microbial counts in Feta cheese:** In Cheddar cheese a decrease in pH is observed up to 7 - 10 days after manufacture due to the fermentation of lactose to lactic acid (Turner & Thomas, 1980), followed by an increase in pH due to release of proteolytic breakdown products like ammonia (Fox, 1989). However, in Feta cheese a slow and gradual decrease of pH during storage at 10 °C was detected up to at least six months, even though there was evidence of proteolysis during this period. The explanation for this could be metabolism of residual lactose by starter bacteria or NSLAB. Evidence of presence of residual lactose and formation of lactates in stored Feta cheese partly supported this concept. It was necessary to prove the presence of viable bacteria capable of fermenting lactose to lactic acid in conditions of high salt in moisture (S/M) concentration and low pH of Feta cheese. The following experiment was performed to find evidence for this.

Starter bacteria (*Lactococcus lactis* subsp. *cremoris* strains - 2%, *Streptococcus thermophilus* - 0.1% and *Lactobacillus casei* - 0.1%) used during cheesemaking were individually inoculated in selected media to determine the growth patterns. Growth of all three starter bacteria took place in M17 broth at 30°C incubated for 2 days. Growth in MRS (pH 5.3) at 30°C in anaerobic conditions for two days was limited to only *Lactobacillus casei*. M17 broth and MRS (pH 5.3) were therefore used to estimate the total counts and *Lactobacillus casei* counts respectively in cheesemilk and cheese (Table 6.12).

Total microbial counts and *Lactobacillus casei* counts in cheesemilk had multiplied by 4-6 generations and 2-3 generations respectively in cheese (before brining). Thereafter, there was a rapid decrease of streptococci and only a slow decrease in *Lactobacillus casei* until two weeks. By four weeks the bulk of the total microflora appeared to be only *Lactobacillus casei* (or NSLAB).

There was growth of starter bacteria during the manufacture of cheese but growth of *Lactobacillus casei* was less. The streptococci did not survive for long in cheese after brining while the *Lactobacillus casei* strain was able to survive during storage at 10°C in conditions of high salt and low pH. This may be primarily responsible for the proteolysis of large casein peptides arising from the enzymatic action of rennet. *Lactobacillus casei* also probably metabolises lactose to lactic acid. It is likely that the activity of *Lactobacillus casei* is very low due to conditions of high salt and low pH. This may explain the gradual decrease in pH of cheese with increase in storage time. The production of lactic acid by NSLAB can not be ruled out, particularly because the growth of NSLAB has been shown to dominate at a concentration of 6% S/M in Cheddar cheese (Turner & Thomas, 1980). However, the high S/M (> 9.0%) and low pH of cheese that inhibits the growth of starter bacteria would also be expected to inhibit the growth of NSLAB (Lawrence & Gilles, 1982). Tests on lactates, acetates and citrates have shown that NSLAB is unlikely to be active.

Table 6.12 Microbial counts in cheesemilk and Feta cheese (during storage)

Particulars	Total counts of starter bacteria in M 17 broth (30°C/2 days)		Estimate of Lactobacilli counts in MRS broth (pH 5.3/ 30°C/2days/anaerobic)	
	I	II	I	II
Inoculated milk (cfu/ml)	2.2X10 ⁷	1.3X10 ⁷	2.1X10 ⁵	1.5X10 ⁵
Adjusted milk solids in milk ^a (cfu/ml)	6.7X10 ⁷	4.0X10 ⁷	6.4X10 ⁵	4.0X10 ⁵
Cheese before brining (cfu/g)	1.2X10 ⁹	2.8X10 ⁹	3.5X10 ⁶	1.2X10 ⁶
Cheese/3 d (cfu/g)	2.3X10 ⁸	3.5X10 ⁸	2.2X10 ⁶	1.2X10 ⁶
Cheese/16 d (cfu/g)	3.1X10 ⁶	1.3X10 ⁷	1.5X10 ⁶	9.5X10 ⁵
Cheese/28 d (cfu/g)	1.6X10 ⁶	5.0X10 ⁶	1.1X10 ⁶	2.0X10 ⁵
Cheese/66 d (cfu/g)	1.8X10 ⁶	1.8X10 ⁵	8.5X10 ⁴	1.6X10 ⁵
Cheese/124 d (cfu/g)	n.d.	1X10 ⁵	n.d.	n.d.

^a The microbial counts in milk have been increased in proportion to the solid levels in cheese (before brining) for a better comparison;
n.d. = not determined.

(f) **Water activity (A_w):** A_w of Feta cheese and its exudate were identical. This indicated that the exudate was in equilibrium with the aqueous phase of cheese.

Marcos *et al.* (1981) reported that the A_w of soft cheeses with a moisture content above 40% can be predicted from the NaCl molality (M) in the total water of the product by the equation $A_w = 1 - 0.033 M$. Using this equation the A_w of cheeses were determined and were found to be greater than the estimated values (Fig. 6.8). The lower values of the A_w of Feta cheese may be attributed to the formation of low molecular weight protein breakdown products due to proteolysis. A_w of the cheeses mostly ranged from 0.911 to 0.943 during storage. In comparison, reported values of A_w in other cheeses are: processed Cheese = 0.93, natural Cheese = 0.93,

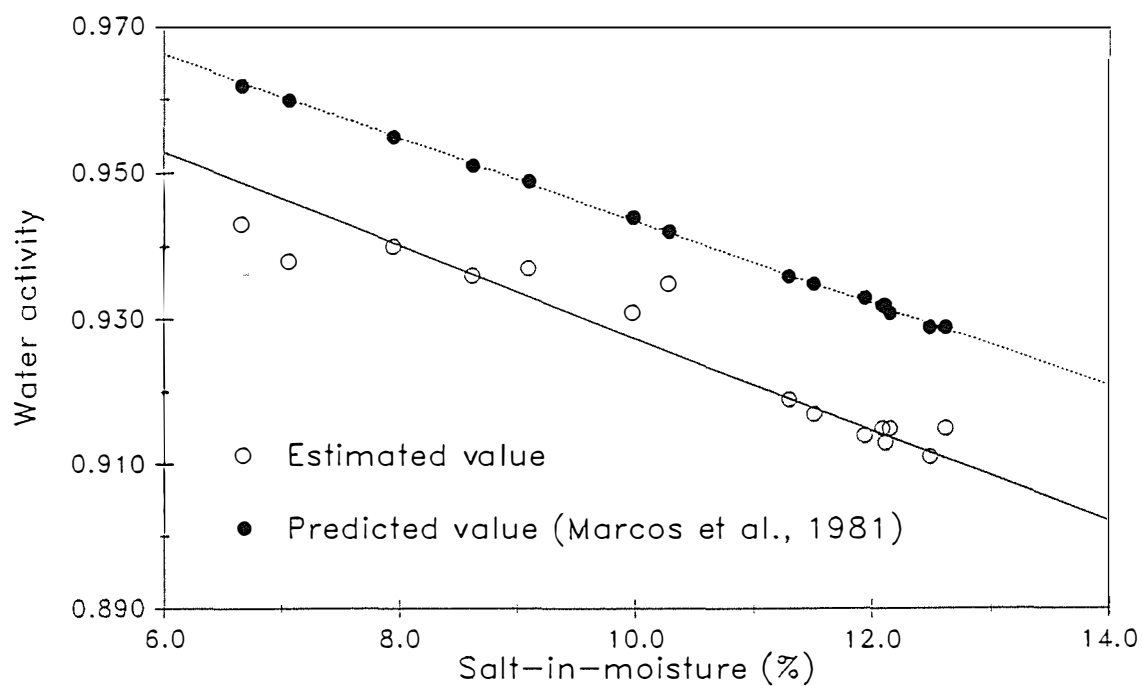


Fig. 6.8 Relationship between water activity and salt-in-moisture in Feta cheese.

Note: The predicted line is based on the findings of Marcos *et al.* (1981). They reported that the water activity (A_w) of soft cheeses with a moisture content above 40% can be predicted from the NaCl molality (M) in the total water of the product by the equation $A_w = 1 - 0.033 M$.

Muenster cheese = 0.94, Swiss cheese = 0.94, Provolone cheese = 0.92, 8% NaCl = 0.95, 12% NaCl = 0.92 (Troller & Christian, 1978).

No relationship between the estimated values of A_w and age of the Feta cheese could be established. A number of factors may contribute to A_w of cheese. Increase in soluble nitrogen with proteolysis of cheese would decrease A_w , provided that the breakdown products are of very low molecular weight. A_w generally tends to decrease with decreasing moisture in cheese (Marcos *et al.*, 1983). A decrease in moisture content of cheese due to exudation should therefore reduce the A_w . However, with proteolysis some of the water bound to the protein may be released. This may lead to an increase in free moisture. The increase in available free water is expected to increase the A_w . It is therefore difficult to predict the net effect on A_w of Feta cheese during storage.

Relationship between A_w and S/M: Estimated values of A_w of Feta cheese and their respective S/M ratios were inversely related regardless of the age of the cheese (Fig. 6.8). The correlation coefficient (r) was - 0.979 (constant = 0.99, slope = - 6.34). Thus NaCl content of the cheese influenced the A_w more than other solubles.

(g) Differential Scanning Calorimetry on Feta cheese

Cheese contains water in various states ranging from that entrapped in pores and cavities within the cheese to that tightly bound to charged groups in the protein. Water which is tightly bound to charged or polar groups within the cheese will be highly ordered and will not freeze when the temperature is lowered. Typically proteins bind around 50 g water per 100 g protein. Only the water entrapped in the gels or loosely associated in the protein matrix is available for exudation. It is of interest to know whether there is a change in the proportions of the various types of water as the cheese ripens.

Differential Scanning Calorimetry (DSC) has been used to determine the unfreezable water in caseins (Ruegg *et al.*, 1974), whey protein concentrates (Berlin *et al.*, 1973), soy proteins (Muffett & Snyder, 1980) and several model systems

(Ross, 1978). No previous study appears to have been reported on application of DSC to estimate the unfreezable water in cheese. In the present study the DSC was applied to Feta cheese and its exudate to determine the amount of unfreezable water.

The reproducibility of the calorimetry was tested with 8 replicates from the same lot of grated cheese. The results are reported in Table 6.13.

Table 6.13 Reproducibility of DSC analysis of Feta cheese

	Heat transfer ^a				Peak
	J/g cheese		f.p.	m.p.	temp. ^b

	Warming	Cooling	K	K	K

Range	85.0 - 94.7	92.0 - 100.0	250.0 - 252.2	240.0 - 236.3	269.1 - 268.1
Mean	88.6	95.1	251.3	238.2	268.6
S.D.	2.8	2.5	0.72	1.3	0.32

^a Latent heat transfer for phase conversion of water in cheese;

^b Temperature at which heat transfer is maximum;

m.p. = melting point; f.p. = freezing point; S.D. = Standard deviation.

Feta cheese was subjected to two rates of cooling, 5 K/min and 10 K/min, to determine if supercooling was occurring. The two rates of cooling produced identical thermograms which suggests that supercooling does not occur. For convenience, the samples were cooled at 10 K/min.

Details of the method used for analysis are described in Chapter 4. A typical DSC thermogram for the heating of Feta cheese is shown in Fig. 6.9. The area under the curve represents the total amount of energy required for warming the cheese

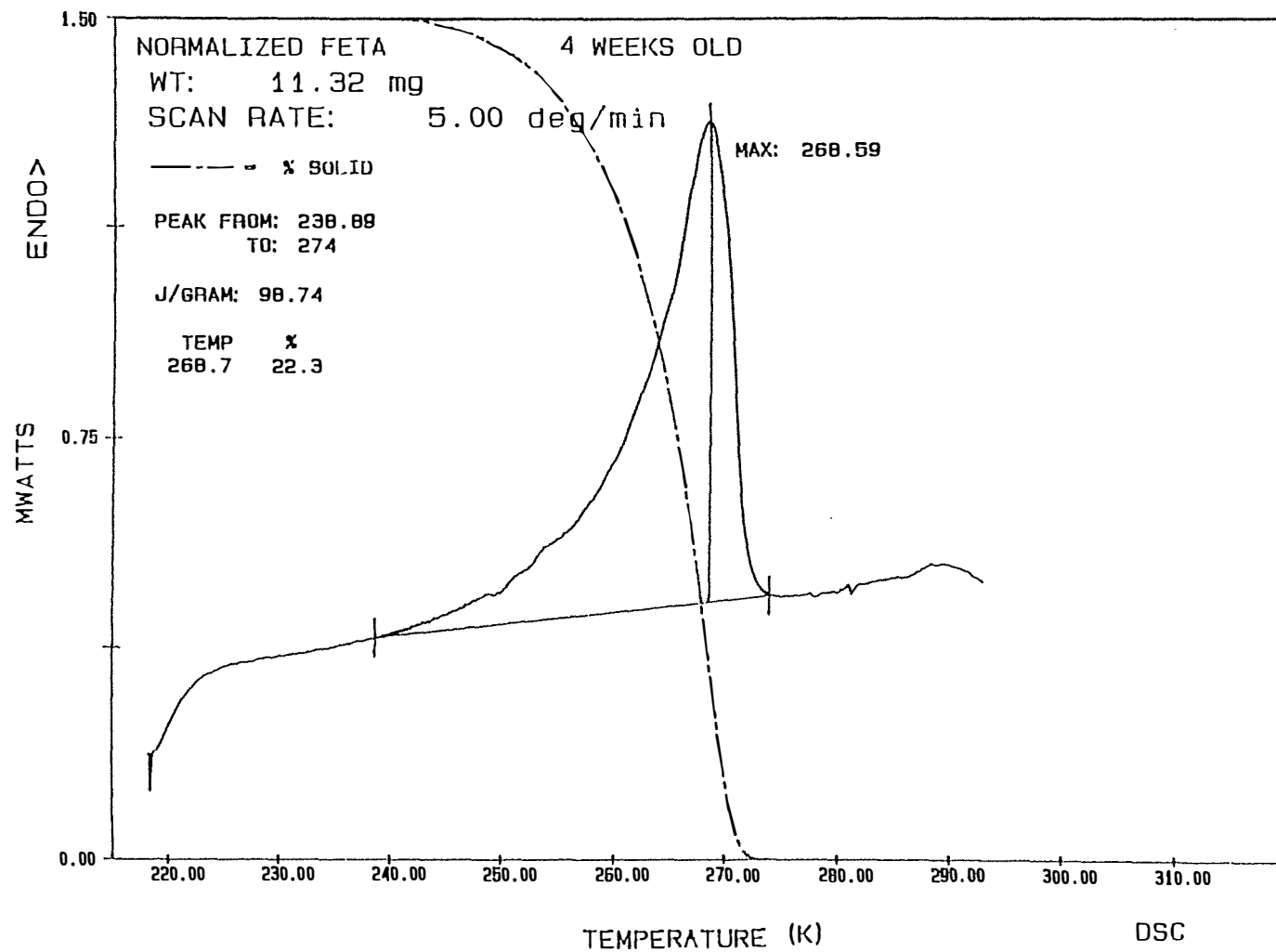


Fig. 6.9 DSC thermogram showing a typical heating phase (220 K - 285 K) of Feta cheese.

from 220 K to 285 K. The sharp rise in energy is attributed to the latent heat required for phase conversion of ice to water in the cheese. The latent heat for the phase conversion was calculated from the area of the peak above the extended baseline. The heat required to convert ice to water is 333.5 J/g. The measured value was 331.8 indicating that the DSC has been correctly calibrated. Energy requirement for phase changes for other components in cheese, particularly fat, is negligible in the range 220 - 290 K. It is assumed that the amount of unfreezable water in cheese can be calculated directly from the amount of heat energy absorbed, given that 333.5 J is required to melt 1 g of ice.

Effect of water soluble components: Investigations on the effect of major components in exudate on the amount of freezable water were carried out. The extent to which the components contribute to the amount of the unfreezable water is shown in Table 6.14. Polyethylene glycol (molecular mass 20,000 daltons) was used as a substitute for protein breakdown material. The unfreezable water in simulated exudate solution (Table 6.14) is considerably less than the unfreezable water in the actual exudate (Table 6.15). The simulated exudate solution failed to match the actual exudate. This may be attributed to the presence of protein breakdown material of variable molecular mass, mostly lower than 20,000 daltons, in the exudate. It is apparent that the low molecular weight protein breakdown material increase the unfreezable water in Feta cheese.

Table 6.14 Results from the DSC thermograms on the study of effect of major components in exudate: heating phase

Particulars	m.p. (K)	Latent heat(J/g)	Unfreezable water (%)
Milli-Q water	273.3	331.8	0.5
Lactic acid (1%)	272.3	308.8	6.5
Calcium lactate (1%)	271.3	314.7	2.5
Lactose solution (1.8%)	272.3	310.6	5.2
NaCl solution (9%)	251.5	287.5	5.3
SES without PEG ^a	249.0	253.4	10.6
SES with PEG ^b	249.1	232.3	14.6

Milli-Q water: Water filtered by reverse osmosis and Millipore treatment; SES: Simulated exudate solution;

^a Moisture = 85%; ^b Moisture = 81.6%.

Effect of storage time : Proteolysis is expected to increase the unfreezable water because of the production of the low molecular weight protein breakdown material and the water-binding properties of the newly formed amino and carboxyl groups. The results of DSC analyses on Feta cheese and its exudates at different periods of storage are reported in Table 6.16 and Table 6.15, respectively. The effect of aging on the unfreezable water in cheese and exudate is shown in Fig. 6.10. The amount of unfreezable water increased with aging of cheese. The results are not conclusive because of lack of sufficient data points and experimental errors. The major source of experimental error appears to have been in drawing a representative sample of 10 mg from Feta cheese, which is not homogeneous. Another source of error is the estimation of the peak limits in the thermogram for determination of area. It is difficult to detect the cut-off points at the base-line as the peak becomes broader, particularly in complex mixtures as that of Feta cheese. As a result the areas tend to be underestimated. A slight decrease of unfreezable water in exudate up to about 20 weeks of storage may have been due to a corresponding decrease in salt-in-moisture concentration. It is probable that the low molecular weight protein breakdown material has an effect on the unfreezable water only after being reduced to a specific size. Such reductions may be occurring after about 20 weeks of storage of cheese. No definite relationship could be established between the unfreezable water and the amount of exudate released.

The amount of unfreezable water in cheese is markedly higher than in exudate. The exudate is in equilibrium with the aqueous phase of cheese as evidenced by their similar water activities (reported earlier in this section). Thus, the effect of water soluble components on the unfreezable water may be assumed to be identical both in exudate and cheese. The higher amount of unfreezable water in Feta cheese in comparison to that in exudate may therefore be attributed to the water bound to the casein gel.

The freezing points of cheeses of varying ages were also estimated using the DSC thermograms. The changes in the freezing points were small and a definite trend in their relationship was not detected. More detailed study is required to establish the application of DSC technique with cheese.

Table 6.15 Results from the DSC thermograms on the study of exudates from Feta cheese of varying ages - heating phase

Age of cheese from which exudate was obtained	Composition of exudate			Results from DSC thermograms			
	Moisture	NaCl	S/M	m.p. (K)	Latent heat		Unfreezable water (%)
	%	%	%		(J/g material)	(J/g water)	
4 weeks	82.4	9.98	12.1	240.8	217.4	263.8	20.9
14 weeks	81.6	9.61	11.8	238.4	223.0	273.3	18.1
26 weeks	82.0	7.53	9.2	238.7	220.3	268.6	19.5
65 weeks	81.5	7.02	8.6	240.2	158.2	193.9	41.8

Table 6.16 Results from the DSC thermograms on the study of Feta cheese of varying ages

Age of cheese	Composition of cheese			Results from DSC thermograms				
	Moisture	NaCl	S/M	f.p. (k)	m.p. (K)	Latent heat		Unfreezable water (%)
	%	%	%			(J/g material)	(J/g water)	
1 day	52.7	3.6	6.8	254.5	259.3	107.6	204.2	38.8
4 weeks	49.9	4.2	8.4	250.0	259.3	98.7	197.8	40.7
15 weeks	49.1	4.0	8.1	250.8	259.3	93.4	190.2	43.0
26 weeks	49.5	4.1	8.3	249.7	258.7	96.7	195.4	41.4
33 weeks	49.2	4.1	8.3	251.0	258.0	88.0	178.9	46.4

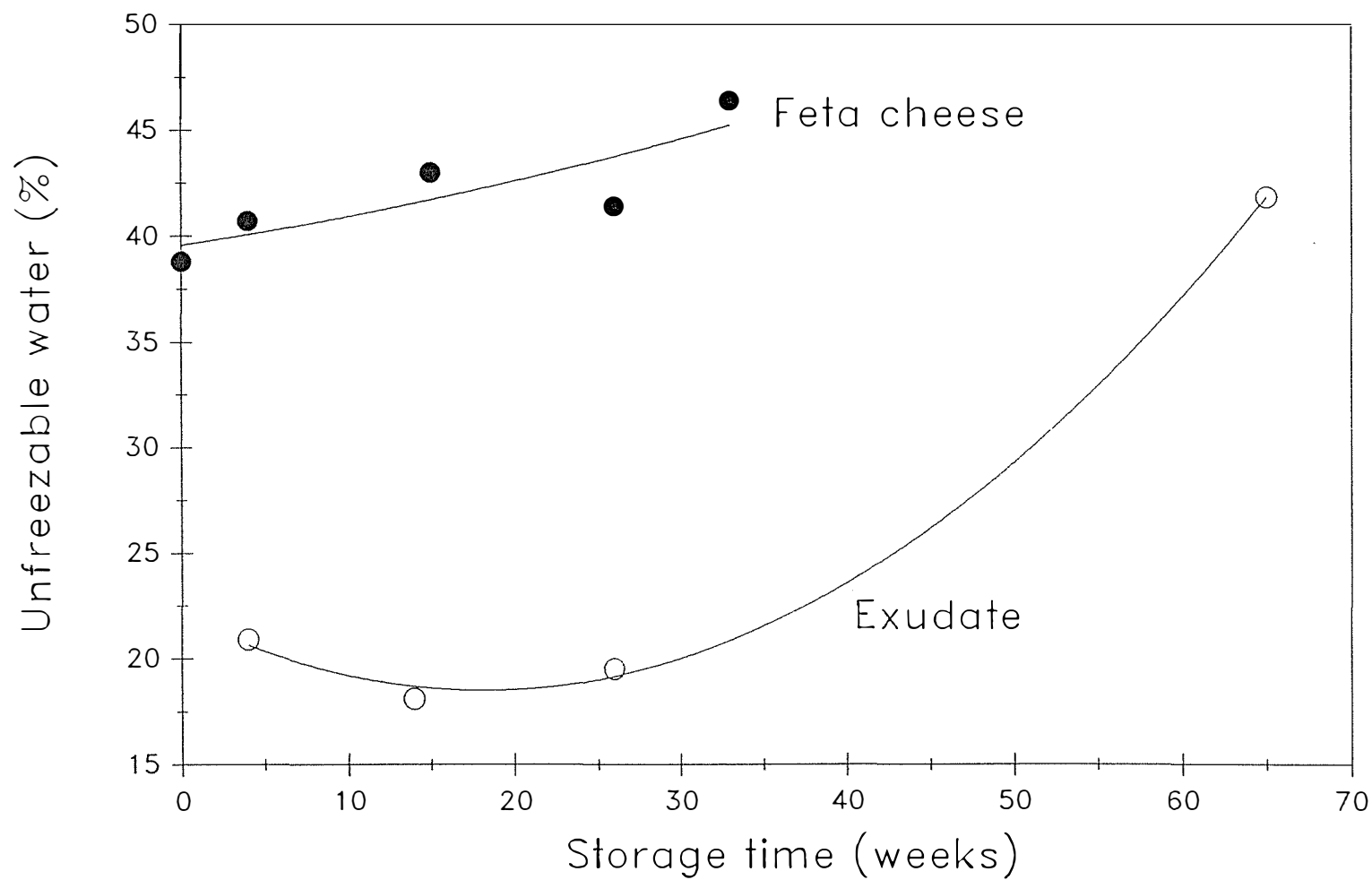


Fig. 6.10 Unfreezable water in Feta cheese and exudate during storage (estimated using DSC technique).

SECTION THREE

6.4 EFFECT OF SELECTED MANUFACTURING VARIABLES ON EXUDATION FROM FETA CHEESE

The effect of selected manufacturing variables on exudation was investigated with the following objectives:

- To determine practical measures to reduce the extent of exudation.
- To gain an insight into the mechanism of exudation.

6.4.1 Experimental plan: The selected manufacturing variables chosen for the study are reported in Table 6.17.

Table 6.17 Selective manufacturing variables and their respective levels of variation chosen for studying the effects on exudation of whey from Feta cheese during storage

Treatments	Levels	Reference in the text
Replicates		A
First 9 trials	1	First replicate
Next 9 trials	2	Second replicate
Protein to fat ratio		B
	0.56	Low
	0.73	Control
	0.90	High
Homogenisation pressure (psi) ^a		C
(single stage)	600	Low
(single stage)	1000	Control
(two stage)	2000/500	High
Priming time (min)		D
	15 ^b	Low
	30 ^c	Control
	45 ^d	High
Curd pH at draining		E
	6.1	Low
	6.2	Control
	6.3	High
Storage time (weeks)		S
	1, 3, 5, 7, 9, 12, 15, 20, 26, 39	

^a 1 psi = 6.89 kPa;

^{b c d}: Corresponding setting times were 60, 45, 30 min, respectively.

(i) Basis of selection of manufacturing variables and the levels of variation: The criteria involved in the selection of variables was that they should be readily controllable by the cheesemaker. The limiting values of the variables were chosen with the objective of having maximum variation, so as to clearly detect the effect on exudation, without however affecting the quality of cheese.

Protein to fat ratio (P/F ratio): P/F ratio was selected as a variable because the commercial practice of cheese manufacture is generally based on the standardisation of casein to fat ratio of cheesemilk. This was expected to provide evidence about the role of protein, fat, and total solids. A variation of P/F ratio from 0.58 to 0.90 in cheesemilk resulted in variation of fat content from 4.2 to 6.5% in cheesemilk and 20 to 30% in cheese, respectively.

Homogenisation pressure: The homogenisation process was necessary in order to prepare 'manufactured cream' using FFMR and RSM. An increase in homogenisation pressure was expected to decrease the size of fat globules, increase the total number of fat globules and increase the total area of fat globule surface in cheese. Variation in homogenisation pressure was expected to provide evidence on the role of fat globules and the fat-protein interaction on exudation of whey during storage. The upper limit was arbitrarily chosen as two-stage homogenisation at a pressure of 13,780/3,445 kPa (2000/500 psi). Single stage homogenisation of milk at 4,134 kPa (600 psi) was chosen as the lower limit of homogenisation because at a further lower pressure it was difficult to obtain a reasonably good emulsion of FFMR.

Priming time: A variation in priming time alters the mineral balance of milk (Creamer *et al.*, 1985). It was intended to study the effect of minerals on the exudation by variation of priming time. A variation only in priming time would affect the curd strength, coagulation time and pH of whey at draining; and this would complicate interpretation of results on exudation. To avoid interactions with the other factors and determine the effect of priming time *per se*, cheeses were made with a fixed total time of priming and setting. A variation in the priming time, with a fixed total time for priming and setting, did not significantly affect curd strength (subjective analysis by slicing the curd with fingers) and other

manufacturing processes. This was expected to result in nearly identical cheese in terms of moisture and pH while providing an indication to the effect of the priming time on the mineral balance and exudation of whey from cheese.

Curd pH at draining: This is an important step in the manufacture of most types of cheese (Lawrence *et al.*, 1983) as it affects the residual rennet and mineral balance (Lawrence & Gilles, 1987), residual lactose (Czulak *et al.*, 1969), and moisture in cheese. After cutting, the time taken for attaining curd pH values of 6.3, 6.2, 6.1 were 50-60 min, 80-90 min and 110 - 120 min, respectively.

Storage study: Preliminary studies clearly indicated an increase in the amount of exudate with an increase in storage temperature. It was therefore decided to have only one storage temperature, *i.e.* 10°C, which is of practical relevance. Selected periods of storage intervals were chosen as specified (Table 6.17).

(ii) Statistical design: The experiment was fractional-factorial designed. The trials were carried out using the combinations of 'low' and 'high' levels of four variables (P/F ratio, homogenisation pressure, priming time, and curd pH at draining). Each trial was split at the time of study of the variable 'storage time'. The trials were conducted with the following variables (refer Table 6.17 for nomenclature):

Control, bcde, BCde, BcDe, BCDE, BcdE, bCdE, bCDe, bcDE

Note: Upper case refers to the higher level and lower case refers to the lower level for the respective manufacturing variables.

The above trials were replicated twice. Trials in each replicate were randomised.

6.4.2 Experimental: Feta cheeses were manufactured using the procedure outlined in Section One. The variations were applied as described in Table 6.17.

6.4.3 Analytical methods: 'Manufactured cream' and reconstituted skim milk (RSM) were tested for fat and protein by the Milko-Scan. Standardised milk and whey obtained after draining were analysed for fat, protein, lactose, total solids by the Milko-Scan; and calcium by complexometric method. Feta cheese at four

weeks was analysed for pH, fat (Babcock), moisture, protein (Kjeldahl), NaCl (potentiometric titration), and calcium (complexometric method). Exudate of Feta cheeses were analysed for total solids, NaCl (potentiometric method), calcium (complexometric method) and protein (Kjeldahl). The distribution pattern of peptides in exudates from four week old Feta were analysed by HPLC. Proteolysis was determined in control cheese and exudate from Feta cheese at different storage periods by urea-PAGE and SDS-PAGE, respectively. The amount of exudate were measured at the selected storage intervals. Details of all the above methods are outlined in Chapter 4 and Appendix 4.1.

6.4.4 Sensory evaluation: The product was evaluated by a sensory panel at eight weeks after manufacture for flavour and textural characteristics. Details of these are reported in Chapter 4.

6.4.5 Results and Discussion

This is described under the following headings:

- (a) Composition of milk, whey, cheese and exudate.
- (b) Manufacturing aspects and quality of cheese.
- (c) Effect of manufacturing variables on exudation.

(a) Composition of milk, whey, cheese and exudate

(i) Composition of standardised milk (or cheesemilk) (Table 6.18): The milk was standardised to the desired P/F ratios.

The composition of milks for the replicates were different. This was primarily due to experimental error involved in standardising 'manufactured cream' and adjusting the total solids in reconstituted skim milk. While the P/F ratio of milk was standardised accurately, the level of total solids was not adjusted to a constant value and this affected the composition of cheese. Ideally, a slightly concentrated

Table 6.18 Composition of milks for Feta cheeses manufactured (with respect to manufacturing variables)

Treatment & levels	Fat (%)		Protein (%)		Lactose (%)		TS (%)		Ca ²⁺ (mM/kg)		P/F ratio	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A		34.8***		26.4**		8.37*		21.62**		20.52**		7.67*
First	5.64		3.87		5.44		15.37		34.9		0.719	
Second	5.39		3.74		5.32		14.87		36.3		0.727	
B		3105***		46.5***		18.57**		347.6***		2.61		12637***
High	4.35		3.89		5.47		14.12		35.91		0.89	
Low	6.69		3.71		5.29		16.11		35.4		0.55	
C		0.32		0.01		0.00		0.14		11.75*		1.64
High	5.51		3.79		5.38		15.09		36.2		0.725	
Low	5.53		3.80		5.38		15.13		35.1		0.721	
D		0.74		0.79		0.03		0.29		2.36		0.08
High	5.50		3.79		5.38		15.09		35.9		0.723	
Low	5.54		3.81		5.39		15.15		35.4		0.724	
E		1.48		0.01		0.00		0.20		4.04		2.87
High	5.49		3.80		5.38		15.09		35.3		0.726	
Low	5.54		3.79		5.38		15.14		36.0		0.721	
Control	5.18		3.80		5.34		14.73		35.6		0.732	

A = Replicates; B = P/F ratio of milk; C = Homogenisation pressure for 'manufactured cream';
D = Priming time; E = Curd pH at draining; S = Storage time;

LSM = Least-square mean; F = F ratio;

*** p < 0.001; ** p < 0.01; * p < 0.05;

F values without asterisks denote 'not significant'.

milk with the desired P/F ratio should have been prepared first and then diluted to the pre-determined level of total solids.

The mean diameter of fat globules in standardised milk decreased with increase in homogenisation pressure (Table 6.19).

Table 6.19 Effect of variation of homogenisation pressure on the mean diameter of fat globules in 'manufactured cream' (FFMR + RSM)

Level	Homogenisation pressure ¹	Mean diameter (μm)
High	Two stage (2000/500 psi)	0.53
Control	Single stage (1000 psi)	0.83
Low	Single stage (600 psi)	0.99

¹ 1 psi = 6.89 kPa

(ii) Composition of whey (Appendix 6.3): Most of the treatments did not have any effect on the composition of whey. Calcium content of whey was slightly higher for the lower 'curd pH at draining'. This difference was however not detected in 'entire whey' collected over 17 h following draining. This was probably because the variation in pH of curd at the time of draining was not retained by overnight draining.

(iii) Composition of Feta cheese at four weeks after manufacture: (Table 6.20)

Fat and moisture contents in the cheese were significantly affected by the process treatments. High fat content in the cheeses of first replicate was due to a corresponding high fat in the standardised milk. Variation in protein and fat contents for the P/F ratio was due to the treatment effect. Increased calcium content in cheese made from milk with higher P/F ratio was due to the higher solids-not-fat (SNF) content. When calcium is represented in terms of Ca^{2+} /SNF (g/100 g), this difference was not detected.

Table 6.20 Effect of manufacturing variables on the composition of four week old Feta cheeses

Treatment & levels	Fat (%)		Moisture (%)		Protein (%)		NaCl (%)		pH	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A		28.17**		21.98**		2.75		0.11		7.71*
First	26.13		48.94		15.63		4.6		4.71	
Second	23.97		50.85		15.18		4.6		4.76	
B		289.0***		45.1***		150.58***		2.81		6.28*
High	21.59		51.27		16.91		4.7		4.72	
Low	28.50		48.52		13.91		4.5		4.76	
C		33.28		25.58		6.69*		0.28		1.32
High	23.88		50.93		15.06		4.6		4.75	
Low	26.22		48.86		15.69		5.4		4.73	
D		4.98		6.63*		3.96		0.22		0.22
High	25.50		49.37		15.63		4.6		4.74	
Low	24.60		50.42		15.18		4.5		4.73	
E		8.10*		12.54**		0.63		3.57		0.22
High	24.47		50.62		15.31		4.5		4.73	
Low	25.63		49.17		15.50		4.7		4.74	
Control	24.25		51.48		15.50		4.3		4.73	

A = Replicates; B = P/F ratio of milk; C = Homogenisation pressure for 'manufactured cream';
D = Priming time; E = Curd pH at draining;

LSM = Least-square mean; F = F ratio;

*** p < 0.001; ** p < 0.01; * p < 0.05;

F values without asterisks denote 'not significant'.

Table 6.20 continued

Treatment & levels	Ca ²⁺ (mM/kg)		FDM (%)		S/M (%)		MNFS (%)		Ca ²⁺ /SNF (g/100g)	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A		4.78		29.00**		1.49		6.47		2.81
First	84.1		50.95		9.32		66.29		1.35	
Second	95.1		48.59		9.04		66.92		1.51	
B		11.63*		635.8**		0.13		101.3***		0.11
High	98.3		44.24		9.14		65.37		1.45	
Low	81.0		55.29		9.22		67.84		1.41	
C		0.01		35.33**		1.44		7.21*		0.00
High	89.9		48.46		9.04		66.94		1.43	
Low	89.4		51.07		9.32		66.28		1.43	
D		0.06		4.00		1.76		5.62*		0.03
High	90.2		50.21		9.33		66.32		1.44	
Low	89.0		49.33		9.02		66.89		1.42	
E		0.59		3.96		9.76*		13.84**		0.66
High	91.6		49.33		8.81		67.07		1.46	
Low	87.7		50.20		9.54		66.15		1.39	
Control	98.2		50.0		8.4		68.0		1.62	

A = Replicates; B = P/F ratio of milk;

C = Homogenisation pressure for 'manufactured cream';

D = Priming time; E = Curd pH at draining;

LSM = Least-square mean; F = F ratio;

*** p < 0.001; ** p < 0.01; * p < 0.05;

F values without asterisks denote 'not significant'.

An increase in moisture was detected in cheeses made with 'manufactured cream' homogenised at a higher pressure. This was possibly due to the increase in the amount of casein coated to the newly created fat globule surface area (Chapter 8).

Slight variations of fat, moisture, FDM and MNFS for two levels of priming time may have been due to the variation in gel strength of the coagulum that was not detected visually during manufacture. There was no effect of 'priming time' and 'curd pH at draining' on calcium contents in Feta cheese and whey. Variation in priming time did not affect calcium levels possibly due to having a constant total time of priming and setting. This resulted in a constant draining pH for all the trials. Lawrence *et al.* (1983) had suggested that calcium level in Cheddar type cheeses can be controlled by the priming time. Present findings suggest that variation in priming time *per se* (without affecting the draining pH) does not influence calcium content in cheese. The effect of priming on calcium levels or mineral balance is due to an effect on draining pH.

Cheese drained at lower curd pH had lower moisture. This is consistent with the findings of increased syneresis in renneted gels as a function of low pH (Patel *et al.*, 1972; Walstra *et al.*, 1985; Walstra & Vliet, 1986). Slight increase in S/M ratio in cheese made with higher curd pH at draining is surprising because normally cheese with higher moisture would be expected to have a higher S/M ratio (Geurts *et al.*, 1980) due to a higher diffusion coefficient.

The statistical analysis shows a significant variation for some of the treatment variables. However, the variations are small and are not of much practical concern.

(iv) Composition of exudate from four week old Feta cheese (Table 6.21): The composition of exudate was mostly unaffected by the process variables. The effect of increased protein content at the higher P/F ratio may be attributed to the treatment itself. Increased NaCl (also high S/M) values for 'lower curd pH at draining' was due to an increased salt content in cheese.

Table 6.21 Effect of manufacturing variables on the composition of exudate from Feta cheese after 4 weeks of storage.

Treatment & Levels	TS (%)		Ca ²⁺ (mM/kg)		Protein ¹ (%)		NaCl (%)		S/M (%)	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A										
First	16.29	0.70	122.1	0.01	2.98	1.76	7.73	0.00	9.2	0.04
Second	16.08		122.7		3.15		7.72		9.2	
B										
High	16.37	1.93	120.6	0.34	3.25	8.62*	7.76	0.19	9.3	0.39
Low	16.01		124.3		2.88		7.69		9.2	
C										
High	16.17	0.02	117.6	2.35	3.02	0.51	7.71	0.05	9.2	0.05
Low	16.21		127.3		3.11		7.74		9.2	
D										
High	16.32	1.07	122.6	0.00	3.08	0.05	7.78	0.49	9.3	0.58
Low	16.06		122.3		3.05		7.68		9.1	
E										
High	15.90	5.14	124.3	0.34	3.04	0.18	7.36	25.8**	8.7	23.3**
Low	16.48		120.6		3.09		8.10		9.7	
Control	15.85		121.7		3.36		7.11		8.4	

¹ This is an estimate of the nitrogenous material in the exudate. Total nitrogen content in the exudate was multiplied by a factor 6.38 to obtain this protein content.

A = Replicates; B = P/F ratio of milk; C = Homogenisation pressure for 'manufactured cream';

D = Priming time; E = Curd pH at draining; LSM = Least-square mean; F = F ratio;

** p < 0.01; * p < 0.05; F values without asterisks denote 'not significant'.

HPLC studies on exudate: The HPLC profiles of exudates did not show much variation in the nature of peptides and their quantities. This implied that the pattern of degradation of large peptides in the cheeses were similar. The range of peptides and their sizes indicated that a steady and substantial proteolysis had occurred in Feta cheese by the end of four weeks. The whey proteins did not appear to have undergone any change. It was concluded that the selected manufacturing variables did not influence the proteolysis.

(v) Composition of Feta cheese at six months (Appendix 6.4): The major change detected in all cheeses was the decrease in pH value to ≈ 4.5 when compared to composition of cheese at four weeks (Table 6.20). The change in pH was however not affected by the treatment variables. Changes in moisture, MNFS, fat and FDM were due to the exudation of whey from Feta cheese during storage.

(b) Manufacturing aspects and quality of cheese

(i) Cheese manufacture: Standardised milks made using 'manufactured cream' homogenised in two stages at pressures of 13,780/3,445 kPa (2000/500 psi) had no cream layer. The milk appeared satisfactory when manufactured cream was homogenised at a pressure of 6,890 kPa (1000 psi). A thin cream layer was visible when milk was homogenised at 4,134 kPa (600 psi). Typically, the pH profile during cheese manufacture was as follows: 6.65 for standardised milk; 6.54 after addition of starter, 6.42 at the time of cutting, ≈ 4.9 for cheese before brining, ≈ 4.75 for cheese after brining, and ≈ 4.65 after four weeks of storage. A typical temperature profile during cheese manufacture was: 34 °C for cheesemilk, 33.5 °C after the addition of starter, 33 °C at the time of adding rennet, 32 °C at the time of cutting, 31.5 °C at stirring and 28-29 °C at the time of draining whey. When the P/F ratio was low the curd was softer, the whey had more fines and some curd floated in whey till draining. Before brining, cheeses made with low curd pH at draining appeared more elastic and pliable, and cheeses made with high curd pH at draining were relatively crumbly and brittle. However, these differences were not detected in cheeses after brining.

(ii) Sensory evaluation of Feta cheese (Appendix 6.5): Increased acidity for the second replicate may be attributed to possible increased microbial activity at a high moisture in cheese. Cheese made with lower curd pH at draining was perceived salty which is consistent with the increased salt in cheese. Bitter and oxidised flavours were mostly not detected as evidenced by the lower scores allotted by the panel of judges.

Increased smoothness of the cheeses made at lower P/F ratio was due to the increased fat content. The structure (sliceability) was more crumbly for cheese made with lower curd pH at draining and the first replicate. This was probably due to lower moisture in the respective cheeses.

Overall, the cheeses were considered acceptable and their quality not significantly altered by the manufacturing variables.

(iii) Quality of cheese: The quality of the experimental cheeses was of high standard, as evidenced from the microbiological results (described earlier in this Chapter) and the sensory evaluation. This could be attributed to the use of: raw material of uniform and good quality, active starters of defined strains, sanitised equipment, clean and hygienic pilot plant facilities, and good process control.

(c) Effect of manufacturing variables on exudation of whey from Feta cheese during storage: The effect of selected manufacturing variables on the exudation of whey from Feta cheeses during storage is shown in Table 6.22.

(i) Effect of replicates: The amount of exudate was significantly different between the replicates (Table 6.22). This variation was probably due to the variation in the composition of standardised milks and composition of Feta cheeses (moisture, MNFS and FDM) for the replicates, as discussed earlier in this section. Increase in the amount of exudate was observed with increased moisture, increased MNFS and decreased FDM. It was not intended to study the effects of replication as a variable. However, the results showed an increase in the amount of exudate with increased moisture and decreased fat contents in cheese. The effect of an increase

Table 6.22 Effect of manufacturing variables on the exudation^a of whey from Feta cheeses during storage at 10°C

Source of variation & levels	Exudate in g/kg cheese		Exudate in g/kg cheese moisture		Final MNFS (%) in cheese		% reduction in MNFS of cheese	
	LSM	F	LSM	F	LSM	F	LSM	F
A		6.61*		4.90#		0.27		4.1
First	39.9		79.8		65.9		1.39	
Second	49.6		95.5		66.4		1.62	
B		18.8**		12.57*		103***		31.4**
High	51.6		98.6		64.8		1.82	
Control	44.2		84.8		67.5		1.34	
Low	38.0		76.8		67.5		1.20	
C		0.96		0.20		5.76#		0.01
High	46.3		89.1		66.5		1.50	
Control	44.2		84.8		67.5		1.34	
Low	43.2		86.3		65.8		1.52	
D		0.04		0.28		5.33#		1.04
High	45.1		89.3		65.8		1.56	
Control	44.2		84.8		67.5		1.34	
Low	44.5		86.1		66.5		1.45	
E		7.18*		5.26#		10.18*		2.25
High	49.0		94.7		66.6		1.59	
Control	44.2		84.8		67.5		1.34	
Low	40.6		80.7		65.7		1.43	
S		249***		273***		132***		273***
1	15.2		30.0		66.8		0.49	
3	20.5		40.4		66.7		0.66	
5	26.5		51.9		66.6		0.86	
7	32.6		63.7		66.4		1.06	
9	39.8		77.8		66.3		1.31	
12	48.1		94.2		66.1		1.60	
15	55.3		108.3		65.9		1.86	
20	61.1		119.6		65.8		2.09	
26	66.8		130.8		65.6		2.29	
39	81.8		160.2		65.2		2.87	
Interactions								
B X S		9.24***		7.45***		7.58***		18.4***
C X S		1.01		0.55		0.14		0.21
D X S		0.23		0.34		0.30		0.74
E X S		3.91***		3.52***		1.30		2.34*
S.D.	4.89		9.07		0.155		0.160	

^a Equations to calculate exudation values are provided in Appendix 4.2;

A = Replicates; B = P/F ratio of milk; C = Homogenisation pressure for 'manufactured cream';

D = Priming time; E = Curd pH at draining; S = Storage time;

LSM = Least-square mean; F = F ratio; S.D. = Standard deviation;

*** p < 0.001; ** p < 0.01; * p < 0.05; # p < 0.10; 'F' values without asterisks denote 'not significant'.

in the amount of exudate for cheese with increased moisture is consistent with the findings of Pedersen *et al.* (1971) who reported that Blue cheeses made with higher overnight drainage temperature released a reduced amount of exudate due to a reduction of the moisture in the cheese.

(ii) Effect of 'P/F ratio' (0.56 & 0.90) on exudation: An increase in the amount of exudate was observed from cheese made using milk at a higher P/F ratio (Table 6.22). A lower MNFS content in cheese was of no avail. This is in contrast to the effects of P/F ratio on exudation from Cream cheese (Chapter 5). Thus increased available protein in the cheese was not effective in reducing the amount of exudate during storage of cheese. The two major changes taking place in Feta cheese during storage are proteolysis and a slow decrease in pH. The effect can not be attributed to pH because the pH values were similar for all cheeses after six months of storage (Appendix 6.4). Proteolysis therefore is likely to have caused the difference in the amounts of exudate. It is possible that proteolysis cleaves the protein matrix (mainly casein), and the moisture held in the casein gel structure is released with the solubles as exudate. Increase in the amount of exudate at the higher P/F ratio may also have been due to reduced fat content (and FDM) in cheese. It was shown earlier with the replicates that increase in the amount of exudate was observed in cheeses with an increased MNFS and decreased FDM. These results are comparable to decreased syneresis in renneted milk gels with increased fat content (Storry *et al.*, 1983). The role of fat as a mechanical barrier (Lelievre & Creamer, 1978), or coating of fat globules with casein may be causing the reduction of the amount of exudate (discussed in Chapter 8).

(iii) Effect of pressure of homogenisation for 'manufactured cream' (13,780/3445 kPa & 4,134 kPa) on exudation: Exudation of whey from Feta cheese was not affected by the selected levels of homogenisation for manufactured cream (Table 6.22). In renneted milk gels Vaikus *et al.* (1970) observed that syneresis was inversely proportional to the extent of homogenisation of milk or cream. Increase in the area occupied by the fat globule surface and the increase in protein-fat interaction (Vliet & Dentener-Kikkert, 1982; Walstra & Vliet, 1986) due to the increase in pressure of homogenisation was expected to reduce the amount of

exudate. Absence of such a trend suggests that either these factors have no effect on exudation, or that the differences that existed in cheesemilk due to the variation in homogenisation pressures did not exist in cheese.

(iv) Effect of priming time (15 & 45 min) on exudation: No significant difference in the amounts of exudate was detected at the different levels of priming time (Table 6.22). Priming time was chosen as a treatment variable to determine the effect of minerals in cheese on the exudation of whey. The variation in priming time was performed with corresponding variation in setting time so that the curd pH at draining was not affected. However, Feta cheese and whey at the different levels did not show any difference in calcium level. Thus priming time *per se* did not influence the mineral balance of cheese and the exudation. The role of calcium in cheese on the exudation could not be ascertained from this study.

(v) Effect of curd pH at draining (6.1 & 6.3) on exudation: The exudation of whey from Feta cheese was significantly influenced by the curd pH at draining (Table 6.22). The effect could be due to a combination of factors as curd pH at draining is known to influence the mineral balance (Dolby *et al.*, 1937), residual rennet (Holmes *et al.*, 1977; Lawrence *et al.*, 1983; Singh & Creamer, 1990) and the moisture content. There is some uncertainty about the effect of variation in time of holding the curd in the whey on the residual lactose in cheese (Lawrence & Gilles, 1982).

More calcium was found in whey drained at lower pH of draining. However, the lack of any difference in calcium contents in 'whole' whey (pooled from all the whey drained overnight) suggests that the difference in calcium levels at the time of draining was possibly restored during the overnight drainage of whey. Lack of any difference is further evident from similar pH and calcium values in cheese before brining. Thus it is unlikely that the observed difference in the amounts of exudate was related to calcium content.

Similarly, any difference in residual rennet in curd at draining is likely to have been restored during overnight draining of whey. This is supported by attainment of a

similar pH value for all cheeses. Further, the lack of any variability in proteolysis of exudates indicates a similar residual rennet level in all cheeses.

Czulak *et al.* (1969) found that in addition to the pH at draining the rate of acid development and the time the curd is in whey were critical factors in controlling the quality of Cheddar cheese. They observed that when a high lactic acid was developed slowly, the curd did not cheddar well. On maturing, even though the pH was within normal limits up to 3 weeks, the cheese became acid, crumbly and liquid separation (exudation) occurred at two months age. However, in this experiment there is no indication of a difference in pH of cheese as observed at four weeks and twenty-six weeks of storage. Residual lactose in cheese and pH of cheese during storage are therefore not likely to have influenced exudation.

A significantly higher moisture at the higher pH at draining (resulting in higher MNFS and lower FDM) is likely to have caused the increase in the amount of the exudate.

An important implication from the above findings is that, in contrast to hard varieties of cheese, variations in the manufacturing variables 'priming time' and 'curd pH at draining' are unlikely to have the desired effect in cheeses such as Feta that are drained overnight.

(vi) Effect of Storage time: Storage time had a highly significant effect on exudation of whey (Fig 6.11). The amount of exudate increased steadily up to about 20 weeks. Thereafter, the increase in the amount of exudate was slower. Rapid rise in the amount of exudate at the start of storage was caused by the external mechanical effect of vacuum packing. The increase in the amount of exudate during storage may be attributed to increase in proteolytic activity or to the spontaneous expulsion of liquid from gels with aging (Matz, 1965).

(vii) Interactions of storage time with P/F ratio: Interactions of storage time with P/F ratio (Fig. 6.12) and pH at draining (Fig. 6.13) significantly affected exudation.

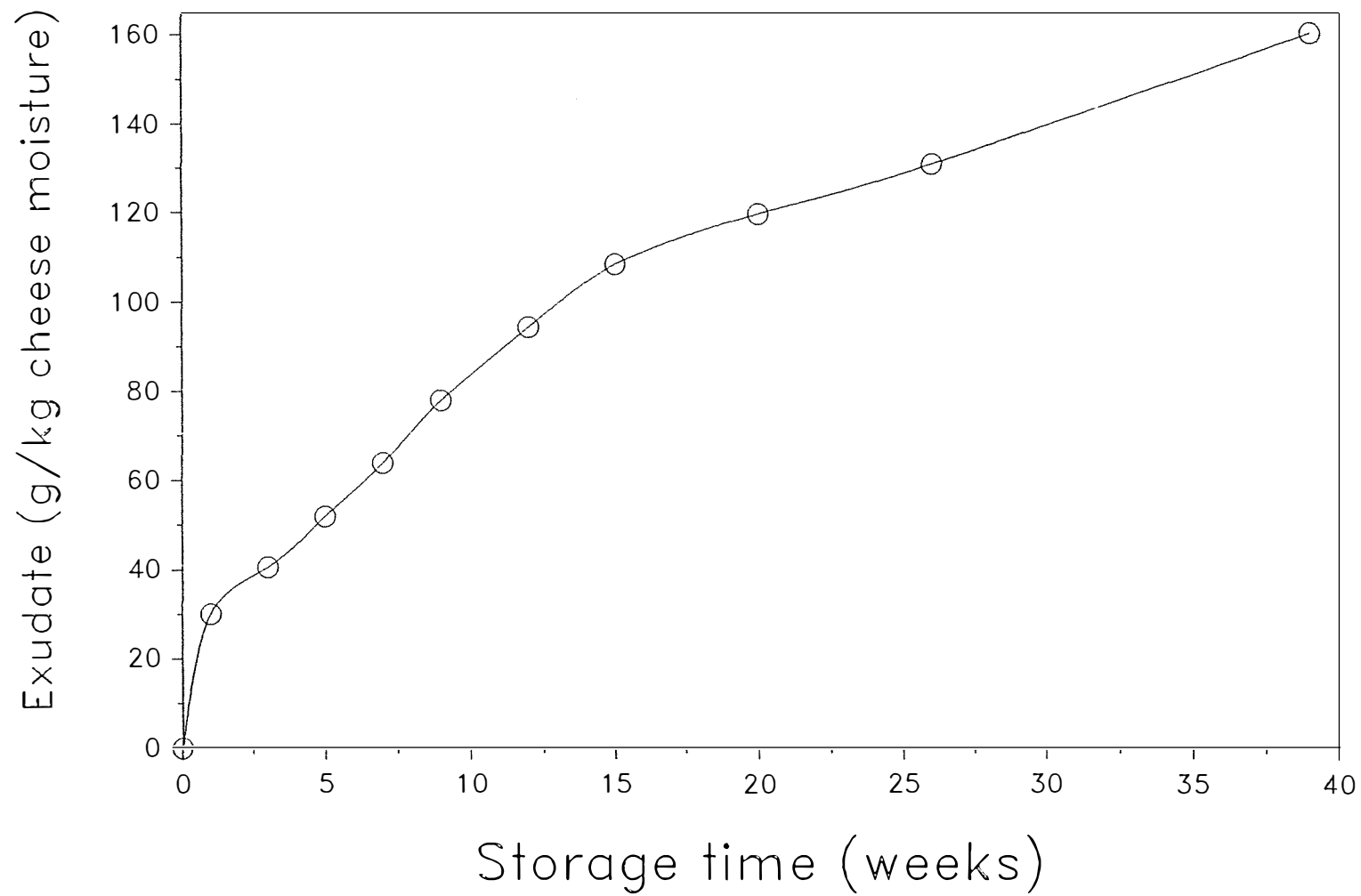


FIG. 6.11 EXUDATION OF WHEY FROM FETA CHEESE DURING STORAGE

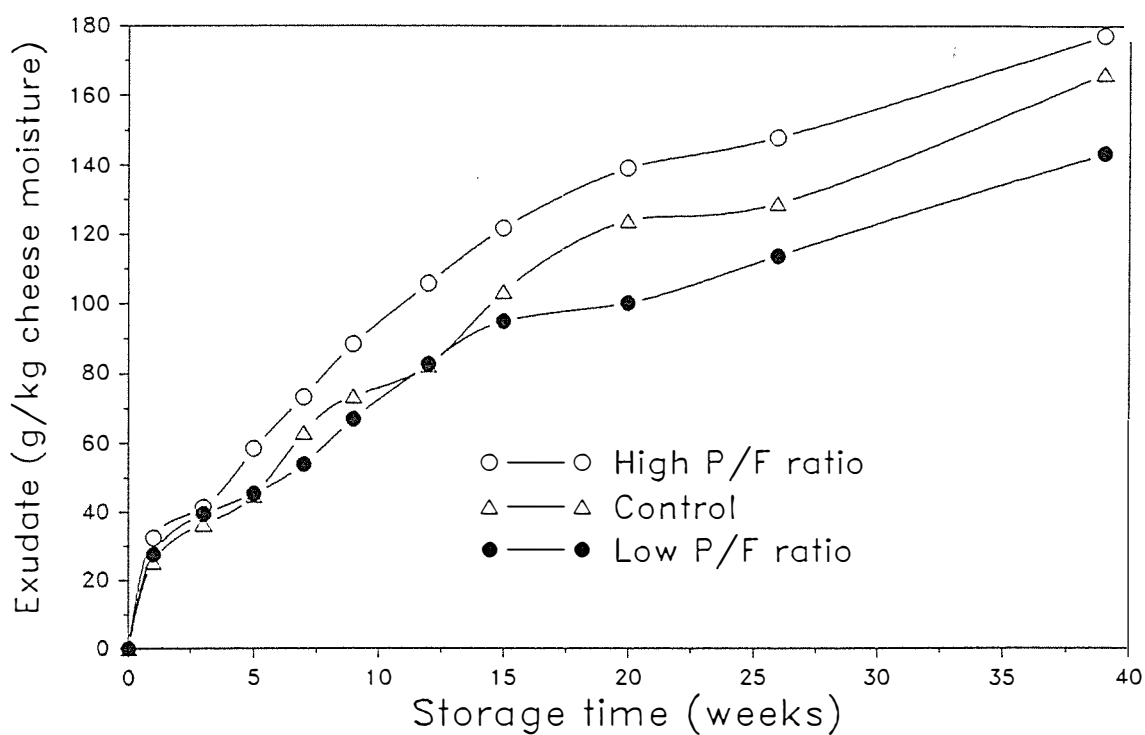


Fig. 6.12 Effect of protein to fat ratio (P/F ratio) on the exudation of whey from Feta cheese during storage.

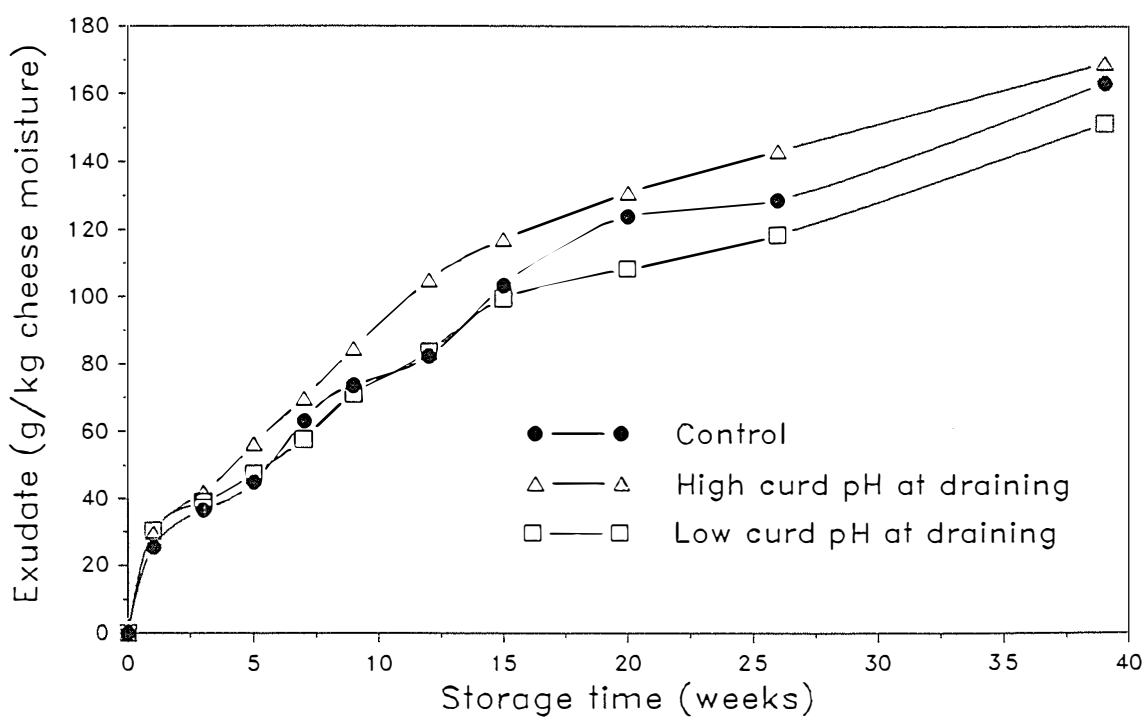


Fig 6.13 Effect of 'curd pH at draining' on the exudation of whey from Feta cheese during storage.

Increase in the amount of exudate was more for the high P/F ratio with increase in storage time. Likewise, the increase in the amount of exudate at higher curd pH at draining was more with increase in storage time. This further emphasised the significant individual effects of the treatment variables P/F ratio and curd pH at draining on exudation.

6.5 SUMMARY AND CONCLUSION TO CHAPTER 6

Vacuum packaging of Feta cheese reduced the rate of exudation by blocking the the ends of the capillaries of the protein matrix in cheese. However, an increase in the number of times of repacking (with vacuum) the same block of cheese increased the amount of exudate. Piling of the blocks of cheese one over the other did not affect the exudation. The thermal shocks imparted to the product during storage increased the amount of exudate.

The amount of exudate from Feta cheese increased with: increased P/F ratio, increased curd pH at draining, increased storage time and increased storage temperature. Variations in 'homogenisation pressure for manufactured cream' and 'priming time' had no effect on the extent of exudation. Variations brought about by process treatments (priming time and curd pH at draining) on mineral balance, residual lactose, and residual rennet in curd at the time of draining were apparently neutralised during the overnight draining of whey from curd.

Increased moisture (and MNFS) and decreased fat content in Feta cheese resulted in increase in the amount of the exudate. The effect of fat in reducing the amount of exudate may be similar to its role as a mechanical barrier during syneresis of renneted gels (Lelievre & Creamer, 1978).

Protein content in cheese may be primarily responsible for the initial water-holding capacity in cheese. However, an increase in the amount of exudate coincided with an increase in proteolysis. Proteolysis was rapid in the first four weeks and continued during the storage. α_{s1} -casein was degraded rapidly and most of it was hydrolysed by six months. β -casein was more resistant and $\approx 50\%$ of it was still

intact after six months of storage. These results are comparable to the proteolysis in Gouda cheese (Visser & Groot-Mostert, 1977) and in Feta cheese (Alichanidis *et al.*, 1984). The NSLAB did not appear to be active during the storage of cheese.

The water activity of Feta cheese was primarily regulated by the S/M concentration. The amount of unfreezable water increased in both cheese and exudate during storage. However, no relationship could be established between the unfreezable water in cheese and the extent of exudation from cheese.

Substantial amount of residual lactose was detected in Feta cheese after six months of storage. Residual lactose in cheese was metabolised slowly during storage, apparently by *Lactobacillus casei*, resulting in a slow decrease of cheese pH. The slow rate of metabolism was probably due to the high S/M concentration and low pH of cheese. This is consistent with the findings that when Cheddar cheese contained 6% S/M, residual lactose was found up to at least six months (Thomas & Pearce, 1981).

CHAPTER 7

EFFECT OF INCORPORATION OF HEAT-DENATURED WHEY PROTEIN ON THE YIELD AND EXUDATION OF WHEY FROM FETA CHEESE

7.1 Introduction

Partial denaturation of whey protein by heating the cheesemilk is an effective means of reducing the amount of exudate from Cream cheese during storage (Chapter 5). The effectiveness of whey protein in reducing the rate of exudation in ripened cheese, *e.g.* Feta, was unknown. In addition to the possibility of restricting the rate of exudation, incorporation of denatured whey protein had the wider commercial implication of a possible increase in cheese yield.

Various techniques for the incorporation of whey protein into cheese were considered. Native whey protein does not considerably improve the water-binding ability and thus its incorporation was considered unlikely to retard the rate of exudation. Besides, there are reports of native whey protein hindering the activity of plasmin (Hansen, 1990), and lowering the proteolytic activity in cheese (Abrahamsen, 1979).

Incorporation of partially heat-denatured whey protein by high heat treatment of milk was another option. However, heating milk is known to impair the rennet coagulation (Hoodyonk *et al.*, 1987). The commercial feasibility of this procedure has not been reported. Although this method has the potential for reducing the rate of exudation, doubts about the suitability of the process for cheese manufacture, and lack of adequate equipment and facilities were the reasons for not experimenting. Another method that could have been tried was the application of ultrafiltration to concentrate milk to a level such that draining of whey during cheesemaking is not necessary, *e.g.* Cast Feta. However, the primary objective of this project was to find a solution to the problem of exudation in Cheshire cheese. The 'Cast Feta' method of cheesemaking, which involves no drainage of whey, is different from the traditional methods of cheesemaking and is not suitable. The option left was to try the incorporation of heat-denatured whey protein by the

Centriwhey method (Walker, 1970). This method has the advantages of utilising the greater water-binding ability of denatured whey protein and the avoidance of any difficulty with rennet coagulation.

The literature on aspects related to incorporation of whey protein is reported in Chapter 2.

The scope of this experiment was to study the effect of incorporation of heat-denatured whey protein on: the exudation of whey during storage, the yield of cheese, the product acceptability and the proteolysis of Feta cheese.

7.2 Experimental plan

The procedure for preparation of heat-denatured whey protein suitable for cheese manufacture was optimised based on the principle involved in the *Centriwhey* method (Walker, 1970). The *Centriwhey* method involves pH adjustment of whey, heat-denaturation of whey proteins and centrifugal separation of the denatured whey proteins. In the present study the retentate from ultrafiltered acid casein whey was taken as the base material, heat treated, diluted to required concentration and milled in a colloid mill. Details of the method are outlined in the following section (Chapter 7.3).

It was expected that the increase in cheese yield, and the reduction in the amount of exudate, would depend partly on the quantity of incorporated denatured whey protein. Therefore, the quantity of denatured whey protein to be added was chosen as one of the variables for study.

It has been recommended that the fat level in the cheese should be increased in proportion to the amount of denatured whey protein incorporated (Walker, 1970), in order to maintain the quality of cheese. Accordingly, this factor was chosen as a variable for study.

particle size of the whey proteins. Size reduction was achieved by passing the product through a colloid mill. Further size reduction was accomplished by homogenisation (Pang, 1989). Homogenisation of heat-denatured whey protein has also been reported to result in slight increased yield (Krashenin *et al.*, 1974). As homogenisation formed a part of the process for preparation of cheesemilk, there was a possibility of homogenising the slurry (containing denatured whey protein) in combination with homogenisation of reconstituted skim milk (RSM) and fresh frozen milkfat for recombining (FFMR) while making 'manufactured cream'. Therefore, the effect of homogenisation of denatured whey protein on the product characteristics, yield and exudation of Feta cheese was selected as another variable for study.

Addition of slurry containing heat-denatured whey protein lowered the pH of cheesemilk from 6.67 to about 6.45, depending on the quantity of denatured whey protein added. If cheeses were to be made from these milks without adjustment of pH to a fixed value it would have resulted in variations in gel strength during rennet coagulation, and variations in draining pH. Gel strength affects syneresis while draining pH influences MNFS, mineral balance and residual rennet in cheese. It was necessary to minimise these effects and maintain uniformity in all cheeses. This was achieved by adjusting the pH of cheesemilk to a constant value of 6.55 at the start of the process by addition of NaOH or HCl, and having a fixed time (from inoculation of starter until draining of whey) for all the trials.

Experimental design: Details of the variables and their respective levels of variation are listed in Table 7.1. The experimental design was a 2^3 factorial with 3 control trials. A total number of 15 trials (one trial for each combination of process variables involving 'incorporation of heat-denatured whey protein', 'fat adjustment', and 'homogenization' plus three control trials) was performed in a random sequence. For each of the 15 trials, 10 blocks of cheese were packed and stored, and one block was used for each selected storage time. The process variables for the control runs did not have any addition of heat-denatured whey protein, and therefore did not have fat adjustment or homogenisation. Analysis of variance was performed using SAS (SAS Institute Inc., 1985).

Table 7.1 Treatment variables, and their respective levels of variation, chosen for study of the effects of incorporation of heat-denatured whey protein on yield, product characteristic and exudation of whey from Feta cheese during storage.

Treatment variables	Levels of variation	Abbreviated as
Quantity of heat-denatured whey protein added [on dry matter (DM) basis] per kg cheesemilk	0	Control
	4.5 g	W 1
	9.0 g	W 2
Fat adjustment in proportion to the denatured whey protein incorporated (adjustment of P/F ratio)	Not adjusted	F 1
	Adjusted (0.74)	F 2
Homogenisation of slurry containing heat-denatured whey protein	No homogenisation	H 1
	Homogenised separately (2,000 psi ¹ , 60 °C)	H 2
	Homogenised with FFMR and RSM (during preparation of manufactured cream)	H 3
Storage time (weeks)	2 weeks	S 2
	4 weeks	S 4
	6 weeks	S 6
	9 weeks	S 9
	12 weeks	S 12
	15 weeks	S 15
	20 weeks	S 20
	26 weeks	S 26
	39 weeks	S 39

¹ 1 psi = 6.89 kPa

7.3 Experimental

7.3.1 Preparation of slurry containing heat-denatured whey protein: Retentate from ultrafiltered acid casein whey was used as the source of whey proteins. This retentate was obtained from Tui Milk Products Ltd., Palmerston North. Typically the retentate had 24% total solids. The retentate was heated to 85°C in a water bath and held for 30 min¹. It was then diluted with water to approximately 20% total solids, cooled to about 70°C and milled in a colloid mill² with one-pass. It was then diluted with water to about 15% total solids. A portion of this slurry (unhomogenised) was stored at 2°C until addition to cheesemilk. The rest of the slurry was homogenised at 60°C/2,000 psi (13,780 kPa) in single stage. The homogenised slurry was stored at 2°C until used.

7.3.2 Cheesemaking

The experimental procedure for making Feta cheese was similar to that described in Chapter 6, except for the following differences.

- (i) Cheesemilk consisted of RSM, manufactured cream, and the slurry containing heat-denatured whey protein (not added in control).
- (ii) When the slurry containing heat-denatured whey protein was to be homogenised in combination with manufactured cream, the calculated amount of

¹The viscosity of the retentate increased with increase in temperature during heat treatment, presumably due to heat-denaturation of whey proteins. It was not possible to stir the highly viscous product beyond a temperature of 85°C. Instead of attempting a further rise in temperature, the retentate was therefore held at 85°C for 30 min. It is assumed that most of the whey proteins are heat denatured due to this heat treatment. In the text heat denatured whey protein refers to whey protein denatured at a heat treatment of 85°C/30 min.

² A Probst und Class colloid mill (Model JV10, power rating 9.2 kW) was used. The colloid mill has a rotor that revolves at a high speed within the stator. This creates a large amount of mechanical stress, shear, tangential and frictional forces with impact stress on the product.

slurry was mixed with FFMR and RSM, and was homogenised by a procedure similar to the standard practice of manufactured cream.

(iii) The pH of cheesemilk was adjusted to 6.55 using 0.1% NaOH or 0.035% HCl. The possible localised effect of acid/alkali on milk protein was minimised by continuous and thorough agitation of milk during the addition of acid/alkali.

(iv) The quantity of acid or alkali to be added was unknown as it depended upon the buffering index of milk and the pH of the slurry containing denatured whey protein. Provision was made for the dilution effect of the added acid/alkali by using a slightly concentrated RSM (ratio of SMP and water was adjusted to 1:7.95 ratio instead of 1:8.5). A calculated amount of water up to 2 kg was then added at the end to bring the RSM concentration to the required value. An example of the calculations for this is provided in Appendix 7.1.

(v) 12 ml of rennet was added per 100 kg milk instead of the standard practice of 16 ml per 100 kg cheesemilk. The amount of rennet was reduced to negate the effect of a reduction in the pH of cheesemilk due to the addition of slurry containing denatured whey protein. This aspect was investigated earlier in a separate study (Pang, 1989).

(vi) Incorporation of denatured whey protein has been reported to result in cheese with high moisture (Abrahamsen, 1979; Furtado & Partridge, 1988; Pang, 1989). In the present study it was planned to have uniform MNFS in all cheeses so that MNFS was not a factor affecting exudation. In order to attain the MNFS level in cheeses incorporated with denatured whey protein similar to that in the control cheese, the following modifications were used: 9 mm knives instead of 12 mm, and cooking temperatures of 34 °C for the lower level of whey proteins and 36 °C for the higher level.

(vii) The draining pH was 6.15.

7.3.3 Mass balance studies: To support the findings on cheese yield mass balance studies were performed for fat, protein, total solids, calcium and moisture.

7.4 Methods of analysis

Slurry containing heat-denatured whey protein was analysed for total solids by microwave analyser at various stages of preparation. Final slurry containing denatured whey proteins was analysed for total solids (gravimetrically), total nitrogen (Kjeldahl), and calcium (complexometric).

Cheesemilk and whey were analysed for total solids (gravimetric), fat (Rose-Gottlieb), total nitrogen (Kjeldahl), and calcium (complexometric).

Cheeses before brining and after four weeks of storage were analysed for fat (Schmidt-Bonzynski-Ratzlaff), total nitrogen (Kjeldahl), moisture, calcium (complexometric) and pH. Cheeses were analysed for NaCl (potentiometric) after four weeks. Proteolysis in cheeses at four weeks and six months of storage were determined by SDS-PAGE. Exudation of whey was measured at the stipulated storage intervals. Exudates were analysed for total solids, total nitrogen (Kjeldahl), calcium (complexometric), NaCl (potentiometric) and distribution pattern of low molecular weight peptides (HPLC).

Details of the analytical methods for the above analyses are provided in Chapter 4 and Appendix 4.1.

7.5 Sensory evaluation

Cheeses were evaluated by a panel after four and eighteen weeks of storage. Details of the product testing and grading are reported in Chapter 4.

7.6 Results and discussion

The results are described under the following headings:

- (i) Composition of slurry, milk, whey, cheese and exudate.
- (ii) Manufacturing aspects and quality of cheese.
- (iii) Mass balance and cheese yield.
- (iv) Proteolysis in cheese.
- (v) Exudation from cheese.

7.6.1 Composition of slurry, milk, whey, cheese and exudate.

Slurry containing denatured whey protein: Composition of the slurries were in the following range: Total solids = 14.0 - 15.0%, Protein = 11.10 - 12.38%, Ca^{2+} = 16 - 19 mM/kg, pH = 4.7 - 4.8.

Cheesemilk: Variation in the fat and protein of cheesemilks for the different levels of process treatments was in accord with the experimental design. Details of the composition of cheesemilk (including added denatured whey protein) are provided in Appendix 7.2.

Whey (Appendix 7.3): A slight increase in fat loss into the whey was detected when cheese was made with higher amount of heat-denatured whey protein, and also in cheese with higher level of fat. Fat loss into the whey was lower when the slurry containing the heat-denatured whey protein was homogenised separately. However, none of these variations are of any practical concern. Slightly increased calcium level of whey was detected when cheese was made with higher level of fat. This may have been due to a corresponding lower pH of cheese.

Cheese before brining (Appendix 7.4): A higher percentage of fat and FDM in cheese with fat level adjusted in proportion to the added whey protein were expected. A similar MNFS for all cheeses showed good control during cheese manufacture. As a consequence of having similar MNFS in all cheeses, the moisture was slightly higher in cheese samples manufactured with a higher amount of denatured whey protein.

Cheese after four weeks of storage (Appendix 7.5): A slight shift in moisture and MNFS in cheeses were noted in comparison to the respective values of cheese before brining. This may be attributed to the effects of brining and exudation. Mostly the composition was typical of Feta cheeses.

Exudate from four week old Feta cheese: The composition of exudate was mostly uniform and typical (Appendix 7.6). Variations in total solids (of cheese with lower fat), calcium and protein (for cheeses with different levels of added whey protein) of exudate may have been simply due to slight variation in the corresponding amounts of exudates released.

7.6.2 Manufacturing aspects and quality of cheese

Cheese manufacture: All cheeses (including control) appeared firmer in comparison to Feta cheese made in other experiments. This might have been due to the lowering of pH of milk to 6.55 at the start of the cheesemaking process. The curd strength appeared satisfactory for all cheeses and there was no difficulty in cutting the gels. However, the gels appeared firmer and whey turbidity increased with increase in the level of added denatured whey protein. Homogenisation of slurry containing denatured whey protein in combination with manufactured cream resulted in a softer gel and increased moisture in cheese. Too many curd-fines appeared to be lost during manufacture of cheese with a higher level of denatured whey protein. The texture of cheese manufactured with a high level of denatured whey protein appeared to be brittle and soft. In cheeses where the fat level was adjusted in proportion to the added denatured whey protein the curd was seen floating in whey for most of the duration of stirring and the whey appeared turbid.

Sensory evaluation of cheese (Appendix 7.7): The major objectives of the sensory evaluation of the cheeses were to determine the effect of incorporation of heat-denatured whey protein on the acceptability of cheese and the changes in the product quality with increase in storage time.

For most attributes of flavour and texture, cheeses manufactured with heat-denatured whey protein were perceived to be similar to control cheese. A

statistically significant variation was observed for 'acidity'. However, this variation was not of any practical concern. Scores for bitterness and oxidised flavours were in the bottom range, and therefore may be considered as insignificant for all samples. The only significant change detected with increase in storage time was with salt content in cheese. A decrease in saltiness of the cheeses with increase in storage time may be related to the loss of NaCl through the exudate, or to the development of cheese flavour that provided a masking effect on saltiness of the cheese. The texture attributes (mouthfeel, sliceability) and overall acceptability were not affected due to the incorporation of the heat-denatured whey protein, and did not change with increase in storage period.

The major implications of the sensory evaluation was that the quality of Feta cheese manufactured with heat-denatured whey protein was comparable to that of control cheese. Cheddar cheese manufactured from extensively heat treated milk had developed bitter flavour (Banks *et al.*, 1987), while Cheddar cheese manufactured with ultrafiltered cheese whey had developed 'acid' flavour (Brown & Ernstrom, 1982). It was encouraging to note that no such flavour could be detected in this experiment. A general feeling conveyed by the panel was that most cheeses evaluated after four weeks were somewhat dry/crumbly/brittle (except for cheeses from trials 9, 13, 14, and 15). This could have been due to the fact that the cheeses were only four weeks old and had not matured enough. The extent to which the presence of heat-denatured whey protein could have contributed to the dryness of cheese could not be ascertained because the control cheeses fared no better. With the benefit of hindsight, it now appears that lowering the pH of cheesemilk at the beginning of cheese manufacture could have contributed to the dryness as it resulted in generally lowering the level of moisture, and thus affected the cheese texture. Remarks by the panel for cheese at eighteen weeks were much more favourable, even though the dryness was still perceived. The Feta cheeses had definitely obtained a more 'cheesy' flavour. Some of the best rated cheeses were with added heat-denatured whey protein.

It was concluded that the incorporation of heat-denatured whey protein did not adversely affect the organoleptic properties of cheese.

7.6.3 Mass balance and cheese yield

(i) **Mass balance studies:** Mass balances were carried out for fat, protein, total solids, calcium and moisture. The calculations were based on the method reported by Lelievre *et al.* (1983). The input comprised of milk (including slurry containing denatured whey protein), starter and rennet. The output was cheese (before brining) and whey. Appendix 7.8 provides: (a) the data on quantities of input and output material, the calculated values of mass balance, yields and recoveries of the milk solids for each of the 15 trials; (b) an example of mass balance calculation; (c) the effect of process treatments on the mass balance of the selected milk constituents; (d) justification for variations in mass balances of selected milk constituents.

Mass balance values of the milk constituents for the individual trials were mostly reasonable. Instances where the values were very high or low, it was mostly due to the specific nature of process variable. Increased loss of fat occurred in trials with combined homogenisation of denatured whey proteins and manufactured cream. This was possibly due to the increased adsorption of denatured whey protein to the fat globules. Increased loss of protein was detected when the denatured whey protein was added without homogenisation. Mass balance for calcium and moisture were reasonable for all trials. Variation in mass balance for total solids was related to the variations in fat and protein in the individual trial.

The mass balance was satisfactory and supported the findings, particularly with respect to cheese yield.

(ii) **Cheese yield**

Table 7.2 shows the yield of cheese as affected by the process variables. A comparison of the 'control' trials with that of all other trials showed a significant increase in yield with incorporation of denatured whey proteins.

Table 7.2 Effect of process treatments on yield of cheese^a

Source of variation and levels	Yield			
	kg cheese per 100 kg milk		kg cheese (adjusted to 50.0% moisture) per 100 kg milk	
	LSM	F	LSM	F
Control	18.42	11.96	17.24	19.59
Others	20.13	*	18.34	*
Added whey protein (W)		12.71*		7.69*
W 2	20.92		18.65	
W 1	19.34		18.03	
Fat (F)		3.15		37.12**
F 2	20.52		19.02	
F 1	19.74		17.66	
Homogenisation (H)		0.83		0.07
H 1	19.79		18.33	
H 2	20.11		18.40	
H 3	20.49		18.30	
S.D.	0.766		0.386	

^a All data relate to weights of cheese as determined before brining

Control = Cheese prepared without any added whey protein;
'Others' = Includes all trials (12 out of 15) in which denatured whey protein was incorporated;

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;

W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein;

F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;

H 2 = Denatured whey protein homogenised separately;

H 3 = Denatured whey protein homogenised in combination with manufactured cream;

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;

F ratios without asterisks denote 'not significant'.

Effect of process variables on the yield of cheese

Increase in quantity of denatured whey protein added: The yield of cheese increased with increase in amount of denatured whey protein incorporated in cheesemilks.

Fat adjustment in proportion to the added whey protein: A significant increase in yield was obtained when fat content in cheesemilk was increased in proportion to the added denatured whey protein.

Method of homogenisation of slurry with denatured whey protein: Cheese yields were similar with different treatments of homogenisation.

Effect of process variables on the recovery of milk solids in cheese

The variation in percentage of milk solids (fat, calcium, protein and nitrogen) retained in cheese due to the process variables are reported in Table 7.3. The recovery of milk solids has been expressed in two ways; firstly as a percentage of input (milk solids present in milk) which accounts for the losses during cheesemaking, and secondly as a percentage of output (milk solids in cheese and whey) which ignores handling losses and is more realistic when handling losses are not entirely due to process treatments.

Recovery of fat: Recovery of fat was not greatly influenced by any of the process variables. Although some treatments show a statistical significance, the variation does not appear to be of much practical relevance. The trends indicate that the fat loss increased marginally due to the incorporation of denatured whey protein, increased slightly with increased fat level, decreased slightly when whey protein was added without homogenisation, and increased when the whey protein was incorporated by homogenising it along with the manufactured cream.

Recovery of protein: The recovery of protein decreased with an increase in the amount of denatured whey protein incorporated. The recovery of protein was not influenced by the fat levels and was minimum when the added whey protein was not homogenised.

Table 7.3 Effect of process treatments on the recovery of milk constituents in cheese

Variations & levels	Percent recovery of milk constituents (w/w)							
	Fat ^a		Fat ^b		Calcium ^a		Calcium ^b	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	99.6		98.9		53.4		53.2	
Added whey protein (W)		0.08		14.21*		3.68		5.51
W 2	97.4		98.7		55.1		54.7	
W 1	97.7		98.9		52.3		51.5	
Fat (F)		4.72		0.11		6.59		12.15*
F 2	96.5		98.7		51.9		50.7	
F 1	98.6		98.8		55.5		55.5	
Homogenisation (H)		2.08		7.14*		2.64		1.90
H 1	98.9		98.7		51.6		52.2	
H 2	97.2		98.9		55.6		55.0	
H 3	96.5		98.7		53.9		52.1	
S.D.	1.69		0.11		2.47		2.36	

Variations & levels	Percent recovery of milk constituents (w/w)							
	TS ^a		TS ^b		Protein ^a		Protein ^b	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	59.6		60.6		80.4		79.0	
Added whey protein		0.77		20.18*		4.36		2.66
W 2	61.8		63.3		78.1		80.7	
W 1	61.4		62.0		80.2		80.0	
Fat (F)		12.3*		29.1**		0.01		0.05
F 2	62.4		63.5		79.2		80.3	
F 1	60.8		61.8		79.1		80.4	
Homogenisation (H)		1.62		0.73		8.36*		3.84
H 1	61.0		62.4		76.7		79.7	
H 2	61.9		62.9		79.2		80.1	
H 3	61.9		62.6		81.7		81.2	
S.D.	0.79		0.54		1.72		0.80	

^a Expressed as a percentage of input (milk + slurry containing denatured whey protein + starter)^b Expressed as a percentage of output (cheese + whey)

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;

W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein; F 1 = Fat level not adjusted; H 1 = Denatured whey protein added without homogenisation;

H 2 = Denatured whey protein homogenised separately; H 3 = Denatured whey protein homogenised in combination with manufactured cream; Control = Cheese prepared without any added whey protein; LSM = Least-square mean; F = F ratio; S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; F ratios without asterisks denote 'not significant'.

Recovery of calcium: The process treatments did not cause a great variation in calcium levels.

Recovery of TS: Recovery of total solids increased with increase in the amount of denatured whey protein incorporated, and increase in the fat level. There was no difference between cheeses with high and low levels of incorporated whey protein in terms of % TS recovered. However, it was encouraging to note that the % TS recovered had not decreased with the increase in the amount of whey protein incorporated.

It is interesting to note that the total solids recovered in cheeses incorporated with whey protein are higher than 'control' even though the percentages of fat and protein recovered in control cheeses were higher. The cheeses manufactured with heat-denatured whey protein retained moisture in excess of control. Additional soluble solids and salts appear to have been retained with this extra moisture. This resulted in an overall increase in recovery of TS even though fat and protein contents recovered were slightly low. It is thus likely that the cheeses with added whey protein retained a greater amount of lactose.

Conclusion: Incorporation of heat-denatured whey protein [on dry matter (DM) basis] up to 9.0 g/kg cheesemilk resulted in an increase in yield of Feta cheese. The increase in yield was due to retention of: a proportionate amount of added whey protein and fat, extra water (retained in proportion to the incorporated heat-denatured whey protein to obtain a constant MNFS), and additional soluble solids (probably lactose) retained along with the extra water. These results are consistent with the reported increase in yield and satisfactory quality of semi-hard cheeses with incorporation of heat-denatured whey protein (DM basis) up to 4.0 g/L milk (Anon, 1969a; Walker, 1970; Krasheninina *et al.*, 1974).

Adjustment of fat level in proportion to the increased denatured whey protein resulted in an increase of cheese yield. Addition of denatured whey protein without any homogenisation resulted in a slightly lower yield than when homogenised. From the yield point of view, the heat-denatured whey protein may be incorporated by homogenising it in combination with manufactured cream.

7.6.4 Proteolysis in cheese

SDS-PAGE: The objective was to determine the effect of incorporation of heat-denatured whey protein on the proteolytic pattern of Feta cheese. In order to determine the rate of proteolysis the results have been expressed as the ratio of areas of individual proteins/peptides (α_{s1} -casein, β -casein and the breakdown bands) to the area of para- κ -casein. Para- κ -casein was chosen as the reference protein because it resolved well in the gel, it was isolated from other bands in the gel, and it did not appear to have been affected by proteolysis.

Proteolysis in cheese at four weeks: The pattern of proteolysis was similar in all cheeses and was typical of Feta cheese (Table 7.4). The rate of breakdown of α_s -casein (α_{s1} - & α_{s2} -) was mostly similar in all cheeses. β -casein was more stable, though it was difficult to accurately determine whether β -casein was being proteolysed. Cheeses incorporated with denatured whey protein had increased amounts of whey protein, as detected from the density of β -lactoglobulin bands. However, the increase in β -lactoglobulin was not in proportion to the increase in the amount of denatured whey protein incorporated. This was due to experimental error involved in the recovery of all the whey proteins. It was found that not all of the added denatured whey proteins were soluble when cheese was dispersed in SDS sample buffer, and some sedimented during the centrifugation operation involved in protein extraction from cheese. In subsequent analyses this was overcome by adding dithioerythritol (DTE) to cheese and sample buffer, heat treating (95°C/10 min), and then centrifuging. Addition of DTE before heat treatment and centrifugation helped reduce the disulphide linkages in the denatured whey proteins and solubilised the insoluble whey protein. There was no indication of a reduced proteolysis in cheeses manufactured with denatured whey protein. The ratio of peptide representing the first breakdown band to para- κ -casein was identical in all cheeses. The ratio of peptide representing the second breakdown band to para- κ -casein was slightly higher in cheeses manufactured with denatured whey protein. This suggested a slightly faster proteolysis, a trend similar to that observed by Sakharov (1975). However, this is very unlikely as denatured whey proteins have been reported to have little effect on α_{s1} -casein degradation, and to retard β -casein proteolysis (Harper *et al.*, 1989). It is possible that in control cheeses the peptide representing the second band was

Table 7.4 Effect of process treatments on the proteolysis in four week old Feta cheese. Proteolysis has been expressed in terms of ratio of areas of selected proteins/peptides to that of para- κ -casein. The areas have been estimated using the densitometer plots of SDS-PAGE.

Variation	Ratio of areas							
	β -lg / para- κ -cn		α_{s1} -casein / para- κ -cn		Peptide ¹ / para- κ -cn		Peptide ² / para- κ -cn	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	0.64		2.36		0.38		0.46	
Added whey protein (W)		2.44		0.41		0.12		2.46
W 2	1.02		2.31		0.40		0.53	
W 1	0.83		2.38		0.39		0.55	
Fat (F)		3.76		0.24		0.11		4.41
F 2	0.80		2.37		0.39		0.55	
F 1	1.04		2.32		0.40		0.53	
Homogenisation (H)		0.51		0.55		1.49		6.21
H 1	0.91		2.41		0.40		0.52	
H 2	0.85		2.36		0.42		0.57	
H 3	1.00		2.26		0.37		0.54	
S.D.	0.211		0.202		0.043		0.02	

¹ First breakdown product.

² Second breakdown product.

para- κ -cn = para- κ -casein;

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;

W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein;

F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;

H 2 = Denatured whey protein homogenised separately;

H 3 = Denatured whey protein homogenised in combination with manufactured cream;

Control = Cheese prepared without any added whey protein;

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;

F ratios without asterisks denote 'not significant'.

proteolysed rapidly to smaller molecular weight peptides or amino acids leading to the observed effect. The other possibility could be the difficulty in recovering all proteins as described earlier. It was decided that a better assessment of the rate of proteolysis could be made with storage study on cheese at a later stage.

Proteolysis in cheese at six months: The pattern of proteolysis was similar in all cheeses (Fig. 7.1 & Table 7.5). Substantial amount of α_s -casein (α_{s1} - & α_{s2} -) had been extensively proteolysed. It was difficult to establish the extent to which β -casein had been hydrolysed. Most of it however appeared intact. There are reports that the activity of plasmin is retarded by denatured β -lactoglobulin (Grufferty & Fox, 1986; Rollema & Poll, 1986; Harper *et al.*, 1989). β -casein is usually hydrolysed by plasmin. In this experiment it is difficult to ascertain the extent to which the presence of denatured β -lactoglobulin would have retarded proteolysis of β -casein. This is because proteolysis of β -casein may have been partly retarded in Feta cheese even in the absence of denatured whey proteins (control cheese) due to low pH and high salt in cheese. Para- κ -casein, β -lactoglobulin and α -lactalbumin did not appear to have been proteolysed during storage. The rate of proteolysis appeared to be about the same in all the cheeses as there was no difference in ratios of α_s -casein to para- κ -casein and the ratio of breakdown products with para- κ -casein.

The ratios of β -lactoglobulin with para- κ -casein in the cheeses indicated the following: increase in amount of β -lactoglobulin in proportion to the increased incorporation of denatured whey protein in cheesemilk, increase in β -lactoglobulin when fat level was adjusted in proportion to the added whey protein, increase in β -lactoglobulin when the slurry containing heat-denatured whey protein was homogenised with manufactured cream, and a decrease in β -lactoglobulin when the slurry containing heat-denatured whey protein was added without homogenisation. These findings are in agreement with the results of mass-balance studies. One of the important implications of this study is that the added denatured whey protein did not hydrolyse during storage and did not influence the proteolysis in cheese. It is assumed here that the amount of β -lactoglobulin retained in cheese provided a proportional representation of the whey proteins in cheese. Appendix 7.9

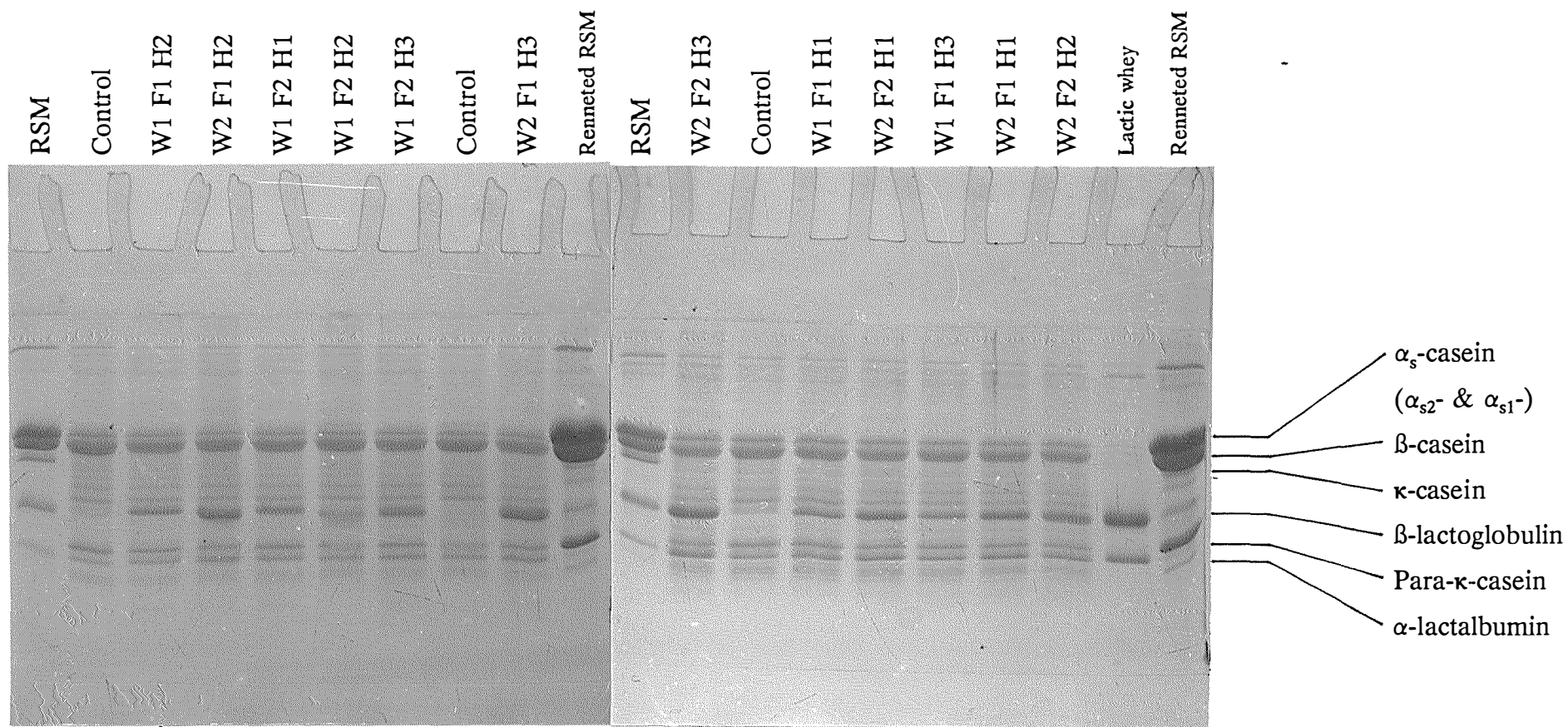


Fig. 7.1 SDS-PAGE on six month old Feta cheeses incorporated with heat-denatured whey protein.

W2 = Incorporation of heat-denatured whey protein (on dry matter basis) at the rate of 9.0 g/kg cheesemilk;

W1 = Incorporation of heat-denatured whey protein (on dry matter basis) at the rate of 4.5 g/kg cheesemilk;

F2 = Fat level in cheesemilk adjusted in proportion to incorporated whey protein;

F1 = Fat level in cheesemilk not adjusted (and therefore higher protein/fat ratio in cheesemilk);

H1 = Slurry containing denatured whey protein added to cheesemilk without any homogenisation treatment;

H2 = Slurry containing denatured whey protein was homogenised at 13,780 kPa (single stage) and then added to cheesemilk;

H3 = Slurry containing denatured whey protein homogenised along with manufactured cream;

RSM = Reconstituted skim milk; Control = Cheesemilk without incorporation of heat-denatured whey protein.

Table 7.5 Effect of process treatments on the proteolysis in six month old cheese. Proteolysis has been expressed in terms of ratio of areas of selected proteins/peptides to that of para- κ -casein. The areas have been estimated using the densitometer plots of SDS-PAGE.

Variation	Ratio of areas							
	β -lg / para- κ -cn		α_{s1} -casein / para- κ -cn		Peptide ¹ / para- κ -cn		Peptide ² / para- κ -cn	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	0.45	341	1.02	0.00	0.90	0.25	0.78	1.19
Others	2.21	***	1.02		0.81		0.67	
Added whey protein (W)		212		1.13		0.51		0.0

W 2	2.83		0.98		0.76		0.67	
W 1	1.58		1.06		0.87		0.67	
Fat (F)		12.0*		0.83		2.07		0.0
F 2	2.35		1.06		0.92		0.67	
F 1	2.06		0.99		0.70		0.67	
Homogenisation (H)		9.39		0.92		0.86		0.12
		*						
H 1	1.96		0.97		0.73		0.66	
H 2	2.24		1.09		0.95		0.65	
H 3	2.41		0.99		0.75		0.70	
S.D.	0.15		0.13		0.26		0.15	

¹ First breakdown product.

² Second breakdown product.

para- κ -cn = para- κ -casein;

Control = Cheese prepared without any added whey protein;
Others = Includes all trials (12 out of 15) other than the control trials;

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;

W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein;

F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;

H 2 = Denatured whey protein homogenised separately;

H 3 = Denatured whey protein homogenised in combination with manufactured cream;

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;

F ratios without asterisks denote 'not significant'.

provides the detailed calculations involved in determining that most of the added β -lactoglobulin did not undergo any change during storage of cheese up to six months.

HPLC profile of peptides in exudate from four week old cheese

HPLC profiles showed that the pattern of proteolysis was similar in all the trials. Slight variation of the peak eluting at about 32.5 s ($\approx$ 4,000 daltons) was observed in a few trials. However, statistical analysis did not show any significant effect of the treatment variables. Therefore, this variation could not be attributed to any process treatment.

Conclusion: It was concluded that the pattern of proteolysis and the extent of proteolysis progressed steadily and fairly uniformly for all the cheeses. Presence of heat-denatured whey proteins did not affect proteolysis in Feta cheese.

7.6.5 Exudation of whey from Feta cheese during storage

Exudation of whey from Feta cheese was expressed in different ways as explained in Appendix 4.2. The general trend in exudation was typical of Feta cheese. The effect of the process variables on the exudation is reported in Table 7.6.

Effect of incorporation of heat-denatured whey protein: The exudation was not significantly affected by the incorporation of heat-denatured whey protein. The denatured whey protein did not improve the water-binding ability of the cheese.

Fat is present in cheese as an inert filler. The study on proteolysis of cheese showed that the heat-denatured whey protein was present also as an inert material. However, unlike fat, the denatured whey protein was not effective in reducing the extent of exudation. From this study it is not clear as to why the denatured whey protein was not effective in increasing the water-binding ability of cheese. The study showed that mere presence of inert material in the cheese may not necessarily be effective in reducing the amount of exudate. Further, the study showed that the form in which the whey protein is incorporated may have an

Table 7.6 Effect of process treatments on the exudation of whey from Feta cheeses (incorporated with heat-denatured whey proteins) during storage at 10°C

Source of variation & levels	Exudate in g/kg cheese		Exudate in g/kg cheese moisture		Final MNFS (%) in cheese		% reduction in MNFS of cheese	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	23.6	0.82	47.4	0.11	67.5	0.07	0.81	0.29
Others	22.9		44.6		67.8		0.75	
Added whey proteins (W)		0.02		0.11		0.07		0.23
W 2	24.0		45.8		68.8		0.72	
W 1	21.8		43.4		66.8		0.77	
Fat (F)		0.93		0.82		0.01		0.68
F 2	20.7		41.1		67.8		0.70	
F 1	25.1		48.1		67.7		0.79	
Homogenisation (H)		0.33		0.17		0.49		0.07
H 1	22.7		44.8		67.5		0.76	
H 2	21.2		41.7		67.4		0.72	
H 3	24.8		47.3		68.5		0.75	

Control = Cheese prepared without any added whey protein;
 'Others' = Includes all trials (12 out of 15) in which denatured whey protein was incorporated;

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;

W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein;

F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;

H 2 = Denatured whey protein homogenised separately;

H 3 = Denatured whey protein homogenised in combination with manufactured cream;

LSM = Least-square mean;

F = F ratio;

F ratios without asterisks denote 'not significant'.

Details of calculation of exudation values are provided in Appendix 4.2.

Table 7.6 continued in next page

Table 7.6 continued

Source of variation & levels	Exudate in g/kg cheese		Exudate in g/kg cheese moisture		Final MNFS (%) in cheese		% reduction in MNFS of cheese	
	LSM	F	LSM	F	LSM	F	LSM	F
Interactions								
W X F		0.66		0.85		2.71		0.18
2 X 2	23.7		45.8		69.7		0.70	
2 X 1	24.4		45.7		67.9		0.74	
1 X 2	17.8		36.4		66.0		0.71	
1 X 1	25.8		50.4		67.6		0.83	
W X H		0.80		0.78		0.51		0.67
2 X 1	25.9		49.2		69.2		0.77	
2 X 2	24.2		46.4		68.4		0.75	
2 X 3	21.9		41.6		68.9		0.65	
1 X 1	19.5		40.3		65.9		0.76	
1 X 2	18.1		37.0		66.4		0.69	
1 X 3	27.7		52.9		68.1		0.86	
F X H		0.96		1.03		0.70		0.76
2 X 1	17.8		36.6		67.1		0.67	
2 X 2	23.4		46.0		68.3		0.77	
2 X 3	21.0		40.7		68.2		0.68	
1 X 1	27.6		52.9		68.0		0.85	
1 X 2	19.0		37.4		66.5		0.67	
1 X 3	28.6		53.8		68.8		0.83	
Storage time (S)								
2 week	13.4	29.04	26.1	33.92	68.0	0.93	0.43	48.43
4 week	14.0	***	27.3	***	68.0		0.45	***
6 week	16.0		31.3		67.9		0.52	
9 week	18.6		36.3		67.9		0.60	
12 week	20.8		40.6		67.8		0.68	
15 week	23.8		46.3		67.8		0.77	
20 week	28.5		55.4		67.7		0.93	
26 week	31.3		60.9		67.6		1.02	
39 week	39.6		77.0		67.4		1.31	
S X W		0.18		0.16		0.01		0.40
S X F		0.86		0.78		0.02		0.89
S X H		0.16		0.14		0.0		0.13
S.D.	4.47		8.1		0.58		0.12	

S = Storage time; W = Added whey proteins; F = Fat level; H = Homogenisation;
W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;
W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;
F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein;
F 1 = Fat level not adjusted; H 1 = Denatured whey protein added without homogenisation;
H 2 = Denatured whey protein homogenised separately;
H 3 = Denatured whey protein homogenised in combination with manufactured cream;
LSM = Least-square mean; F = F ratio; S.D. = Standard deviation of raw data;
*** = $p < 0.001$; F ratios without asterisks denote 'not significant'.

an important role (Lawrence, 1989); and that the complex formation between β -lactoglobulin and κ -casein due to heat-denaturation of whey protein in milk as in acid coagulated milk (Brooker, 1987) may be a prerequisite for improved water-holding ability of whey protein.

It must be noted that the presence of heat-denatured whey protein in cheese did not make the problem of exudation any worse. The whey protein incorporated in cheese were able to hold as much water as other proteins. This might be an useful attribute when considered from other aspects such as increasing the yield of cheese.

Fat content in cheese: Adjustment of fat content of cheesemilk in proportion to the incorporated denatured whey protein resulted in slight reduction of the amount of exudate, though statistically it was not significant. This further confirms the effectiveness of increased fat level in reducing the amount of exudate from Feta cheese.

Homogenisation of heat-denatured whey protein: The different methods of homogenisation of the heat-denatured whey protein did not significantly affect the exudation from cheese. The amount of exudates from these cheeses were also very close to that of control. Homogenisation of denatured whey protein with manufactured cream was less effective when considered strictly from the view point of reducing the amount of exudate from cheeses.

Interaction of the above variables: None of the interactions had a significant effect on the exudation. However, the amount of exudate released was slightly lower in cheeses made with the following combinations.

- Incorporation of denatured whey protein at the lower level with fat level adjusted.
- Incorporation of denatured whey protein at the lower level without any homogenisation.
- Incorporation of denatured whey protein at the lower level with separate homogenisation.
- Addition of unhomogenised denatured whey protein with fat level adjusted.

- Homogenisation of the added whey protein separately without adjusting the fat level.

Some of these interactions which show a decreasing trend with the amount of exudate might have a pronounced effect in reducing the rate of exudation in other cheeses, *e.g.* Cheshire, where the moisture level is lower and the pH is higher. It is likely that the statistical analysis has not picked up differences that may be considered real or of practical importance. It is possible that if replication or a larger number of trials were performed, some of these differences might have been detected significant. It is not clear at this stage which of these combinations may have a practical significance. The reader is advised to exercise caution in making any definite conclusions.

Effect of Storage time: There was a steady and significant rise in the amount of exudate with increase in storage time. The initial high amount of exudate was due to the mechanical effect of vacuum packing the cheese. The interactions of storage time with process treatments had no significant effect on the exudation.

7.7 Summary

Incorporation of heat-denatured whey protein (on DM basis) up to 9.0 g/kg cheesemilk resulted in a significant increase in the cheese yield, and had no effect on the exudation of whey from Feta cheese. Incorporation of heat-denatured whey protein did not lead to any adverse effect on the acceptability of cheese. Hydrolysis of incorporated denatured whey protein was not detected up to six months. There was no indication of proteolysis of casein in Feta cheese being retarded due to the presence of heat-denatured whey protein. Adjustment of fat content of the cheese milk in proportion to incorporated heat-denatured whey protein resulted in an increase in yield and slight reduction of the amount of exudate from Feta cheese. While incorporation of heat-denatured whey protein in cheesemilk by homogenising it in combination with the manufactured cream did not affect the cheese yield, it resulted in a slight increase in the amount of exudate.

CHAPTER 8

EFFECT OF HOMOGENISATION, SOURCE OF MILK SOLIDS AND FAT EMULSIFICATION ON THE EXUDATION OF WHEY FROM FETA CHEESE DURING STORAGE

8.1 Introduction

A variation in the pressure of homogenisation from single stage, 4134 kPa (600 psi) to double stage, 13780/3445 kPa (2000/500 psi) during preparation of 'manufactured cream' did not show any noticeable effect on the exudation of whey (Chapter 6). However, this did not indicate whether the homogenisation process *per se* has any effect on the exudation. A number of reports suggest that homogenisation of milk leads to a decrease in syneresis in renneted gels during manufacture of cheese (Vaikus *et al.*, 1970; Emmons *et al.*, 1980; Storry *et al.*, 1983; Walstra *et al.*, 1985; Fox, 1987a). It is reasonable to expect a similar effect of homogenisation on exudation. Thus it became necessary to establish whether homogenisation of cream affected the exudation during storage of Feta cheese.

All the experiments related to Feta cheese have been performed using recombined milks. Fresh frozen milkfat for recombining (FFMR) and skim milk powder (SMP) were the source of milk solids. The fat in the recombined milk differs from the fat in fresh milk due to the absence of milk fat globule membrane (MFGM) and the effects of homogenisation. The properties of solids-not-fat in recombined milk may differ from solids-not-fat in fresh milk due to the concentration and drying effects during powder making. The extent to which these variations affected exudation was uncertain. The role of combinations of milksolid from fresh milks and recombined milk on exudation was another factor that needed to be studied.

Study on the exudation of whey from Feta cheese (from recombined milk) during storage (Chapter 6) revealed that increase in fat level decreased the amount of exudate. However, the mechanism of this was not clear. It was also uncertain whether fat would be effective in reducing the amount of exudate when cheese was made from unhomogenised milk. Syneresis in renneted gels during cheese

manufacture is reduced by increase in fat content (Storry *et al.*, 1983). This may be explained as possibly due to fat mechanically blocking casein-casein interactions, that are likely to cause syneresis (Lelievre & Creamer, 1978); or fat globules acting as filler particles in an aqueous matrix of swollen proteinaceous material (Luyten, 1988). The decreased syneresis due to increased fat may be similar to the effect of fat impeding the salt diffusion in brined cheese (Geurts *et al.*, 1974). It is proposed that the effect of fat on the exudation of whey from Feta cheese may be considered similar to that during syneresis in renneted gels during cheese manufacture. The effect may, however, be diminished due to the considerable increase in concentration of milk solids and the decrease in moisture content.

Casein provides the basic structure in cheese (Lawrence *et al.*, 1983). In low pH cheeses water is held within the interstitial structure of the three-dimensional casein network in an inert state (Creamer & Olson, 1982). In cheese from unhomogenised milk, fat globules are trapped between the casein chains and function as inert filler. However, in homogenised milks the fat particles predominantly contain casein at the fat-water interface (Mulder & Walstra, 1974) and therefore participate in the formation of the casein matrix in cheese (Vliet & Dentener-Kikkert, 1982; Walstra & Vliet, 1986). Such fat particles function as permanent cross-links (Vliet & Dentener-Kikkert, 1982; Walstra & Vliet, 1986). This theory was used to explain the effect of homogenisation in the manufacture of Halloumi cheese and Mozzarella cheese from recombined milks (Lelievre *et al.*, 1990b). The participation of fat globules as part of the casein matrix, due to homogenisation, may be the cause for reduced syneresis during cheese manufacture and the subsequent effect on the extent of exudation during storage of cheese. A study of the role of material adsorbed to fat globules was expected to provide evidence on the proposed role of fat on the exudation.

Section One of this Chapter includes the study of the effect of homogenisation and source of milk solids on the exudation. In Section Two the effect of emulsification of fat with different emulsifying agents on the exudation is described.

SECTION ONE

8.2 EFFECT OF HOMOGENISATION AND SOURCE OF MILK SOLIDS ON THE EXUDATION OF WHEY FROM FETA CHEESE DURING STORAGE

8.2.1 Experimental

Feta cheese was manufactured using the procedure outlined in Chapter 4 except for variations in the combinations of 'creams' and 'skim milks' from different sources as listed in Table 8.1. Raw whole milk was obtained from the bulk supply from Tui Milk Products Ltd., Palmerston North. Skim milk and fresh cream (25% fat) were obtained by separation of whole milk. Cream was homogenised at a pressure of 6,890 kPa (1,000 psi), wherever applicable. After standardisation of recombined milk to a protein to fat ratio of 0.73, the milk was diluted with water so that the protein content in the milk was close to the average protein in whole milk ($\approx 3.3\%$).

The experiment was based on one-way classification of analysis of variance. A total of 10 trials, spread over two weeks, were performed using two replications of each combination. The trials were randomly selected in each replicate. The sources of variation were 'replicates' and 'source of milk solids'. The data were analysed using the statistical package of SAS Institute Inc. (1985).

8.2.2 Analytical methods: Milk and whey were analysed for fat, protein, lactose and total solids by the Milko-Scan. Milk was also analysed for calcium (complexometric method) and mean diameter of fat globules (spectroturbidimetric method). Feta cheese at three weeks was analysed for pH, fat (Schmidt-Bonzynski-Ratzlaff method), protein (Kjeldahl), moisture, NaCl (potentiometric titration) and calcium (complexometric). Exudate from Feta cheese at three weeks was analysed for total solids, NaCl (potentiometric), calcium (complexometric) and protein (Kjeldahl). The distribution pattern of low molecular weight peptides in exudate from four week old Feta was estimated by HPLC. Proteolysis in Feta cheese and exudate at four weeks was estimated by Urea-PAGE. The proteins adsorbed to fat globule surface in Feta cheeses were extracted and identified by SDS-PAGE (after brining

and at four weeks after manufacture). Exudation of whey from cheese was measured at selected intervals.

Details of the above analytical methods are outlined in Chapter 4 and Appendix 4.1.

Table 8.1 Variables used in cheese manufacture : combinations of creams and skim milks from different sources used for preparation of cheesemilk

Source of creams	Source of skim milks	Reference in the Tables/Appendices
Manufactured cream ¹	Reconstituted skim milk	A (control)
Pasteurised, homogenised cream ²	Reconstituted skim milk (RSM)	B
Pasteurised, unhomogenised cream ²	Pasteurised skim milk	C (unhomogenised milk or cream)
Manufactured cream ¹	Pasteurised skim milk	D
Pasteurised, homogenised cream ²	Pasteurised skim milk	E

¹ Prepared from FFMR and RSM (1:3 ratio);

² Fresh cream.

8.2.3 Sensory evaluation

Cheese was evaluated by a sensory panel at eight weeks after manufacture for flavour and textural characteristics. Details of the procedure for sensory evaluation are provided in Chapter 4.

8.2.4 Results and discussion

This is described under the following headings:

- (i) Manufacturing aspects and quality of cheese.
- (ii) Composition.
- (iii) Proteolysis in cheese during storage.
- (iv) Proteins adsorbed to surface of fat globules.
- (v) Exudation.

(i) **Manufacturing aspects and quality of cheese**

Cheese manufacture: There were no major problems in cheesemaking. All the cheeses were very similar and typical of Feta cheese (Chapter 6.3), except for that made using unhomogenised fresh cream. Cheese made using unhomogenised fresh cream was elastic and continuous. During the manufacture of cheese there was increase in the syneresis of whey and the whey was turbid in appearance.

Sensory evaluation (Appendix 8.1): The 'control' cheese (reconstituted skim milk & manufactured cream) was perceived significantly more acidic than other cheeses. There might have been an increased microbial activity because of a slightly lower salt-in-moisture (S/M) concentration in cheese. The control cheese was also rated as slightly oxidised and bitter. This could reflect the panel's reduced preference for a recombined milk cheese (control) in comparison to the other cheeses. Cheese made using unhomogenised fresh cream was described as 'tough', which may be attributed to the reduced moisture in non-fat substance (MNFS) in the cheese.

In general, except for cheese made using unhomogenised fresh cream, all other cheeses resembled typical Feta cheese.

(ii) **Composition**

Composition of cheesemilk: Composition of cheesemilk was alike for the replicates and treatments, except for calcium and fat globule size (Appendix 8.2). The variation in Ca^{2+} was not noticed when expressed as $\text{Ca}^{2+}/\text{SNF}$ (g/100 g). The variation in the calcium contents may therefore be attributed to the use of

milk-solids from different sources. As expected, the cheesemilk using unhomogenised fresh cream had a significantly higher mean diameter of fat globules in comparison to all other cheesemilks. Most other variations are not of practical concern and may be attributed to experimental error.

Composition of whey: Appendix 8.3 shows the composition of whey obtained during manufacture of cheeses. Whey obtained during manufacture of cheese made using unhomogenised fresh cream had higher fat. The mechanism of reduced fat loss in whey with the use of homogenised creams may be attributed to the increased retention of casein-coated fat globules in the casein matrix of curd.

Composition of cheese: The composition of cheeses before brining, after brining, and at three weeks is shown in Appendices 8.4, 8.5 and 8.6 respectively. Cheese made with unhomogenised fresh cream had increased syneresis of whey during manufacture of cheese. This is consistent with other findings (Vaikus *et al.*, 1970; Emmons *et al.*, 1980; Storry *et al.*, 1983). The increase in syneresis resulted in a significantly reduced moisture and MNFS content in the cheese. Cheeses made using reconstituted skim milk (RSM) had slightly higher moisture. This may be attributed to a reduced syneresis during cheese manufacture due to the presence of more heat denatured whey protein in RSM. Cheeses made using skim milks had low moisture in comparison to cheeses made using RSM, and consequently a higher FDM content.

Composition of exudate: Composition of exudates was generally typical. Variations in composition of exudates were mostly observed in replicates (Appendix 8.7). The cause of variation in the protein content of replicates was not clear. Exudate available was insufficient to allow analyses in duplicate, and therefore the results could not be verified.

(iii) Proteolysis in cheese during storage

Urea-PAGE on cheeses at four weeks: The protein bands in the electrophoretogram showed a similar pattern of proteolysis in all cheeses. The ratio of areas of α_{s1} -casein to β -casein determined by densitometry was used to

obtain an approximate idea of the rate of proteolysis in cheeses (Table 8.2). It has been observed before that β -casein does not proteolyse in the first four weeks of storage. A higher ratio of α_{s1} -casein to β -casein would therefore represent reduced hydrolysis of α_{s1} -casein and a decreased proteolysis. Reduced proteolysis was observed in cheese made using unhomogenised fresh cream. This could have been due to a significantly lower moisture in cheese. Reduced proteolysis was also observed in cheese made using a combination of skim milk and homogenised cream. The cause of this is not clear.

Table 8.2 Results from Urea-PAGE on four week old cheeses to assess the rate of proteolysis (Densitometer readings)

Source of variation	Ratio of areas of α_{s1} -casein to β -casein (Least squared mean values)
Replicates	
1	0.81
2	0.86
Treatments	
A (control)	0.76
B	0.77
C	0.96*
D	0.77
E	0.90*
S.D.	0.04

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

S.D. = Standard deviation of raw data; * = $p < 0.05$.

HPLC profiles of exudates: The low molecular weight profile (500 to 10,000 daltons) of peptides in exudates from four week old cheeses showed no significant variations in either the area or size of the peaks, or the total number of peaks. This indicated a similar pattern of degradation of high molecular weight peptides for all cheeses.

It was concluded that the pattern of proteolysis was similar in all cheeses. The rate of proteolysis was slower in cheese made using unhomogenised fresh cream.

(iv) Proteins adsorbed to surface of fat globules

Proteins adsorbed to surface of fat globules in cheese were estimated at two stages; after brining and after four weeks of storage. Table 8.3 provides an estimate of the extent to which casein proteins are adsorbed to the fat globule surface. The units for the area are arbitrary and are for the same amount of fat in cheese.

No change in the type and the concentration of proteins adsorbed to the surface of fat globules at brining and four weeks was detected. SDS-PAGE of fat globules from cheeses made using homogenised creams showed the presence of casein and whey proteins, which was similar to that observed from homogenised milks (Anderson *et al.*, 1977; Darling & Butcher, 1978). Markedly more protein was adsorbed to the fat globule surface in cheese made using manufactured cream than those made from cream (fresh). Cheese made using unhomogenised fresh cream had little or no protein adsorbed to the fat globule surface. This observation may be explained by the amount of milk fat globule membrane (MFGM) material present, and the relatively small surface area of the fat globules. Manufactured cream prepared from FFMR has little MFGM material, and therefore most of the fat globule surface are coated with milk proteins during homogenisation. MFGM in fresh cream is mostly intact, and provides a coating to the surface of the fat globules. However, in homogenised fresh cream, since the amount of available MFGM material is not sufficient to cover the increased fat globule surface area due to homogenisation, skim-milk proteins adsorb to some of the newly created fat globule surface (Darling & Butcher, 1978; McPherson & Kitchen, 1983). The

MFGM material is intact in unhomogenised fresh cream and does not allow other milk proteins to be adsorbed onto the fat globule surface in cheese.

Table 8.3 Effect of homogenisation and 'milk solids source' on the amount of casein proteins adsorbed to surface of fat globules in cheese (densitometer readings of SDS-gel)

Source of variation	Total area of casein proteins ¹ (Least square mean)
Replicates	
1	1.02
2	1.19
Treatments	
A (control)	2.24
B	0.73**
C	0.00**
D	2.06
E	0.51**
Storage time	
0 week	1.06
4 weeks	1.15
S.D.	0.43

¹ The units for area are arbitrary and are for the same amount of fat in cheese;

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

S.D. = Standard deviation of raw data; ** = $p < 0.01$.

(v) Exudation in cheeses during storage

Least-squared means and 'F ratios' for the amount of exudate from Feta cheeses as affected by source of milk solids and homogenisation at different periods of storage are shown in Table 8.4.

There was no difference in the amount of exudate between the replicates. Exudation from all cheeses, except for cheese made using unhomogenised fresh cream, was similar. The amount of exudate from all the cheeses increased with time. There was no significant effect of interaction of treatments on the exudation during storage.

Cheese made using unhomogenised fresh cream released significantly more amount of exudate than the control (FFMR & RSM). This difference was noticed when exudation was expressed in terms of 'final MNFS' or '% reduction in MNFS'. Exudation was not found to be statistically significant when expressed as 'g exudate/kg cheese' or 'g exudate/kg moisture in cheese' because the initial moisture content (and MNFS) of cheese made using unhomogenised fresh cream was significantly low.

In the cheeses made using homogenised creams, fat globules coated with casein (Mulder & Walstra, 1974) formed a part of the casein matrix (Vliet & Dentener-Kikkert, 1982; Walstra & Vliet, 1986) and were effective in blocking or retarding the flow of moisture in the casein matrix.

Cheeses made using manufactured cream contained more fat-bound protein than cheeses made using homogenised cream (Table 8.3). If participation of fat globules in the casein matrix of the cheese was the only factor to restrict the process of exudation, the cheeses made with manufactured cream should have had the least amount of exudate. As this did not happen, it indicates that exudation is influenced by other factors. There is evidence that in addition to casein and whey proteins, the surface of the fat globule from homogenised milk consists of MFGM material (Anderson *et al.*, 1977; Darling & Butcher, 1978). It is therefore expected that fat globules in cheeses made using homogenised cream (fresh) will also consist of

Table 8.4 Effect of homogenisation of cream and selected sources of milk solids in cheesemilk on the exudation^a of whey from Feta cheese during storage

Source of variation	Exudate in g/kg cheese		Exudate in g/kg moisture in cheese		Final MNFS (%)		% reduction in MNFS	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		3.68		3.45		6.86		0.21
1	39.8		78.9		67.6		1.38	
2	42.9		83.9		69.1		1.36	
Treatments		1.44		0.90		8.43*		9.52*
A(control)	44.4		82.8		69.5		1.32	
B	41.9		79.3		69.6		1.20	
C	39.4		84.6		65.1**		1.68*	
D	39.3		77.6		68.7		1.30	
E	41.8		82.8		69.0		1.35	
Storage time		152.5***		157.4***		146.9***		143.4***
2 weeks	19.6		38.8		68.9		0.64	
4 weeks	24.4		48.3		68.8		0.79	
6 weeks	29.0		57.4		68.7		0.95	
9 weeks	37.3		73.5		68.5		1.22	
12 weeks	43.8		86.1		68.3		1.44	
15 weeks	49.6		97.2		68.2		1.63	
20 weeks	62.3		122.4		67.9		2.10	
26 weeks	65.0		127.8		67.8		2.20	
Interaction								
Treatments X Storage time		1.48		1.08		0.66		0.59
S.D.	4.33		8.33		0.105		0.153	
C.V. (%)	10.46		10.23		0.15		11.18	

^a Details for calculation of exudation values are provided in Appendix 4.2;

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

F = F ratio; LSM = Least-square mean; S.D. = Standard deviation of raw data;

C.V. = Coefficient of variation;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;

F values without asterisks are 'not significant';

Individual LSM values marked with asterisks show their significant variation in comparison to control.

MFGM material. Indirect evidence for this has been already shown earlier in this Chapter in the form of absence of proteins on the fat globule surface. MFGM material are known to contain phospholipid as a principal component. It is possible that phospholipid-phospholipid interaction keeps the fat globules close to each other, or may be in contact with each other. This chain or cluster of fat globules may restrict the flow of moisture and retard the process of exudation. This is consistent with a reduced rate of exudation from Feta cheese due to coating of fat globules with lecithin, a phospholipid (described in Section Two).

Variation in the source of skim milks (fresh or reconstituted) did not appear to have any effect on the exudation.

8.2.5 Summary and conclusion

Feta cheese made using homogenised creams had reduced syneresis of whey during manufacture, and consequently a significantly higher amount of MNFS than cheese made using unhomogenised fresh cream. The amounts of exudate from both these cheeses were similar when expressed as a percentage of the weight of cheese. However, it was apparent that the cheese made using unhomogenised fresh cream had a higher rate of exudation when expressed as a percentage of MNFS. Homogenisation of cream (fresh or manufactured) was therefore an effective means of retarding exudation of whey from Feta cheese during storage.

The source of skim milk (fresh or reconstituted) did not affect exudation.

The relative amounts of protein adsorbed to fat globule surface was negligible in cheese made from unhomogenised fresh cream, moderate in cheese made from homogenised fresh cream, and substantial in cheese made from manufactured cream. This showed that the presence of MFGM deters the adsorption of protein onto the fat globule surface.

Exudation is possibly influenced by the size of the fat globules, casein-coating on the fat globules and the type of material adsorbed to the fat globule surface.

SECTION TWO

8.3 EFFECT OF FAT EMULSIFICATION ON THE EXUDATION OF WHEY FROM FETA CHEESE DURING STORAGE

8.3.1 Introduction

The objective of this study was to investigate the mechanism by which fat and homogenisation affect the rate of exudation.

The scope of this study was:

- To determine the influence of material adsorbed to fat globule surface on emulsion stability of fat.
- To study the effect of participation of fat in the protein (casein) matrix.
- To confirm the influence of fat on exudation.

Before proceeding to the major experimental part it was necessary to perform some preliminary studies. These are described in the first part of this section. The second part describes the effect of material adsorbed to surface of fat globule on exudation.

8.3.2 PRELIMINARY STUDIES

The preliminary study involved:

- Selection of a set of emulsifying agents.
- Optimisation of the rate of addition of emulsifying agents.
- Standardisation of the method for preparing 'manufactured cream'.

An emulsifying agent assists in the formation of an emulsion, prevents or inhibits the creaming and coalescence of oil droplets, and contributes to the flow properties and mouthfeel of the product (Marrs *et al.*, 1989). In this experiment both proteins and non-proteins used for emulsifying milkfat in the aqueous phase to form an oil-in-water emulsion have been referred to as emulsifying agents. Caseins are known for their emulsifying abilities (Shimp, 1985), and whey proteins for their

functional properties (Marshall & Harper, 1988). Thus skim milk powder, which contains casein and whey proteins, is referred as an emulsifying agent in the text.

8.3.2.1 Experimental design

Selection of emulsifying agents: This was guided by the following considerations: the material naturally occurring in the MFGM, ability to form a suitable emulsion with FFMR and the commercial adaptability of the process for cheese manufacture.

Skim Milk Powder (SMP) was selected as the 'control'. Lecithin was chosen to represent the phospholipids, a major fraction of MFGM. Sodium caseinate was selected because of its excellent emulsifying properties, and the fact that in homogenised milks casein proteins are known to be preferentially adsorbed (Dickinson *et al.*, 1989a). Whey protein was chosen as an emulsifying agent because it was expected to provide an inert coating on the surface of fat globules. This was expected to provide explanation for the effect of incorporation of denatured whey proteins on exudation (Chapter 7), and also to provide an indication of any preferential adsorption of proteins to the fat globule surface irrespective of the initial coating. A mixture of sodium caseinate and lecithin was expected to be nearest to that of the control. Butter milk powder was chosen because it is cheap, easily available and could be used as 'lecithin-substitute' for commercial applications. Depending upon concentration and type, surfactants are known to displace milk proteins partly or completely (Oortwijn & Walstra, 1979; Walstra & Jenness, 1984; Feitjer *et al.*, 1987). Glycero mono-stearate (GMS), and combinations of Tween-60 & Span-60 were intended to be used as surfactants that have good emulsifying ability but are not naturally present in MFGM.

Preparation of 'manufactured cream': Manufactured cream was to be prepared using a mixture of FFMR, water and the emulsifying agent. The rate of addition of emulsifying agents was to be optimised with the following considerations:

- To have similar fat globule size for all the manufactured creams because the average diameter of the fat globules correlates well with the emulsion stability of cream (Aguilera & Kessler, 1988).

- To have a certain minimum emulsion stability suitable for cheese manufacture.
- To ensure that the addition of protein emulsifying agents resulted in minimal additional protein to the cheesemilk.

8.3.2.2 Experimental

Materials: Low heat SMP from the same batch was used throughout the experiment. Sodium caseinate (Alanate - 180, New Zealand Dairy Board) of the following typical composition was used: protein = 92.7%, lactose = 0.1%, ash = 3.6%, moisture = 3.5%, milkfat = 0.7%, pH = 6.6, sodium = 1.3%, and calcium = 0.02%. A typical concentration of whey protein concentrate (WPC) powder (Alacen - 312, New Zealand Dairy Board) was: Total Nitrogen = 12.83%, Lactose = 5.1%, Fat = 3.93%, moisture = 5.32%, ash = 2.59%, calcium = 105 mM/kg, NPN = 0.78%, NCN = 12.02%. Composition of butter milk powder (made in NZDRI) was: fat = 9.0%, protein = 34.5%, lactose = 45.5%, minerals = 7.2%, moisture = 3.8%. Deodorised lecithin (Blendmax 322, code no. 6230-00, PO Box - 1400, Fort Wayne, Indiana) was used. Tween-60 and Span-60 were obtained from Sigma chemical company, USA.

Preparation of 'manufactured cream': The process for preparation of manufactured cream is outlined in Fig. 8.1. During the preparation of manufactured creams the emulsifying agents were used as follows: lecithin and Span-60 were dissolved in FFMR at 60°C; SMP was dissolved in water at 40°C; and sodium caseinate, WPC powder, Tween-60 and butter milk powder were dissolved in water at 60°C. The desired fat globule size in the cream was targeted to 0.5-0.6 μm , which was the approximate value for the control (using SMP as the emulsifying agent).

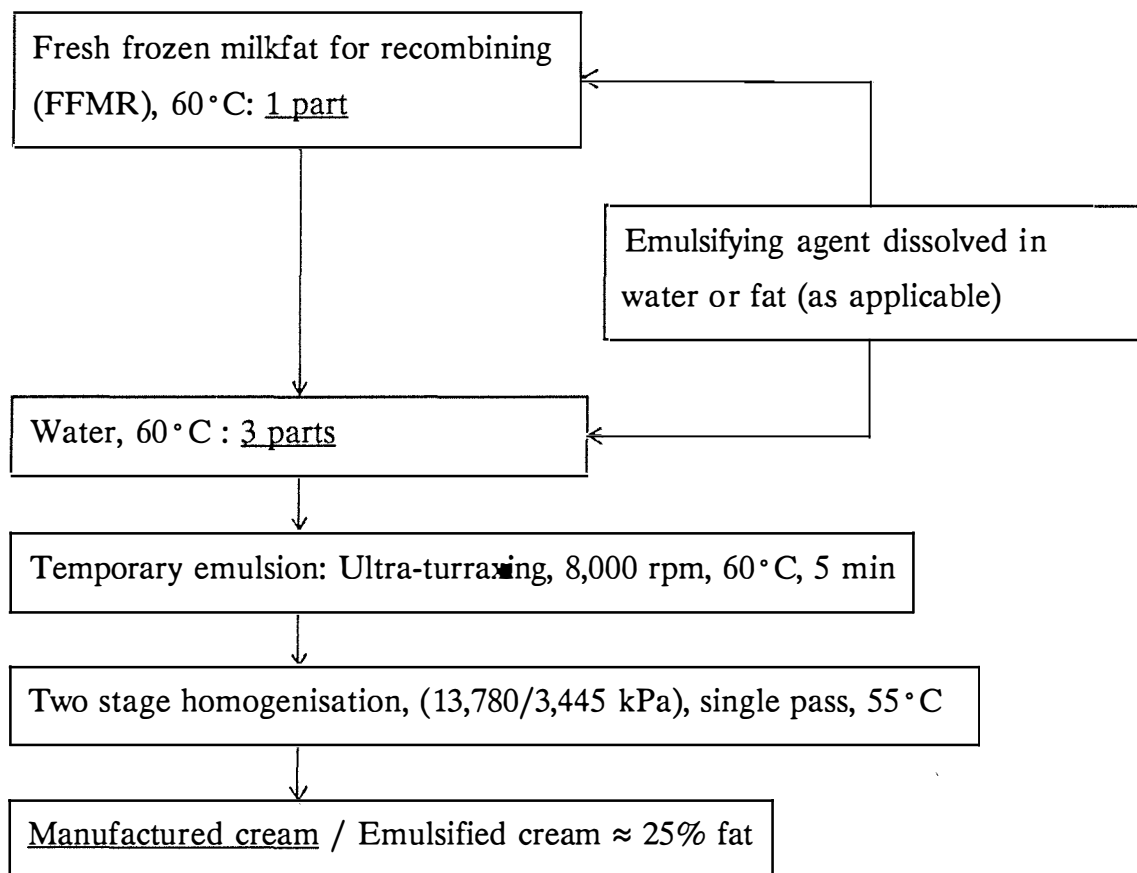


Fig. 8.1 Process for preparation of 'manufactured cream'.

8.3.2.3 Analytical methods: Manufactured cream was tested for average diameter of the fat globules (spectroturbidimetric method) and emulsion stability. Manufactured cream was mixed with reconstituted skim milk (RSM) in proportions of 1:4 ratio to obtain a composition close to that in cheesemilk, and tested for emulsion stability. Aliquots of RSM were incorporated with emulsifying agents and analysed for gel strength (Formagraph). The detailed procedures for the analytical methods are outlined in Chapter 4.

8.3.2.4 Results and discussion

Optimisation of the amount of emulsifying agents: Based on the effect of the use of emulsifying agents (as compiled in Table 8.5) on the properties of manufactured cream (mean diameter of fat globules and the emulsion stability), the rate of

Table 8.5 Effect of use of selected emulsifying agents on the properties of 'manufactured cream' and recombined milk

Emulsifying agent	Emulsifier to FFMR ratio (%)	P/F ratio in cream	Mean diameter of fat globules (μm)	Observation of cream ¹ stored for 18 h at 20°C	Observation of recombined milk stored for 18 h at 20°C
SMP (Control)	31.5	0.11	0.66	Thin layer of fat on top.	Very thin cream layer.
Replicates	31.5	0.11	0.55	No cream plug. A small ring of fat sticking to glass surface.	Very thin cream layer.
	31.5	0.11	0.47	3% cream layer.	slight cream layer.
	31.5	0.11	0.54	No cream layer.	Slight cream layer.
	31.5	0.11	0.54	No cream layer.	Slight cream layer.
Sodium caseinate	5.55	0.051	0.56	49% cream layer.	Thin fat layer.
	4.40	0.040	0.72	40% cream layer.	Thin fat layer.
	2.20	0.020	0.66	43% cream layer.	Thin fat layer.
Butter milk powder	12.5	0.043	1.07	Viscous and thick cream.	Cream separation.
WPC powder	12.5	0.10	0.55	Thin cream layer.	Thin cream layer.
	10.0	0.08	0.54	No cream plug.	Thin cream layer.
	5.0	0.04	0.67	<1.0% cream plug.	Thin cream layer.

¹ Cream layers quoted in percentages indicate the percent of a thick top layer in the entire cream.

Table 8.5 continued Effect of use of selected emulsifying agents on the properties of
'manufactured cream' and recombined milk

Emulsifying agent	Emulsi- fier to FFMR ratio (%)	P/F ratio in cream	Mean diameter of fat globules (μm)	Observation of cream ¹ stored for 18 h at 20°C	Observation of recombined milk stored for 18 h at 20°C
Lecithin	1.0	-	4.05	8% cream layer.	solid fat layer. Phase separation on warming.
	2.0	-	2.37	3% cream plug.	Thick cream layer persists on warming.
	4.0	-	0.80	8% cream layer.	Cream layer on top.
	4.0	-	0.61	7% cream layer.	Thick layer on top.
	8.0	-	0.58	2% cream layer.	Cream layer on top.
	8.0	-	0.58	2% cream layer.	Thick cream on top.

Lecithin & sodium caseinate					
(1 : 5.5)	3.28	0.026	0.56	1% cream layer.	Thin fat layer.
(1 : 9.0)	2.45	0.020	0.59	2.5% cream plug.	Thin fat layer. Dissolved on warming.

Span-60 & Tween-60					
(1 : 0.5)	1.0	-	3.86	1% fat & 11% cream. 3% cream layer.	Fat separation on warming.
(1 : 0.47)	2.0	-	1.21	2% cream layer.	Slight cream layer.
(1 : 1.08)	2.0	-	0.99	1% cream layer.	Slight cream layer.
(1 : 2.57)	2.0	-	0.71	1% cream layer.	Slight cream layer.
(1 : 5.67)	2.0	-	0.89	1% cream layer.	Slight cream layer.
(0 : 1.0)	2.0	-	0.55	1% cream layer.	Slight cream layer.

¹ Cream layers quoted in percentages indicate the percent of a thick top layer in the entire cream.

addition of emulsifying agents in proportion to the FFMR (%) were selected as reported in Table 8.6. Wit *et al.*, (1977) reported that to achieve sufficient dispersion stability in 4% oil-in-water emulsion, a protein concentration to a level of protein to fat ratio of 0.1 is required, and that more protein would be required for 35% oil-in-water emulsions. In this instance the ratios are much lower. This is probably because only a temporary emulsion was required. The emulsion needed to be stable until the drainage of whey during cheese manufacture, which is about 4 h from the time milk is inoculated with the starter.

Butter milk powder was not used as an emulsifying agent because it did not provide a satisfactory emulsion. Addition of Span-60 in admixture with Tween-60 was not necessary as Tween-60 on its own provided a satisfactory emulsion.

An accurate estimate of the mean diameter of the fat globules in manufactured cream made using lecithin could not be determined as the experimental turbidity curve did not fit the theoretical standard curves. This indicated that the fat globules in manufactured cream did not have a monomodal size distribution or that they were aggregated. This is consistent with the findings that milk fat globules associated with appreciable amounts of phospholipids in recombined milk are not very stable (Melsen & Walstra, 1989). This showed the unsuitability of lecithin as an emulsifying agent.

Table 8.6 Proportion of emulsifying agents

Emulsifying agent	Proportion to FFMR (%)	P/F ratio
SMP (Control)	31.5	0.11
Sodium caseinate	2.2	0.02
Lecithin	6.0	-
Sodium caseinate & lecithin (8.8:1)	2.45	0.02
Tween-60	2.0	-
WPC powder	5.0	0.04

Gel strength of renneted 'emulsified milks': The influence of the protein and non-protein emulsifying agents on rennet-induced gels in milk was studied. A Formagraph was used to measure the on-set of gelation and gel-strength of renneted skim milks made with the emulsifying agents as described in Chapter 4.2.9. The Formagraph curves (Fig. 8.2) show the on-set of gel formation, the time taken for the bell-shaped curve to reach a width of 15 mm (expressed as the distance from the point of on-set of gel formation = D_{15}) and the gel-strength 40 min after rennet addition [expressed as the width (L_{40}) of the bell-shaped curve]. The results are tabulated in Table 8.7.

Rennet coagulation of milks with WPC powder, lecithin and SMP (control) occurred at the same time. The coagulation occurred a little earlier with lecithin and Tween-60, and was slightly delayed with sodium caseinate. From the distance measured for attaining a width of 15 mm, it was observed that milks with sodium caseinate and lecithin took a slightly longer time while all others took a similar time. This meant that the gel firmed slowly in these two milks. From the width of the bell-shape after 40 min it was found that milk with Tween-60 had a considerably weaker gel (29.3 mm) and all others were alike (41 - 47 mm).

The above results show that RSM with lecithin and Tween-60 have slightly different gel forming characteristics than control. The effect of lecithin is doubtful because it did not dissolve well in RSM. It was hoped that cheese manufacture would not be significantly affected because of the slight variations in the gel strengths.

Table 8.7 Effect of emulsifying agents on the rennet coagulation properties of skim milk

Emulsifying agent	Distance in mm for a spread of 15 mm ¹ = D_{15}	Width of the bell-shape (mm) after 40 min of rennet addition ² = L_{40}
SMP	9.6	46.5
Sodium caseinate	12.25	42.85
WPC powder	9.15	46.65
Tween-60	9.6	29.3
Lecithin ³	12.3	41.0

¹ The distance is a measure of the time taken from the on-set of coagulation for the coagulation firmness to reach a fixed value.

² The width is a measure of curd firmness after a fixed time (40 min) of addition of rennet.

³ Results may not be very accurate as lecithin did not mix completely in the aqueous phase.

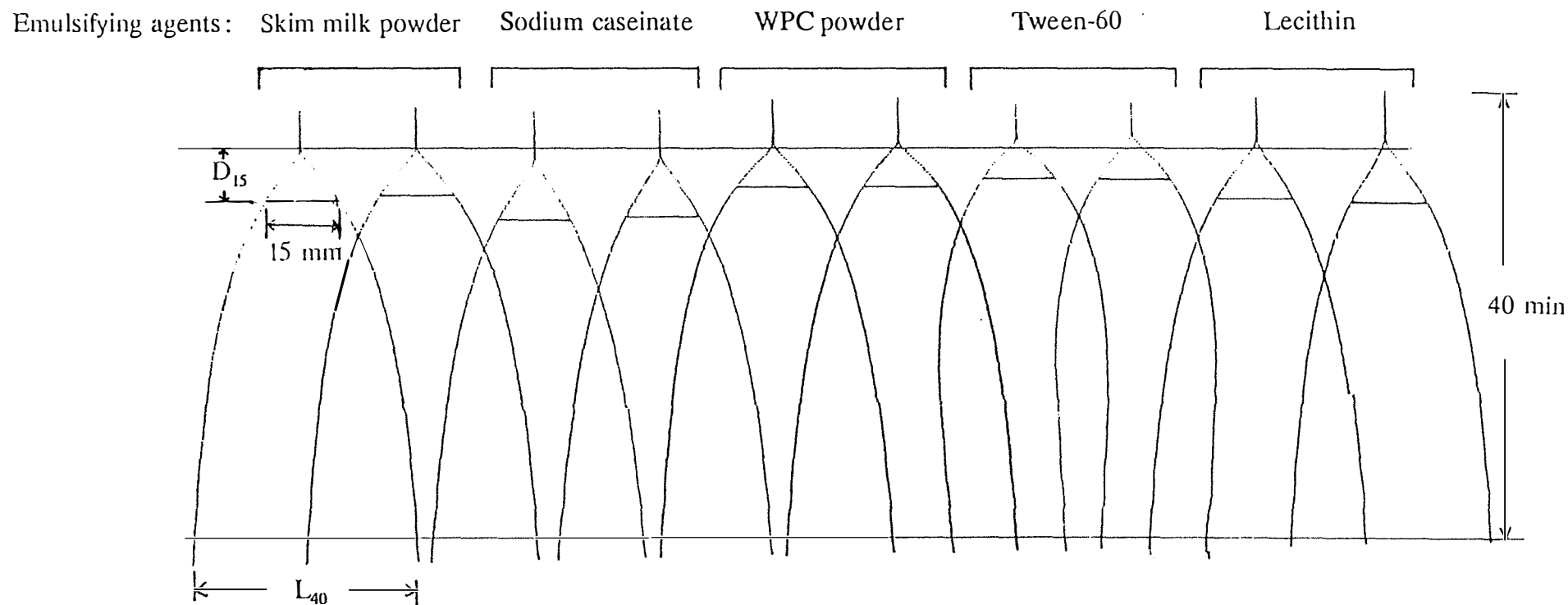


Fig. 8.2 Formagraph curves showing the starting time of gel formation and the subsequent firming of gels in renneted milks prepared with different emulsifying agents. The original curves have been reduced in size.

Note: The point at which the bell-shaped curve starts to form is the time when the gel starts to form. D_{15} indicates the distance in mm (a function of time) until a width of 15 mm is formed. L_{40} indicates the width in mm after 40 min from adding rennet.

8.3.3 EFFECT OF MATERIAL ADSORBED TO SURFACE OF FAT GLOBULE IN FETA CHEESE ON THE EXUDATION

The objective of the following investigation was to gain an understanding of the effect of material adsorbed to fat globule surface on exudation.

8.3.3.1 Experimental

Selected emulsifying agents were used in the proportions established earlier for preparation of manufactured cream (Table 8.6). Details of preparation of manufactured cream have been described in the preliminary studies (Chapter 8.3.2 & Fig. 8.1). The method of manufacture of Feta cheese was similar to that described in Chapter 6 (Fig. 6.1 & Appendix 6.2).

Ingredients used in the preparation of manufactured cream were FFMR, water and the emulsifying agent. SMP was used only for control. Thus, the manufactured creams had a lower amount of milk solids-not-fat than that of control. This reduced amount of SMP was made up by adding concentrated RSM (not applicable to control) while preparing cheesemilk. A sample calculation for this is provided in Appendix 8.8.

As formation of the emulsion was one of the critical steps, mass-balancing of fat was done. The calculations for fat mass balance were similar to the procedure followed earlier for mass balance studies (Appendix 7.8.b).

The experiment was designed on the basis of analysis of variance (one-way classification). A total of 12 trials were randomly performed using two replications for each emulsifier. The sources of variation were 'replicates' and 'emulsifying agents'. The results were statistically analysed using SAS package (1985).

8.3.3.2 Analytical methods

Cheesemilk and whey were analysed for fat, protein, lactose and total solids by the Milko-Scan. Milk and whey were also analysed for fat (Rose-Gottlieb method) and calcium (complexometric method). The mean diameter of the fat globules in the

cheesemilks was determined by the spectroturbidimetric method. Feta cheese after brining and after four weeks of storage were analysed for pH, fat (Schmidt-Bonzynski-Ratzlaff method), protein (Kjeldahl), moisture, NaCl (potentiometric titration) and calcium (complexometric). Exudate from Feta cheeses was analysed for total solids, NaCl (potentiometric), calcium (complexometric), protein (Kjeldahl) and distribution pattern of low molecular weight peptides (HPLC). Proteolysis in Feta cheese and exudate at four weeks was estimated by urea-PAGE. The proteins adsorbed to fat globule surface in Feta cheeses were identified by SDS-PAGE (after brining and at four weeks after manufacture) and by Urea-PAGE (after fourteen months of manufacture). The amount of exudate released from cheese was measured at selected intervals.

Details of these analytical methods are described in Chapter 4 and Appendix 4.1.

8.3.3.3 Sensory evaluation: The product was evaluated by a sensory panel at eight weeks after manufacture for flavour and textural characteristics. Details of the evaluation procedure are provided in Chapter 4.

8.3.3.4 RESULTS

The results are described under the following headings:

- (a) Manufacturing aspects, fat mass balance and cheese quality.
- (b) Composition of milk, whey, cheese and exudate.
- (c) Proteolysis.
- (d) Protein adsorbed to fat globule surface in cheese.
- (e) Exudation.

(a) Manufacturing aspects, fat mass balance and cheese quality

Cheese manufacture: 'Manufactured cream' and 'emulsified milks' (cheesemilks) were generally satisfactory although in some instances the cheesemilk had a slight cream layer similar to that observed during the preliminary studies. Manufacture of Feta cheese did not pose any specific problems. Manufacture of cheese with Tween-60 resulted in a substantial amount of fat being lost in the whey. The cheese had a hard texture. However, the cheesemilk with Tween-60 had

satisfactory emulsion of fat and the renneted curd had the desired gel characteristics. The loss of fat was possibly due to the ability of surfactant Tween-60 to replace the proteins in the oil-in-water type emulsions (Walstra and Jenness, 1984). Coating of fat globules with Tween-60 may have made them behave as water-soluble particles and thereby increased the fat loss into whey. Cheese made using WPC powder as emulsifying agent had a slower rate of draining during manufacture. The renneted coagulum of the cheesemilks made using Tween-60 and WPC powder were weaker (subjective testing) at manufacture. The pH profile at various stages of manufacture was typical.

Mass balance of fat content: The results of mass balance are shown in Appendix 8.9. The variation in fat content was reasonable. Increased recovery of fat with the use of lecithin was due to the additional fat from 'lecithin source'. The mass balance confirms the increased fat loss to the whey with the use of Tween-60 as the emulsifying agent.

Sensory evaluation: All cheeses except those made using lecithin and Tween-60 were rated satisfactory (Appendix 8.10).

Cheese made using lecithin had an undesirable flavour. This originated from the lecithin. Because of the undesirable flavour the panel was advised not to evaluate the flavour of this cheese. The texture of cheese made using lecithin was adjudged smooth, sticky and atypical. Use of lecithin involving a similar method of manufacture with Halloumi cheese resulted in increased stretchability and meltability of the cheese (Lelievre *et al.*, 1990).

Cheese made using Tween-60 was rated very hard. This was due to low fat in the cheese that resulted in low ratio of moisture to casein (Lawrence & Gilles, 1987). The cheese also had a different flavour which was traced back to the emulsifying agent itself. The panel perceived the undesirable flavour as oxidised.

(b) Composition of milk, whey, cheese and exudate

Composition of cheesemilk: The composition of milk was generally satisfactory (Appendix 8.11). The mean fat globule diameters were nearly uniform in the

cheesemilks, except with lecithin where the results are doubtful because the turbidity spectra did not fit well with any standard curve (Table 8.8). Uniformity in the size of the fat globules implied that the emulsification of fat was mostly satisfactory. Slightly increased fat content in milk made with lecithin could be due to some additional fat from 'lecithin source'. Sodium caseinate and WPC powder marginally increased protein and SNF contents in the respective cheesemilks.

Table 8.8 Effect of emulsifying agents on the mean diameter of fat globules in cheesemilk

Source of variation and levels	Mean diameter (μm)	
	LSM	F ratio
Replicate		1.26ns
1	0.61	
2	0.47	
Emulsifying agents		0.17ns
SMP(control)	0.49	
Sodium caseinate	0.62	
Sodium caseinate & lecithin	0.46	
Lecithin	0.61	
Tween-60	0.52	
WPC powder	0.56	
Standard deviation	0.223	

ns = not significant; L.S.M. = Least square mean.

Composition of whey (Appendix 8.12): As reported earlier, considerable loss of fat to the whey occurred during manufacture of cheese with Tween-60. Slightly higher fat loss with the use of emulsifying agent WPC powder is probably an indication of fat not being able form a part of the casein gel matrix as effectively as some other emulsifying agents.

Composition of cheese before brining (Appendix 8.13): Most cheeses had a typical Feta composition. However, fat and FDM values were lower for cheese with Tween-60 because of considerable loss of fat to the whey. Moisture content of cheese made with lecithin was lower which partly contributed to the significantly higher fat level since the sum of the cheese components must equal 100%.

Composition of four week old cheese: The composition of cheeses was fairly consistent and typical (Table 8.9). The general levels of S/M concentration were

slightly higher which was possibly due to the higher moisture level in cheese before brining. The high level of moisture could be due to two-stage homogenisation of manufactured cream.

Reduced moisture in cheese made using lecithin was a result of increased syneresis during manufacture of cheese. Increase in syneresis might have been caused by strong agglomeration of the fat globules in emulsion and consequently larger fat globules in cheesemilk, or incomplete participation of fat globules in the casein matrix of renneted gel.

The low moisture in the cheese with Tween-60 was due to increased syneresis at manufacture. This resulted in significant variations in MNFS and protein contents.

Composition of exudate from four week old cheese: The composition of exudate from cheeses made with the emulsifying agents was mostly uniform (Appendix 8.14). In exudate from cheese with Tween-60 higher total solids was due to the higher salt content, while the lower contents of calcium and protein was probably due to the release of large amounts of exudate.

Table 8.9 Effect of emulsifying agents on the composition of cheese (four weeks)

Source of variation	Fat (%)		Moisture(%)		FDM (%)		MNFS (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicate		1.88ns		1.48ns		0.84ns		0.6ns
1	25.34		49.5		50.1		66.2	
2	25.87		48.8		50.6		65.9	
Emulsifying agent	14.89		5.63		30.32		11.9	
	**		*		***		**	
SMP (control)	25.15		50.28		50.57		67.2	
Sodium caseinate	26.15		49.33		51.60		66.8	
Sodium caseinate & lecithin	26.30		49.11		51.68		66.6	
Lecithin	28.48**		47.12*		53.85**		65.9	
Tween-60	23.25*		47.96*		44.66***		62.5**	
WPC powder	24.30		51.09		49.68		67.5	
S.D.	0.666		0.869		0.799		0.756	

F = F ratio; L.S.M. = Least square mean; S.D. = Standard deviation of raw data;

* = $p \leq 0.05$; ** = ≤ 0.01 ; *** ≤ 0.001 ; ns = not significant; Individual L.S.M. values marked with asterisks denote their significant variation in comparison to the control. Table 8.9 continued.....

.....Table 8.9 continued

Effect of emulsifying agents on the composition of cheese
(four weeks)

Source of variation	Calcium (mMoles/kg)		pH	
	LSM	F	LSM	F
Replicate		1.64ns		0.13ns
1	98.3		4.66	
2	101.6		4.65	
Emulsifying agents		2.91ns		1.63ns
SMP (control)	101.6		4.65	
Sodium caseinate	97.8		4.65	
Sodium caseinate & lecithin	103.2		4.67	
Lecithin	96.2		4.68	
Tween-60	108.0		4.65	
WPC powder	93.0		4.64	
S.D.	4.48		0.016	

Source of variation	NaCl (%)		S/M (%)		Protein (%)	
	LSM	F	LSM	F	LSM	F
Replicate		3.63ns		1.47ns		3.06ns
1	5.89		11.9		15.88	
2	5.64		11.6		16.57	
Emulsifying agents		4.56ns		2.72ns		7.94*
SMP(control)	6.10		12.1		15.31	
Sodium caseinate	5.54		11.2		15.92	
Sodium caseinate & lecithin	5.44*		11.1		16.11	
Lecithin	5.41*		11.5		15.98	
Tween-60	6.11		12.7		18.88**	
WPC powder	6.02		11.8		15.12	
S.D.	0.226		0.53		0.6	

F = F ratio; L.S.M. = Least square mean;

S.D. = Standard deviation of raw data;

* = $p \leq 0.05$; ** = ≤ 0.01 ; *** ≤ 0.001 ;

ns = not significant;

Individual L.S.M. values marked with asterisks denote their significant variation in comparison to the control.

(c) Proteolysis

Urea-PAGE on four week old Feta cheeses: All cheeses showed similar electrophoretic patterns with comparable band densities. Proteolysis was therefore similar in all cheeses. The degree of proteolysis has been expressed as the ratio of the amount of α_{s1} -casein or β -casein in the cheeses to the amount of β -casein of standard casein run in the same gel (Table 8.10). The ratios were calculated using areas under densitometer plots. The amount of β -casein and α_{s1} -casein were greater in cheeses made using Tween-60 and lecithin (ratio = 0.37) than in the control (ratio = 0.27). The other samples had similar composition (ratio = 0.27 to 0.30). The higher casein content in cheese containing Tween-60 could be due to reduced moisture in cheese leading to a reduced rate of proteolysis or due to reduced fat content in cheese leading to a proportionate increase of casein in the total solids. The increase in the density of casein bands in cheese made with lecithin may have been due to a reduced moisture content in cheese, and consequently a slightly reduced rate of proteolysis. It could also be due to experimental error in loading the sample because a corresponding increase in β -casein was detected.

Table 8.10 Effect of emulsifying agents on casein proteolysis of four week old Feta cheese

Sources of variation	Ratio of α_{s1} -casein in cheese to β -casein of standard casein		Ratio of β -casein in cheese to β -casein of standard casein	
	LSM	F ratio	LSM	F ratio
Replicate		0.38ns		0.57ns
1	0.303		0.389	
2	0.316		0.425	
Emulsifying agents		3.82 ns		1.44 ns
SMP (control)	0.269		0.356	
Sodium caseinate	0.281		0.351	
Sodium caseinate & Lecithin	0.267		0.382	
Lecithin	0.371*		0.507	
Tween-60	0.372*		0.486	
WPC powder	0.296		0.362	
Standard deviation	0.082		0.036	

* = $p \leq 0.05$; ns = not significant; LSM = Least square mean; L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

HPLC studies on exudate from Feta cheese stored for four weeks: The distribution pattern of low molecular weight protein breakdown products in all the exudates appeared typical (Table 8.11). The peaks for amino acids, peptides and other protein breakdown products in the molecular mass range 500 - 10,000 daltons was mostly similar in exudates from all the cheeses. The only peak with slight variation was observed at the molecular mass of $\approx 4,000$ daltons at an elution time of 34 s. Exudate from cheese made using WPC powder had a decrease in the amount of breakdown product appearing at 34 s elution time. This variation was however less than the variation between the replicates. It is unlikely that this variation was due to a reduced rate of proteolysis, particularly in view of a higher moisture content in the cheese. The variation is more likely to have been caused by a dilution effect due to increase in the amount of exudate during the initial stage of storage.

Table 8.11 Effect of emulsifying agents on the low molecular weight peptides in exudate from four week old Feta cheese (HPLC technique)

Source of variation & levels	Concentration of peak at 34 s elution time (molecular mass $\approx 4,000$ daltons)	
	Least square mean	F ratio
Replicate		12.39*
1	17.74	
2	19.67	
Emulsifying agents		5.13*
SMP (control)	19.10	
Sodium caseinate	19.55	
Sodium caseinate & lecithin	19.56	
Lecithin	20.39	
Tween-60	17.03	
WPC powder	16.61*	

Standard deviation of raw data 0.952

* = $p \leq 0.05$; LSM = Least square mean; L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

Conclusion: In general, proteolysis was typical of Feta cheese. α_{s1} -casein proteolysed rapidly, while there was no evidence of proteolysis of β -casein in the first four weeks. Pattern of proteolysis was similar in all cheeses. The proteolytic rate could have been slightly slower in cheeses made with Tween-60 and lecithin.

(d) Protein adsorbed to fat globule surface in cheese

SDS-PAGE: The effect of fat emulsification with selected emulsifying agents on the protein adsorbed to the surface of fat globules is described in Table 8.12 (refer Appendix 8.15 for photograph). There was no change in the proteins adsorbed to the surface of fat globules in cheese analysed before brining and at four weeks.

Table 8.12 Effect of emulsification of fat with different emulsifying agents on the protein adsorbed to surface of fat globules in Feta cheese

Emulsifying agents	Identification and comparison of proteins adsorbed to fat globule surface in Feta cheeses made with the emulsifying agents by SDS-PAGE.
SMP (control)	All major milk proteins were present. Increased amounts of proteins (particularly caseins) were detected in comparison to cheeses with other emulsifying agents, as evidenced by the band densities of the electrophoretic patterns.
Sodium caseinate	Electrophoretic pattern was similar as control but the bands were less dense.
Sodium caseinate & Lecithin	This was similar to cheese made with emulsifying agent sodium caseinate.
WPC powder	All major milk proteins were present. Density of casein bands were similar as that of sodium caseinate. There was considerable increase in whey proteins, particularly β -lactoglobulin.
Lecithin	Electrophoretic pattern showed faint bands in the casein region and one or two bands in the high molecular mass region, <i>i.e.</i> > 60,000 daltons.
Tween-60	No proteins were detected.
Unhomogenised milk ¹	No proteins were detected.

¹ Data from Section One.

Urea-PAGE: Proteins absorbed to fat globules in fourteen month old cheeses were extracted in Urea-sample buffer and SDS-sample buffer. These extracts were analysed by Urea-PAGE (Fig. 8.3). Fat and whey protein present in the extracted

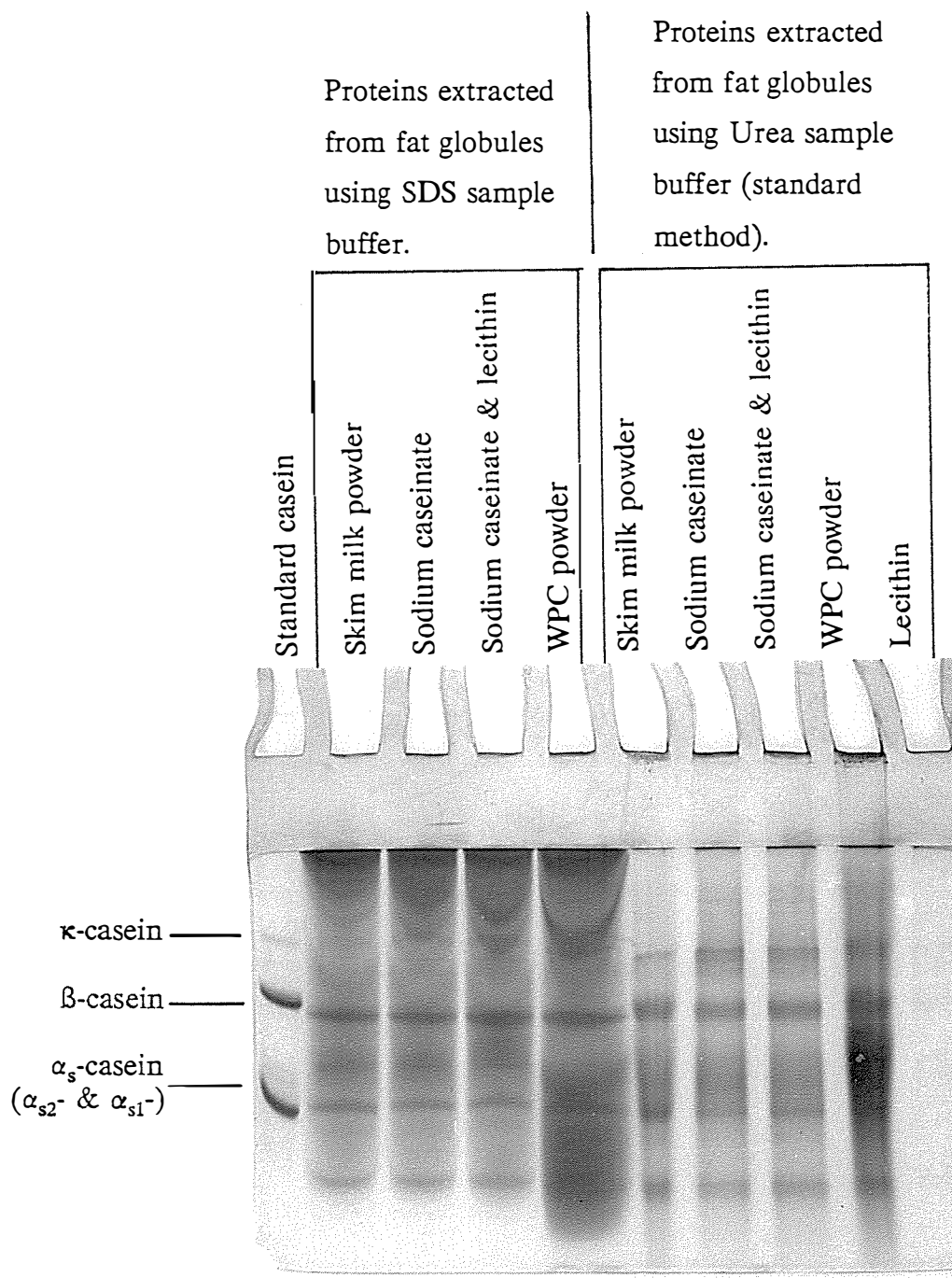


Fig. 8.3 Urea-PAGE showing proteins adsorbed to surface of fat globules in fourteen month old Feta cheeses made using different emulsifying agents.

Note: Urea-PAGE of fat from fourteen month old Feta cheese made using Tween-60 showed no protein bands. It was identical to that of cheese made using lecithin as shown above.

samples appear to have obscured the protein bands in the gel. The electrophoretogram showed β -casein, α_{s1} -casein and possibly α_{s1} -I-casein bands. It was difficult to quantitatively determine the proteins using the densitometer. There were no proteins in fat extracted from cheeses made with Tween-60 and lecithin.

Conclusion: A variation in the amount of casein adsorbed to the fat globules was observed. This provides an estimate of the extent to which fat globules participated in the casein matrix of the Feta cheeses. It is uncertain if the casein adsorbed to the fat globule surface is proteolysed at the same rate as in rest of the cheese.

(e) Effect of emulsifying agents on exudation of whey from Feta cheese during storage (Table 8.13)

The general trend of an increase in the amount of exudate with time was detected up to 9 months of storage. This pattern of exudation is typical of Feta cheese. The pattern of exudation was variable during the initial stages of storage, which steadied after 15 weeks. The rapid increase in the amount of exudate in the first two weeks of storage was partly due to some exudate being drawn out from cheese during vacuum packaging.

Effect of replicates: There was no difference between the two replicates. This indicated that the manufacturing conditions during cheese manufacture did not vary significantly.

Effect of emulsifying agents: The use of emulsifying agent SMP (control) was most effective in reducing the amount of exudate; followed by lecithin, combination of sodium caseinate and lecithin, sodium caseinate, WPC powder and Tween-60. The least square mean values for the amount of exudates from cheeses with different emulsifying agents indicate that, in comparison to control (SMP), the increase in the amount of exudate was: little with lecithin; slight with sodium caseinate, and

Table 8.13 Effect of emulsifying agents on exudation of whey from Feta cheese during storage

Source of variation	Exudate in g/kg cheese		Exudate in g/kg moisture in cheese		Final MNFS (%)		% reduction in MNFS	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		0.75 ns		0.34 ns		2.0 ns		1.9 ns
1	41.5		80.1		67.8		1.2	
2	39.8		78.4		67.1		1.2	
Emulsifiers		13.0 **		18.7 **		8.24 *		53.1 ***
Sodium caseinate	40.3#		78.6*		68.0ns		1.1*	
Sodium caseinate & lecithin	37.8		74.5#		67.6		1.1*	
Lecithin	33.6		68.3		67.3		1.0#	
SMP (control)	32.2		61.9		68.4		0.9	
Tween-60	54.5***		107.1***		64.3**		1.9***	
WPC powder	45.6**		85.1**		69.3ns		1.1*	
Storage Time (weeks)		165 ***		166.5 ***		147 ***		141 ***
2	23.0		44.9		67.8		0.66	
4	25.5		49.7		67.8		0.73	
6	28.8		56.1		67.7		0.83	
9	32.6		63.5		67.6		0.95	
12	37.7		73.5		67.5		1.11	
15	46.0		89.7		67.4		1.36	
20	50.6		98.6		67.3		1.50	
26	55.7		108.6		67.2		1.66	
39	66.0		128.6		66.9		2.0	
Emulsifying agents X storage time		2.22 **		2.18 **		2.38 **		2.47 **
S.D	3.99		7.74		0.089		0.13	
C.V. (%)	9.8		9.77		0.13		11.2	

L.S.M. = Least square mean; F = F ratio; C.V. = Coefficient of variation; S.D. = Standard deviation of raw data;
 # = $p \leq 0.10$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$;
 ns = not significant; LSM values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

combination of sodium caseinate & lecithin; significant with WPC powder; and highly significant with Tween-60.

Interaction of storage time with emulsifying agents: The effect of emulsifying agents on the exudation from Feta cheese became evident during storage (Fig. 8.4). Throughout the storage study cheese made with SMP as the emulsifying agent (control) had the least amount of exudate, and cheeses with emulsifying agents Tween-60 and WPC powder had significantly higher amounts of exudate. The amounts of exudates from cheeses with sodium caseinate, and sodium caseinate & lecithin were significantly higher in comparison to the control only after 15 weeks of storage. There was no difference in the amount of exudates from cheeses with lecithin and SMP. The amount of exudate from cheese made using a combination of sodium caseinate and lecithin was mid-way between that of cheeses with individual emulsifying agents sodium caseinate and lecithin.

8.3.3.5 Discussion

Size of fat globules: It was of interest to determine whether the effect of homogenisation on exudation was due to the reduction in the size of the fat globules (and an increase in the number) or to the protein adsorbed to the fat globules or both. Homogenisation was effective in reducing the amount of exudate (Section One in this Chapter) while a change in homogenisation pressure did not affect exudation markedly (Chapter 6.4.5.c). Homogenisation of cream, even at the lower pressure [single stage 600 psi (4134 kPa)], reduced the size of the fat globules substantially (from $\approx 4.0 \mu\text{m}$ to $\approx 1 \mu\text{m}$) while homogenisation of cream at higher pressure [double stage, 2,000/500 psi (13780 kPa/3445 kPa)] reduced the size of the fat globules to about $0.5 \mu\text{m}$. As the fat globule size is expected to change to some extent during cheesemaking, it is uncertain whether the difference in fat globule sizes obtained in cheesemilks (0.5 to $1.0 \mu\text{m}$) due to homogenisation at different pressures was carried over to the cheese without a change. Evidence from SDS-PAGE on fat globules from cheese made using lecithin and Tween-60 as emulsifying agents suggests that the fat globules were not coated with casein. A reduction in size was presumably obtained due to homogenisation. It is not

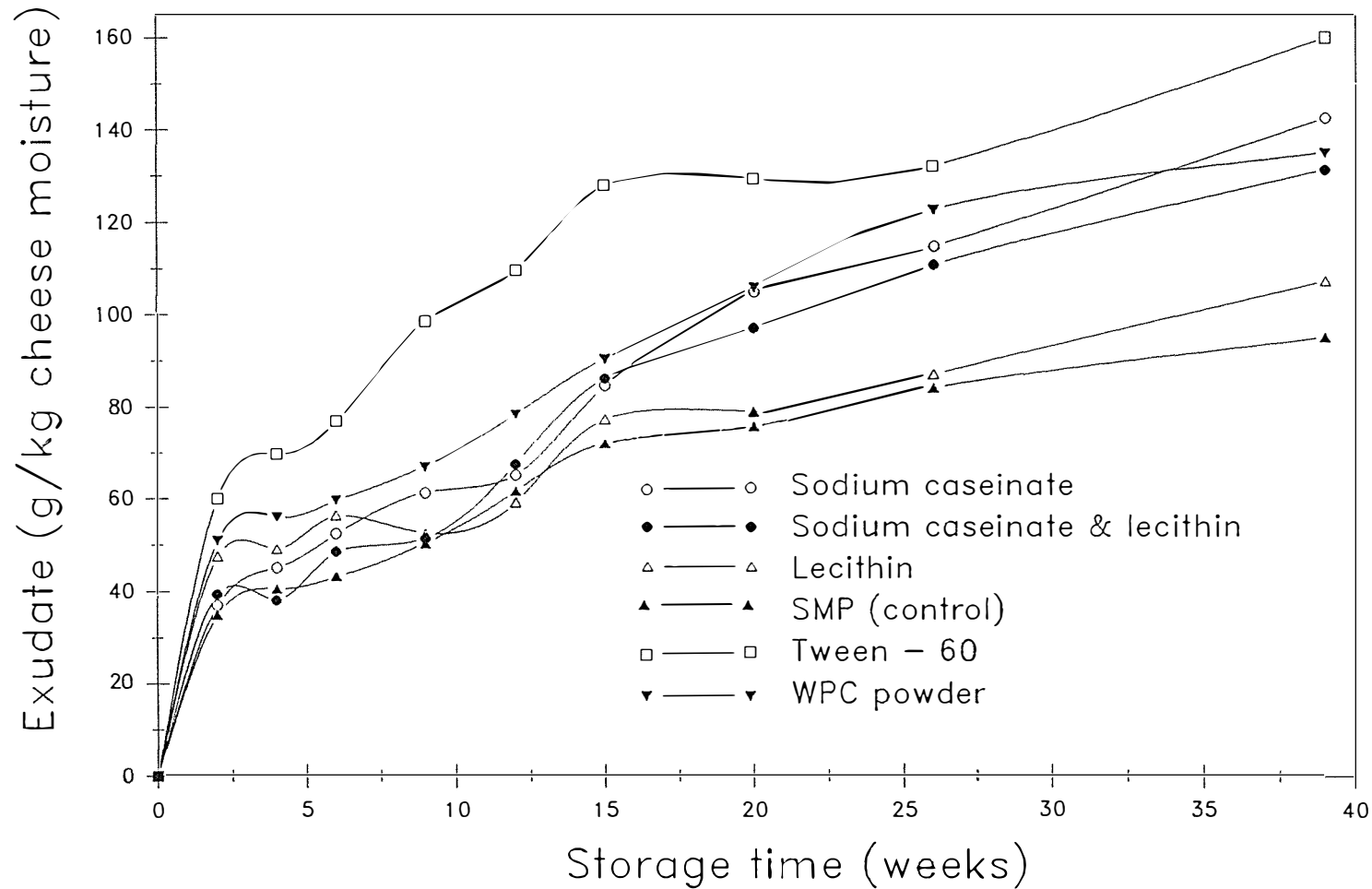


Fig. 8.4 Effect of emulsifying agents on the exudation of whey from Feta cheese during storage.

possible to draw any conclusion from the observations on exudation from cheese made with Tween-60 because of the reduction in the amount of fat in cheese. It is also difficult to draw any conclusion from the observations on cheese made using lecithin as the results have to be considered in light of other possible effects of lecithin, as described later in this section.

When spheres are suspended in a continuous medium and are sparsely distributed such that there is no interaction between them, and the volume concentrations of the media remain unchanged, the diffusion coefficient of the suspension is independent of the size of the suspended particles (Crank, 1983). If the fat globules in cheese are considered as spherical particles embedded in the continuous heterogenous mixture of water and soluble material (exudate), a change in volume of fat would affect the diffusion coefficient. However, when the volume of fat is constant and change in size of fat globules in cheese occurs due to homogenisation, it is difficult to predict whether or not the diffusion coefficient would be affected. This is because in cheese the fat particles are not all spherical, the fat globules are likely to have coalesced and clumped, and the volume fraction of the fat is high.

The available evidence is insufficient to draw conclusion on the effect of a reduction in the size and an increase in the number of fat globules, when the mass of fat is constant, on the exudation of whey from Feta cheese during storage.

Type of material adsorbed to the surface of fat globules: Based on the evidence of the effect of homogenisation (Section One in this Chapter) and of the material adsorbed to the surface of the fat globules (Table 8.12), the following hypothesis is proposed for high-moisture cheeses. The fat globules in cheese are trapped in a three dimensional casein network. In the case of cheese made from unhomogenised milk SDS-PAGE showed little or no protein adsorbed to the fat globule surface, indicating that the MFGM is intact. The MFGM has little affinity for casein as homogenised fresh cream is easily washed free of casein. Electron microscope studies on mature Cheddar cheese (presumably made from unhomogenised milk) showed that the layer adjacent to the fat had the highest content of free water (Kimber *et al.*, 1974). Large proportions of the fat globules

in cheese are likely to be close to each other due to either agglomeration or natural statistical fluctuations. Such clusters create pockets or regions of weak structure in the casein matrix. Water is available in abundance in these regions. In cheese made from unhomogenised milk the water is loosely held and unevenly distributed around the unevenly distributed fat. Fissures, crevices and cracks within the block of Feta cheese further contribute to the lack of homogeneity in the cheese. This unevenly distributed and loosely held water facilitates exudation caused by osmotic pressure difference or other factors within the three-dimensional casein matrix.

In cheese made from homogenised milk the fat globules are coated with casein and form an integral part of the three dimensional casein matrix (Vliet & Dentener-Kikkert, 1982; Walstra *et al.*, 1985; Kebary & Morris, 1990). Casein-coated fat behaves as casein. The effect of fat globules in creating the regions of instability or weakness in the casein matrix is greatly reduced by the casein-coats. This results in a significantly reduced rate of exudation. As proteolysis proceeds, the casein matrix is gradually weakened and the moisture held in the matrix exudes (Chapter 9). Further, the role of fat globules, as part of the network, in hindering the shrinkage of the para-casein matrix (Walstra *et al.*, 1985) may explain the reduced rate of exudation.

The above concept can also be used to explain the pattern of exudation from cheeses made with emulsifying agents SMP and sodium caseinate. The fat globules in the former may be expected to be more firmly embedded in the casein matrix which may have accounted for the difference in the amount of exudates. It is possible that the proteins adsorbed to the fat globule surface hydrolyse as proteolysis progresses (Walstra & Jenness, 1984). If this were true, the amount of caseins adsorbed to the fat globules in cheese made with sodium caseinate, being less in comparison to control, would be hydrolysed sooner. This would undo the effect of casein-coated fat globules on exudation. The fact that a significant variation between the two was noticed only after 15 weeks of storage suggests that this may be happening after a certain storage period when caseins are no longer available to hold the fat in the weakening casein matrix. In the control cheese

some of the caseins adsorbed to fat globules may still be intact and thereby enable fat to remain a part of the casein matrix. However, in an oil-in-water emulsion, peptide bonds in some regions were inaccessible to proteinases (Shimizu *et al.*, 1986), suggesting that the proteins adsorbed to the fat globule surface may not be easily hydrolysed because the peptide bonds susceptible for hydrolysis are not available. The present study on the proteins adsorbed to the fat globule surface showed some proteolysis. However, it is not certain whether the rate of breakdown is the same as in the rest of the cheese.

If all the fat present in cheese were to be assumed as spherical, uniform in size and equidistant from each other, the distance between each fat globule would be about 0.25 μm and 1.2 μm when the diameters of the fat globules are 1 μm and 5 μm , respectively. The calculations to determine the distance between the fat globules are provided in Appendix 8.16. The distance between the fat globules indicates that in cheeses with lecithin, where casein is not adsorbed to the surface of the fat globule, the fat globules may be expected to be very close to each other and in many instances joined to each other. The rate of exudation from cheese made with lecithin was not very different from that of the control. The lecithins are soluble in fat but almost completely insoluble in water. When mixed with water, lecithins hydrate and disperse in the form of spherical globules or *liposomes*, which may be smaller than 1 μm in diameter. The structure within the liposomes is likely to be affected by phospholipid-phospholipid interactions, which are known to occur in soybean lecithin (Kanamoto *et al.*, 1981). It is possible that the closely located lecithin-coated fat globules in cheese interact with each other and form chains, entrapping water molecules within clusters of fat globules. These hydrophobic chains may also repel the flow of exudate in their direction. The tendency of fat globules to cluster was evident in manufactured cream and cheesemilk made using lecithin, where creaming was observed. Cheese made with lecithin also had a sticky texture. Phospholipids are known to form stable colloidal suspensions or emulsions in aqueous solutions and play an important role in the formation and secretion of milkfat (Long & Patton, 1978; Jensen & Clark, 1988). Thus lecithin may have formed water-in-oil type of emulsions with the free water and restricted its release.

Yet another explanation could be that the lecithins interacted with caseins, reduced casein proteolysis and thereby reduced the rate of exudation.

Cheese made using Tween-60 had reduced fat and MNFS. Despite a reduced MNFS exudation continued rapidly during storage. The increased amount of exudate from cheese made with Tween-60 could have been due to a reduced fat content in cheese or to the hydrophilic nature of the emulsifying agent coated on the fat globule surface. Increase in the amount of exudate due to a reduction in the amount of fat in cheese is consistent with the earlier findings of an effect of protein to fat ratio on exudation (Chapter 6). The effect of fat quantity on exudation is also consistent with the earlier explained theory that when spheres are suspended in a continuous medium, a change in volume concentration would affect the diffusion coefficient of the suspension (Crank, 1983). It is possible that an increase in fat level increases the resistance of the casein matrix to contract or change in volume (Geurts *et al.*, 1972), and thereby reduces the amount of exudate.

In cheeses where the casein-coating of fat globules was not pronounced or did not occur, the rate of exudation decreased when the adsorbed material was lipophilic [lecithin, MFGM (refer Section One)], and increased when the adsorbed material was hydrophilic (Tween-60, WPC powder). It is likely the hydrophilic material create regions of weak structure in the casein matrix in cheese, as explained earlier. The material adsorbed to the fat globule surface in cheese made using WPC powder comprised predominantly whey protein and some casein. The whey protein adsorbed to the fat globules is expected to be denatured and insoluble. However, the adsorption of greater amounts of WPC powder to the fat globule surface may have restricted fat globules from becoming part of the casein matrix and thereby created some regions of weak structure and loosely-held water in the casein matrix. Use of WPC powder was therefore not effective in reducing exudation. These results are comparable to the findings that when fat is homogenised into whey instead of skim milk, the decrease in syneresis during manufacture of cheese as a consequence of the homogenisation process was reduced (Emmons *et al.*, 1980). The effectiveness of β -lactoglobulin in providing a long-term stability of emulsion (Darling & Butcher, 1978; Dickinson *et al.*, 1989b), and presumably the absence of

proteolysis of denatured whey proteins may explain slightly lower exudation towards the later stages of storage with cheeses emulsified with WPC powder in comparison to that with sodium caseinate.

8.3.3.5 Conclusion: Adsorption of casein to the fat globule surface in cheese, due to homogenisation, reduces syneresis during manufacture of cheese and subsequently the rate of exudation. When casein does not coat the fat globule surface in cheese from homogenised milk, the type of material adsorbed at the surface of the fat globule (hydrophilic or lipophilic) appears to influence exudation. Hydrophilic substance promotes and lipophilic substance lowers the rate of exudation. Evidence on the effect of size reduction of fat globules and increase in the number of fat globules, as a result of homogenisation, in reducing the amount of exudate from Feta cheese was inconclusive. A decrease of fat content in cheese from homogenised milk increases the amount of exudate. A hypothesis is proposed to explain the effect of homogenisation on exudation.

6.4 OVERALL CONCLUSION TO CHAPTER 8

Homogenisation is an effective process to reduce the amount of exudate. Adsorption of casein to the fat globule surface in cheese, due to homogenisation, reduces the rate of exudation. Evidence on the effect of size reduction of fat globules and increase in the number of fat globules, as a result of homogenisation, in reducing the amount of exudate from Feta cheese was inconclusive.

The properties of the material adsorbed to the fat globule surface influence exudation considerably. Syneresis of whey from cheese during manufacture is also influenced by material adsorbed to the fat globule surface. The amount of exudate decreased with an increase in the fat content in cheese. The use of cheesemilk made from combinations of homogenised creams (fresh and manufactured) and skim milks (fresh and recombined) did not affect the exudation.

CHAPTER 9

INFLUENCE OF PROTEOLYSIS ON THE EXUDATION OF WHEY FROM FETA CHEESE DURING STORAGE

9.1 Introduction

Previous studies indicated that exudation could be related to proteolysis in cheese (Chapter 6). The underlying mechanism for this relationship is postulated to be as follows. Moisture is held in the three-dimensional protein (casein) network that gives cheese its form and substance. The manner and extent of water-protein interactions are affected by various factors such as amino acid composition, protein surface polarity and charge, conformation and topography, pH, ion species and temperature (Kinsella & Fox, 1987). The casein matrix gradually becomes weaker and starts to disintegrate as proteolysis proceeds. The water molecules held in the interstices of the casein gel are released. Unless stopped by some other mechanism, the moisture and soluble material leak out of the cheese as exudate.

Visser (1977b) showed that a variation in residual rennet in cheese resulted in a corresponding variation in the rate of proteolysis in starter-free Gouda cheeses. Rennet is involved primarily in the formation of large peptides in Cheddar cheese (O'Keeffe *et al.*, 1978) and the residual rennet retains its milk clotting activity throughout ripening (Dulley, 1974). It is expected that rennet in Feta cheese will have a similar effect of forming large peptides. Earlier studies in Chapter 6 suggested that *Lactobacillus casei* was proteolytic in Feta cheese during storage even in conditions of low pH and high salt-in-moisture (S/M) concentration. The other microbial strains inoculated through the starter were destroyed in about two weeks after manufacture of cheese. In Feta cheese the larger peptides formed by the action of rennet may be expected to be further proteolysed into smaller peptides and amino acids by the lactobacilli bacteria.

If the proteolytic activity of chymosin is proportional to the amount of residual rennet in cheese (Creamer, 1979), a variation of residual rennet in Feta cheese

should correlate with the rate of proteolysis and exudation. The objective of this study was to determine the effects of varying levels of residual rennet in cheese on proteolysis and exudation.

In general, calf-rennet is referred as rennet throughout this Chapter.

9.2 Experimental plan

Feta cheese was manufactured using three levels of calf-rennet (Table 9.1). Setting time was adjusted according to the amount of calf-rennet added so that the gel-strength at cutting was similar for all the trials. However, the total time from inoculation of starter until draining of whey was kept constant so that curd pH at draining was identical in all cheeses. This was expected to ensure rennet retention in cheese in proportion to the quantity of rennet added to milk. The distribution and mass balance of rennet in cheese and whey were monitored.

The experiment was designed to statistically analyse the results by analysis of variance (one-way classification). A total number of 6 trials were performed using two replications for each level of rennet. The sequence of trials were randomised. The sources of variation were 'replicates', 'level of rennet' and 'storage time'. The data were analysed using the statistical package of SAS Institute Inc. (1985).

Table 9.1 Quantity of calf-rennet (strength = 59 RU/ml) used for manufacture of cheese

Quantity of rennet added to cheesemilk	Reference in the text
8 ml / 100 kg milk	Low rennet
16 ml / 100 kg milk	Control
32 ml / 100 kg milk	High rennet

9.3 Experimental

Feta cheese was manufactured using the procedure outlined in Chapter 6, except for the following changes:

(i) After standardisation of milk to a P/F ratio of 0.73, it was diluted with water so that the protein content was close to the average protein content in whole milk (3.3 - 3.4%).

(ii) The time of renneting milk was varied depending upon the amount of calf-rennet added (Table 9.2).

Table 9.2 Variation in priming and setting time for different amounts of calf-rennet added to milk

Processing step	Cumulative time in minutes		
	Low rennet cheese	Control cheese	High rennet cheese
Inoculation of starter	0	0	0
Addition of rennet	10	30	50
Cutting	70	70	70

9.4 Analytical methods

Cheesemilk and whey were analysed for fat, protein, lactose and total solids by the Milko-Scan. Calcium content in cheesemilk and whey were estimated by complexometric method. The mean diameter of the fat globules in the cheesemilk was determined by the spectroturbidimetric method.

Feta cheeses before and after brining were analysed for moisture, pH and calcium (complexometric method). Feta cheese at three weeks was analysed for fat

(Schmidt-Bonzynski-Ratzlaff method), protein (Kjeldahl), NaCl (potentiometric titration), moisture and calcium (complexometric) at three weeks. Cheese (after brining) and whey were analysed for residual rennet.

Exudate from three week old Feta cheese was analysed for protein (Kjeldahl), total solids, calcium (complexometric), NaCl (potentiometric) and the distribution pattern of low molecular weight peptides (HPLC).

Proteolysis in Feta was determined by urea-PAGE at the following stages: after brining (0 week), 4 weeks, 15 weeks and 26 weeks. Exudation of whey from cheese was determined at selected storage periods as described in Chapter 4.

Details of the analytical methods of all the above analyses are provided in Chapter 4 and Appendix 4.2.

9.5 Sensory evaluation

Feta cheese was evaluated by a sensory panel at eight weeks after manufacture for flavour and textural characteristics. The detailed procedure is described in Chapter 4.

9.6 Results and Discussion

This section is described under the following headings:

- (i) Cheese manufacture; and composition of milk, whey, cheese and exudate.
- (ii) Sensory evaluation of cheese.
- (iii) Distribution and mass balance of rennet.
- (iv) Proteolysis in cheese.
- (v) Exudation from cheese.

9.6.1 Cheese manufacture; and composition of milk, whey, cheese and exudate

There was no specific problems in the manufacture of cheeses with varying amounts of rennet.

The composition of milk (Appendix 9.1) was uniform for all the experiments. The variation in the mean diameter of fat globules for replicates is not of practical significance.

The composition of whey (Appendix 9.2) was not affected by the variation in the quantity of rennet used. The composition of cheeses before brining (Appendix 9.3), after brining (Appendix 9.4) and after 3 weeks of storage (Appendix 9.5) was not affected by the variation in the amount of rennet used for coagulation.

The major variation was in the amounts of residual rennet in cheese and whey, which is described later in this Chapter.

The composition of exudate from three week old Feta cheeses (Appendix 9.6) shows an increase in protein content (protein breakdown products) with the increase in the amount of rennet used during cheesemaking. This may be attributed to the increase in soluble nitrogen, presumably due to increased proteolysis.

9.6.2 Sensory evaluation of cheese (Appendix 9.7)

No difference could be detected for most of the attributes in cheeses made with variable amounts of rennet. However, the panel was of the opinion that cheeses with high rennet had an unusual flavour. In a study on Gouda cheese with increased amount of rennet, bitterness was detected with increased intensity in the early stages of maturation (Visser, 1977a). In this case the panel did not specifically detect bitterness in cheeses with high rennet but indicated a flavour variation in terms of 'lower acidity'. It is possible the high NaCl in cheese masked the bitter flavour.

9.6.3 Distribution and mass balance of rennet

Rennet distribution in cheese and whey (Table 9.3): The desired variation of residual rennet in cheeses was achieved.

Table 9.3 Effect of variation in the amount of rennet used during cheesemaking on the rennet retained in cheese and whey

Sources of variation & levels	Rennet activity (RU/kg)			
	Cheese after brining		Whey	
	LSM	F	LSM	F
Replicates		159.3**		3.86 ns
1	8.87		7.21	
2	10.43		6.85	
Quantity of rennet		4627***		853.3**
Low	3.78**		3.00**	
Control	7.39		5.94	
High	17.78***		12.16**	
S.D.	0.151		0.226	

LSM = Least-square mean; F = F ratio;
 S.D. = Standard deviation; ns = not significant;
 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Mass balance of rennet activity: Details of the calculation of the mass balance of rennet activity are shown in Appendix 9.8. The percentage of total rennet activity (rennet added to milk) recovered in cheese and whey ranged between 64 to 70%. The various sources of losses may be attributed to the following:

- Some rennet activity is lost due to the process treatments. Holmes *et al.* (1977) reported that in Cheddar cheese approximately 35% of the rennet activity was destroyed by the time whey was drained.
- Some whey diffuses out of cheese during brining. Rennet activity would be lost through this whey.
- The method used for measurement of rennet activity (Singh & Creamer, 1990) was standardised with Cheddar cheese. The rennet activity recovered by this method represents approximately 80% of the total rennet activity in cheese. A correction factor is applied to obtain the absolute figures. In this instance the correction factor for Feta cheese is not known and could not be applied. The reported rennet activity for cheese is therefore less than the actual value.

It was concluded that the recovery of rennet activity was reasonably satisfactory.

The rennet activity in the control cheese (6.5 - 8.3 RU/kg) was close to that detected by Singh & Creamer (1990) in Feta cheese. The rennet activity recovered in Feta cheese varied between 11.4 to 17.2%. These figures would be higher if the correction factor was applied. In comparison to this, the recovery of rennet in Cheddar cheese has been reported to be about 6 - 7% of the total rennet added (Dulley, 1974; Holmes *et al.*, 1977; Fox, 1989). Retention of a higher percentage of rennet activity in Feta may be attributed to the high moisture in the cheese.

9.6.4 Proteolysis in cheese

Urea-PAGE on cheeses: Casein proteolysis in Feta cheeses with varying residual rennet levels after selected periods of storage is shown in Fig. 9.1. Increased residual rennet in cheese clearly caused an increased casein proteolysis. Fig.9.2 and Fig.9.3 show the extent of proteolysis of α_{s1} -casein and β -casein, respectively. Proteolysis has been expressed in terms of the ratios of α_{s1} -casein and β -casein in the experimental cheeses to β -casein of the standard casein run in the same gel. The ratios were determined using areas estimated by densitometry. Proteolysis has been expressed as ratios to eliminate possible error due to gel to gel variation. Breakdown of α_{s1} -casein was slow in low rennet cheese and was rapid in high rennet cheese. This is consistent with the findings that breakdown of α_{s1} -casein was proportionately reduced in semi-hard type of cheese with low rennet (Koning *et al.*, 1981). A substantial amount of α_{s1} -casein had been proteolysed in high rennet cheese by the time brining of the cheese was complete. The effect of high rennet was also noticed on the breakdown of β -casein. During the initial stages of storage an apparent increase in β -casein was observed in low rennet cheese which may be attributed to experimental errors, primarily because densitometer plots could not represent the entire protein band in the gel. Proteolysis of β -casein in high rennet cheese was evident after 4 weeks of storage of cheese. It is reported that β -casein in cheese is not hydrolysed by chymosin (Fox, 1989) and that the hydrolysis is strongly retarded in the presence of NaCl (Fox & Walley, 1971; Thomas & Pearce, 1981; Noomen, 1983). β -casein in Cheddar-type cheeses is usually hydrolysed by

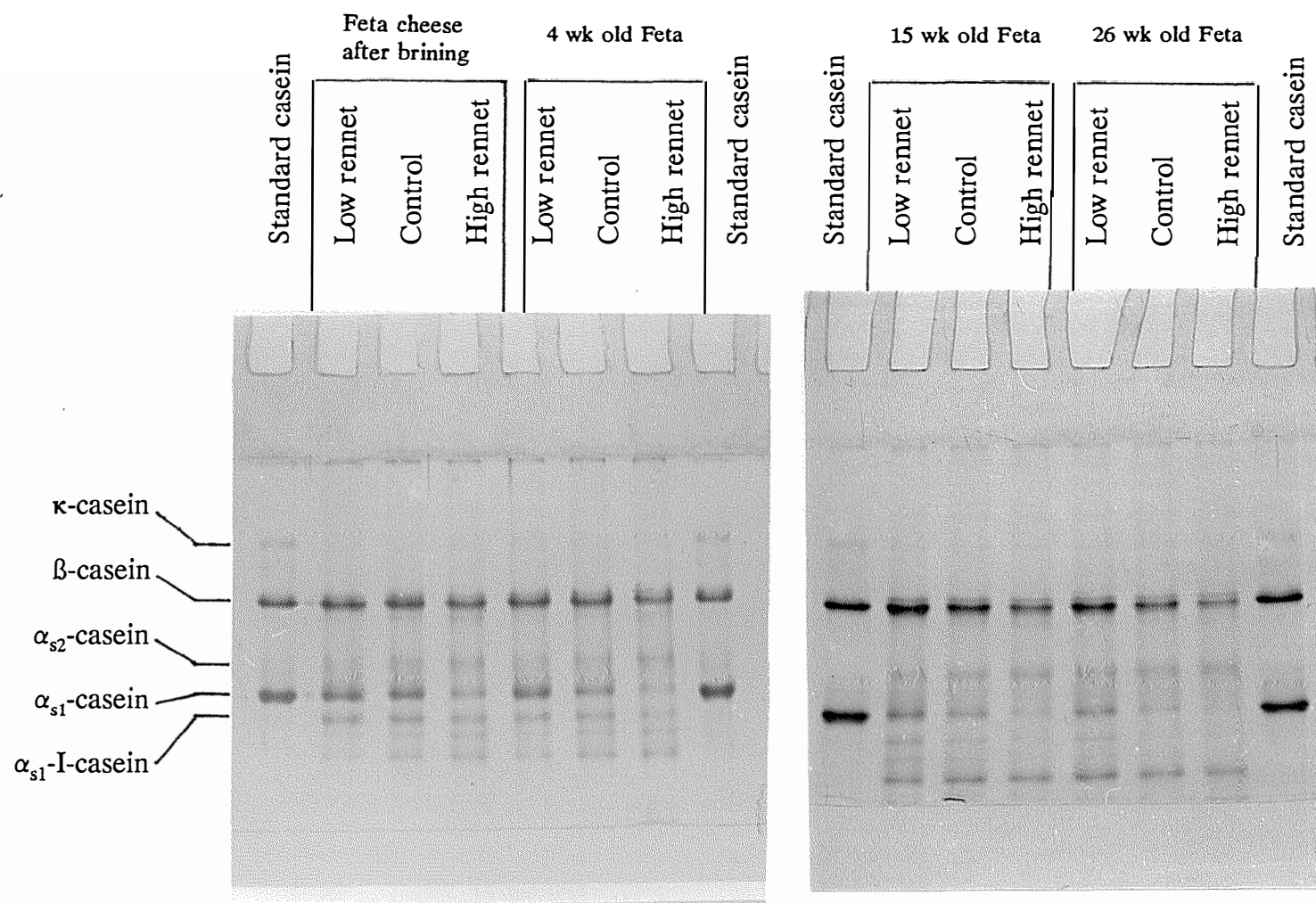


Fig. 9.1

Effect of residual rennet on casein proteolysis in Feta cheese during storage (Urea-PAGE).

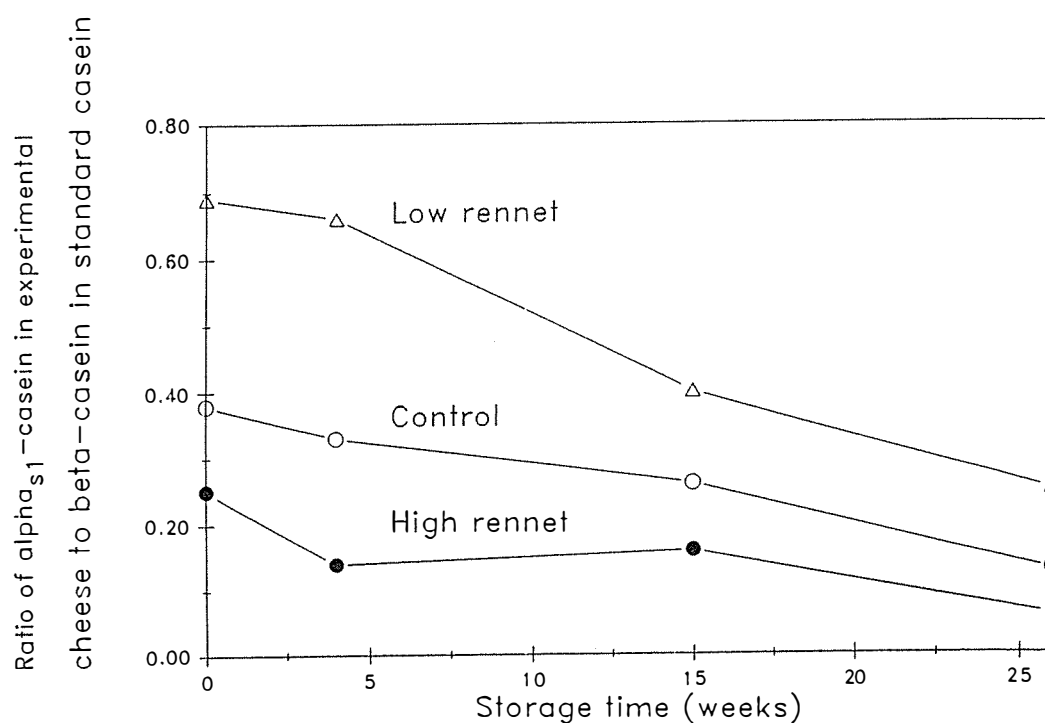


Fig. 9.2 Effect of residual rennet on the hydrolysis of α_{s1} -casein during storage of Feta cheese.

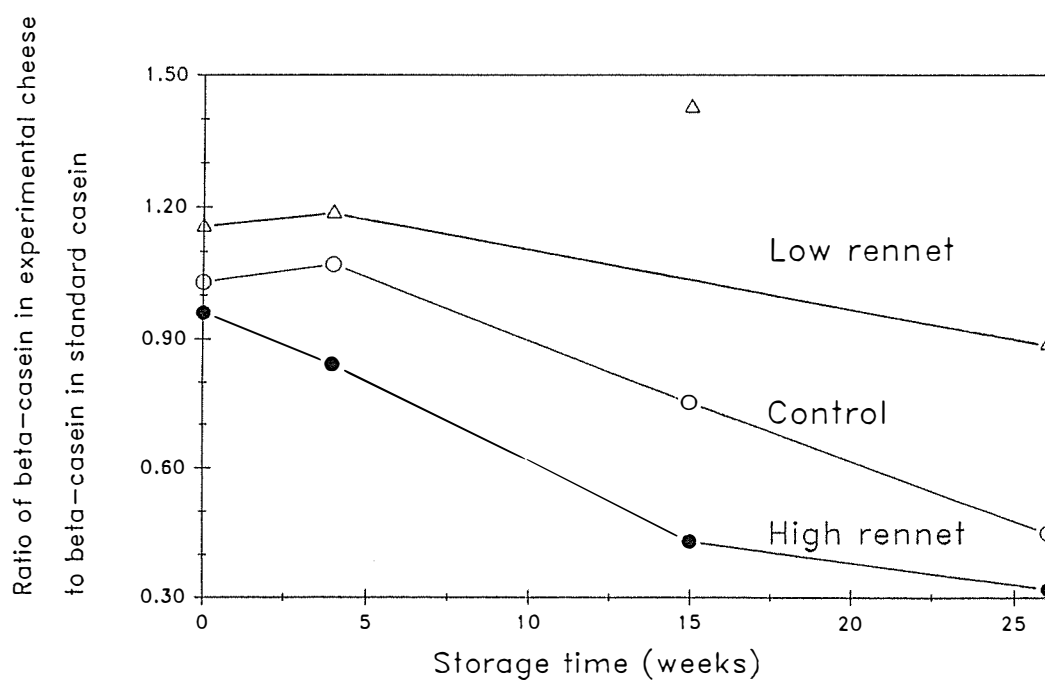


Fig. 9.3 Effect of residual rennet on the hydrolysis of β -casein during storage of Feta cheese.

plasmin (Lawrence *et al.*, 1987) and by starter bacteria enzymes to some extent (Visser & Groot-Mostert, 1977). Present studies clearly showed that a high amount of residual rennet in cheese was effective in proteolysing β -casein in spite of high S/M and low pH of cheese.

It was concluded that the breakdown of α_{s1} -casein and β -casein during storage or ripening of Feta cheese was correlated to the level of rennet in cheese.

HPLC analysis of exudate: The distribution pattern of lower molecular weight peptides/amino acids in exudates from 3 week old Feta cheese is shown in Fig.9.4. An increase in the peak size at the molecular weight of about 4,000 daltons was observed with increased residual rennet in cheese. Storage studies on Feta cheese (Chapter 6) had revealed that this was indicative of increased proteolytic activity. It follows from this that increased proteolysis occurred with increased amount of rennet in cheese. Rennet is primarily responsible for the formation of larger peptides while small peptides and free amino acids are principally produced by the starter (O'Keefe *et al.*, 1978). It is difficult to predict whether this increase in the peptides was the result of direct action of rennet or the action of proteolytic bacteria *Lactobacillus casei* and milk protease on the breakdown product of casein. It is presumed here that the contribution of other starter microorganisms towards proteolytic activity is negligible because they were destroyed in about two weeks after manufacture of cheese (Chapter 6.3.2.e), and in these two weeks large amount of low molecular weight peptides is not available for proteolytic action by the microorganisms. However, variation of peptides at only a specific molecular weight range suggests a relationship with the specific nature of proteolytic action of rennet. The peak sizes increased with an increase in the use of rennet. Whether or not rennet has a role with the production of these peptides, the role of 'quantity of rennet' can not be undermined as the action of rennet clearly stimulates the starter bacteria to accumulate amino acids and low molecular weight peptides (Visser, 1977b). The change in this particular peak size shows that the increased enzyme activity with increased amount of rennet has been specific.

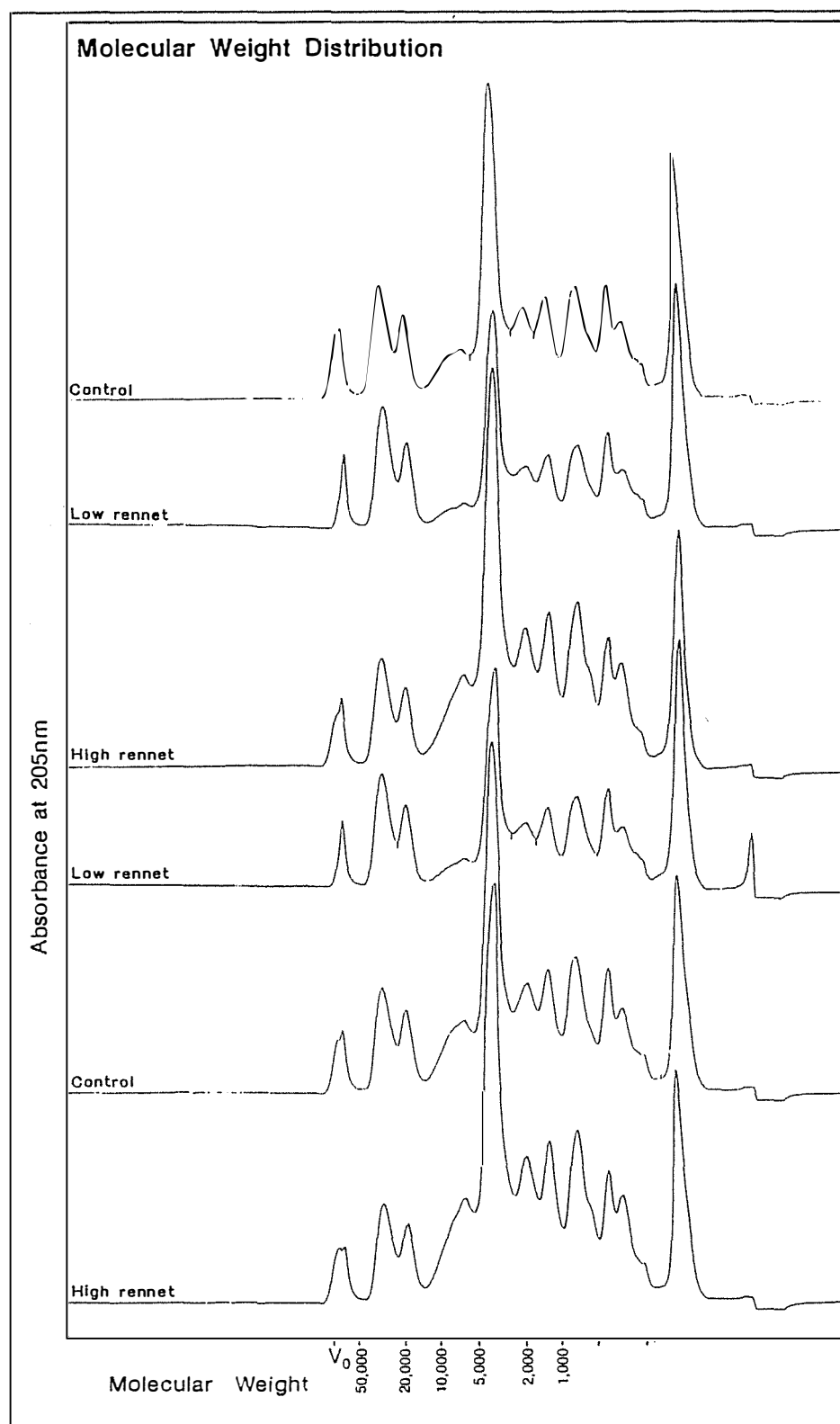
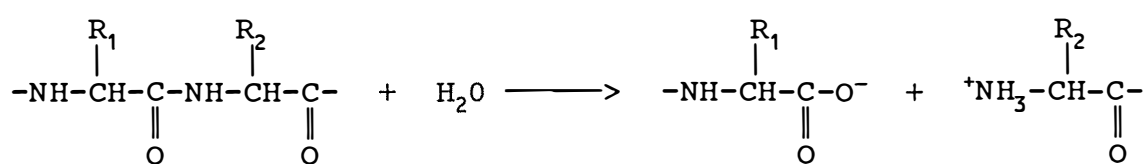


Fig. 9.4 HPLC plots showing the effect of residual rennet concentration on the peptides formed in three week old Feta cheese.

9.6.5 Exudation from cheese

Effect of residual rennet: The effect of variation in the quantity of rennet used in cheesemaking on the exudation of whey from Feta cheeses during storage is shown in Table 9.4. A significant increase in the amount of exudate was observed with an increase in the amount of rennet. It has been reported earlier in this section that increased proteolysis was detected with an increased amount of rennet. Thus, a relationship between proteolysis and exudation appears probable. Occurrence of exudation from other varieties of cheese provides evidence to support a strong correlation between exudation and proteolysis. It is well known that there is extensive proteolysis in blue-veined cheese, and exudation in this cheese is a major problem (Pedersen *et al.*, 1971). Exudate is released from nearly all the blue-veined cheeses during ripening. Further, it is known from practical experience that exudation is often observed in vintage Cheddar cheese (≈ 2 years old). In contrast, proteolysis is much less in Mozzarella cheese, and exudation from the cheese is rare even though it has a high moisture content of about 48%.

It has been suggested that α_{s1} -casein can interact strongly with two, or possibly more, casein molecules (either α_{s1} -casein or β -casein) and can thus be a link in the protein network (Lin *et al.*, 1972). As proteolysis of α_{s1} -casein proceeds, the casein network weakens and gradually disintegrates, releasing moisture held in its interstices as *exudate*. Proteolysis of β -casein may be expected to further disrupt the three-dimensional casein matrix.



As shown in the above chemical equation, when a peptide bond in a protein or peptide is hydrolysed one molecule of water is consumed in the reaction and a new pair of carboxyl and amino groups are formed. It is readily shown that for Feta and other cheeses the water used in the hydrolysis is negligible compared to the

Table 9.4 Effect of variation in the quantity of rennet used in cheesemaking on the exudation¹ of whey from Feta cheeses during storage at 10°C

Source of variation & levels	Exudate in g/kg cheese		Exudate in g/kg cheese moisture		Final MNFS (%) in cheese		% reduction in MNFS of cheese	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		58.69 *		25.45 *		7.07		0.00
1	43.3		82.3		69.4		1.27	
2	48.5		89.2		71.3		1.28	
Quantity of rennet (R)		144.52 **		130.90 **		1.47		16.58 #
Low	39.7		73.8		71.0		1.08	
Control	44.4		82.8		69.5		1.32	
High	53.6		100.5		70.5		1.42	
Storage time (S)		98.40 ***		100.39 ***		105.57 ***		103.4 ***
2 weeks	21.1		39.4		70.8		0.56	
4 weeks	25.6		47.9		70.7		0.68	
6 weeks	31.6		59.1		70.6		0.85	
9 weeks	41.8		78.0		70.4		1.13	
12 weeks	50.5		94.3		70.2		1.40	
15 weeks	57.9		108.2		70.1		1.62	
20 weeks	66.6		124.3		69.9		1.89	
26 weeks	72.1		134.6		69.8		2.06	
Interaction S X R		0.97		0.97		1.22		1.24
S.D.	4.69		8.67		0.094		0.134	

¹ Exudation has been expressed in various ways. Details of calculations are provided in Appendix 4.2;

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

p < 0.10; * p < 0.05; ** p < 0.01; *** p < 0.001;

F values without asterisks denote 'not significant'.

total water in cheese. For example, the average residue weight in paracasein is about 117 daltons. If hydrolysis was complete one mole of water would be used for each mole of peptide bonds or residues. For Feta cheese containing 15% casein protein this amounts to $(15 \times 18) / 117 = 2.3$ g of the 50 g total water in cheese. However, additional water is required to hydrate the new electrically charged terminal groups. No reliable data seems to be available for the acidity constants of these groups but it seems likely that they will be fully charged at the pH of the cheese, typically 4.6. Kuntz (1971) has calculated that some 6 or 7 water molecules are required to hydrate protein side chain carboxyl groups and perhaps 4 or 5 water molecules for the ϵ -amino groups. No information is available for α -carboxyl and α -amino groups. Thus, about 10 water molecules are required for each peptide bond hydrolysed. A total of 30 g of water would be bound in 100 g of completely hydrolysed Feta, a substantial portion of the total 50 g of water.

As hydrolysis of peptide bonds (*i.e.* proteolysis) proceeds in the ripening cheese more and more water-binding groups are formed. A portion of these water-binding groups will be attached to the protein network and the remainder to small peptides and amino acids dissolved in the fluid which permeates the cheese. The first group will inhibit exudation by binding water to the protein network. The effect is inherently small because only a few peptide bonds can be broken before the fragments cease to be part of the protein network. The second group will have a more neutral effect on exudation as the bound water is already part of the continuous aqueous phase of the cheese. It is concluded that binding of newly created terminal groups will have only a small negative effect on exudation.

One way of verifying the above hypothesis would be to analyse the cheese and the exudate for the composition of amino acids, particularly glutamic acid, aspartic acid, histidine, lysine and arginine, which have electrical charged side chains at pH 4.6. If the exudate is relatively rich in these components it would show that with proteolysis the smaller peptides are not a part of the protein network and therefore the water bound to these electrically charged groups does not add to the water-binding ability of the protein network in cheese. The evidence of increased amount of exudate in high rennet cheese is consistent with this view.

Effect of replicates: Variation in the amount of exudates for the replicates may have been due to a variation in the amount of residual rennet in the respective cheeses. Here also an increase in the amount of exudate is observed with increase in residual rennet in cheese. This further shows a direct relationship between residual rennet in cheese and the exudation.

Interaction of residual rennet with storage time: Exudation of whey from cheese increased with increase in residual rennet and increase in storage time (Fig. 9.5). Analysis of variance showed that interaction of rennet levels and storage time did not have a significant effect on the exudation from cheese.

9.7 Summary and conclusion

A change in the amount of rennet added to cheesemilk resulted in a corresponding variation of residual rennet in cheese. Casein proteolysis in cheese was related to the amount of residual rennet in cheese. Degradation of α_{s1} -casein was rapid and increased with increase in the amount of residual rennet in cheese. After 4 weeks of storage proteolysis of β -casein was clearly detected in high rennet cheese.

Exudation of whey from cheese correlated with the residual rennet and proteolysis in cheese. It is likely that with proteolysis the three-dimensional casein network becomes weaker and gradually disintegrates. The water-holding ability of the casein gels is thereby greatly reduced. Moisture is released from the interstices of the casein gel. This free moisture and the soluble material, including peptides and amino acids formed during proteolysis, are released as exudate during storage of Feta cheese.

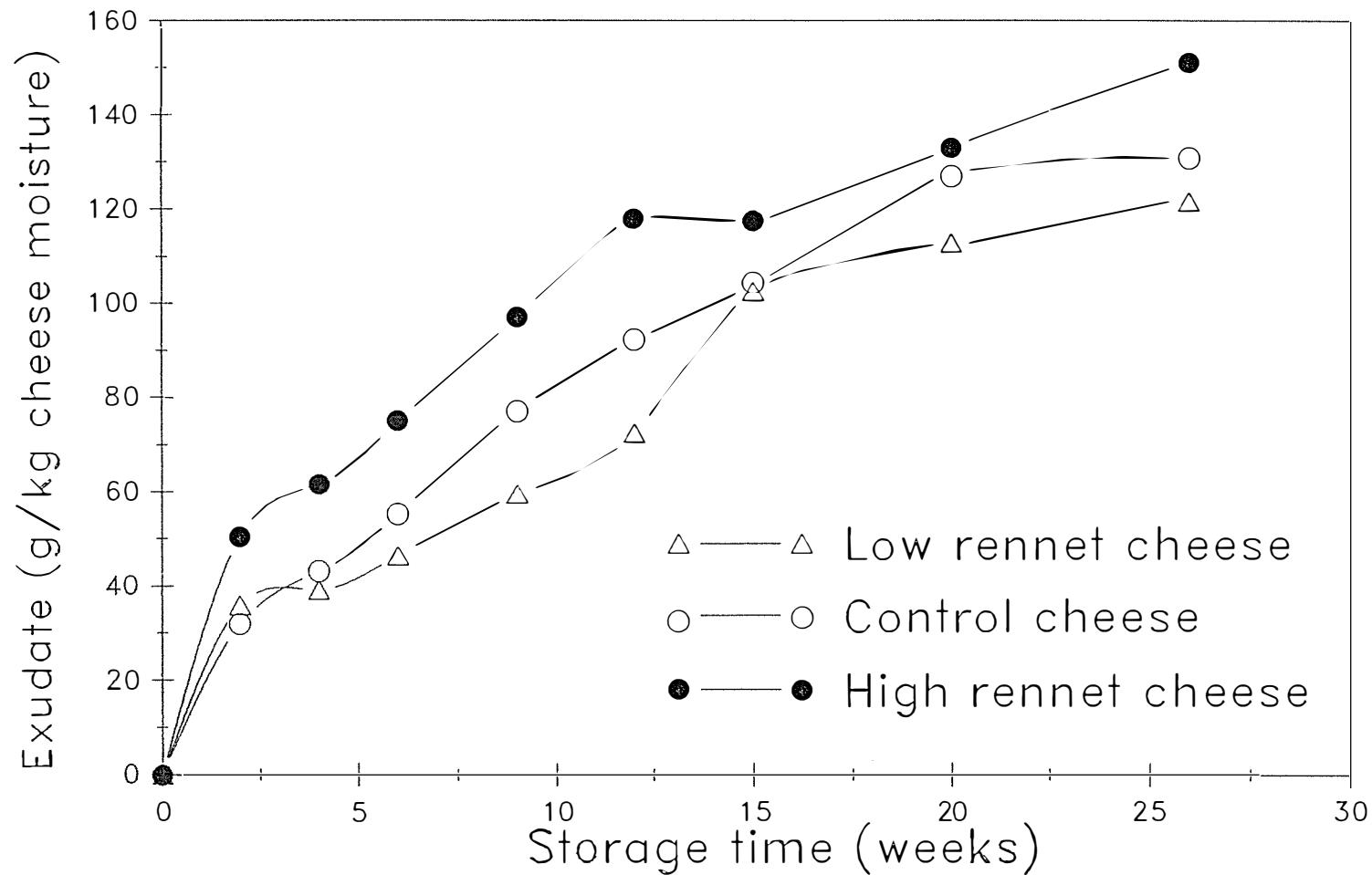


Fig. 9.5 Effect of residual rennet on the exudation of whey from Feta cheese during storage.

CHAPTER 10

OSMOSIS AND DIFFUSION IN FETA CHEESE

10.1 Introduction

Osmosis is the spontaneous flow of solvent from a less to a more concentrated solution when the two liquids are separated from each other by a suitable membrane. Osmosis tends to equilibrate the concentrations on both sides of the membrane and strictly refers to the flow of solvent only. If there is a flow of solute in the opposite direction the behaviour is called *diffusion*. Membranes which permit the passage to some solutes but not others are said to be *semipermeable*. Membranes may discriminate the flow of solutes mainly on the basis of molecular size or on the basis of the electric charge of the solute particle. Membranes with a uniform pore size may show sharp cutoffs, excluding all molecules above a certain molecular mass. If the pores are less uniform, solutes of a range of sizes may pass through the membrane with increasing difficulty.

Cheese consists of a three-dimensional casein network containing fat globules embedded in it. The network is saturated with a complex solution which has a composition similar to that of whey. The cheese may be considered to be a very thick, rather imperfect, semipermeable membrane having a wide range of pores. When cheese is placed in brine, salt penetrates into cheese by the process of diffusion and water is lost from the cheese by the process of osmosis. Some cheese solutes are also lost into the brine.

After cheese is removed from the brine further osmosis and salt diffusion occurs until *osmotic equilibrium* is attained. At this stage cheese and any exudate in contact with it can be assumed to be in equilibrium as they both have the same water activities (Chapter 6.3.2.f).

It is of interest to know the factors affecting the movement of exudate from cheese. Placing cheese in a suitable non-equilibrium solution should either promote exudation or cause the uptake of water by the cheese. Experiments of this type

have been carried out on Gouda cheese by Geurts *et al.* (1972, 1974b, 1980). Unfortunately, such experiments are not practical with small blocks of Feta cheese, as it is not sufficiently coherent. In an effort to overcome this problem an investigation involving packing cheese into dialysis tubing was undertaken. The purpose of the dialysis tubing was to permit the flow of exudate material (water, NaCl and milk salts) as a result of which equilibrium would be attained during dialysis. It was envisaged that the attainment of equilibrium would be influenced by the osmotic effect as well as the other associated factors. The mass transfer during dialysis would therefore indicate the actual movement of exudate from Feta cheese under similar conditions.

The presumption that the mass transfer during dialysis would not be influenced merely by the osmotic effect was supported by the observation from another experiment in which blocks of 3 month old Cheddar cheese (64 g) and 9 month old Feta cheese (506 g) were immersed in water and the change in their weight were measured at selected time intervals. Data on change in weight of cheeses up to 72 h showed a consistent gain in weight of Cheddar cheese (12.3%) and a gradual loss of weight from Feta cheese (3.75%). It is apparent from this that the mass transfer during the process of attainment of equilibrium was influenced by a number of factors.

Following standardisation of the experimental procedure, the effect of selected factors on exudation was investigated.

10.2 Experimental plan

Ideally the cheese inside the dialysis tubing should have been dialysed in exudate obtained from the same cheese. However, the exudate was available only in small quantities. Preliminary experiments were therefore carried out to identify a solution similar in composition to the exudate for use as the external solution when dialysing cheese. This simulated solution will be hereafter referred as "simulated external solution" (SES). SES was prepared by using NaCl (cheese salt), lactose (commercial grade), calcium lactate, lactic acid ($\approx 88\%$), polyethylene glycol (PEG)

of molecular mass 20,000 daltons, milli-Q water¹ and 50% NaOH solution. PEG was used as a substitute for slow or non-dialysing protein/peptides in exudate.

Grated Feta cheese was packed and sealed in dialysis tubings and dialysed in SES. The composition of the SES was varied, one constituent at a time. Alternately, the composition or pH of the cheese was varied by mixing it with an appropriate reagent prior to packing. A large quantity of SES (2-3 kg) was used so that the change in its composition due to diffusion of material from the cheese was negligible. The change in weight of cheese due to mass transfer during dialysis was measured. The influence of NaCl, pH, calcium and proteolysis on weight change during dialysis was investigated.

Initially it was planned to carry out a complete factorial designed experiment and use statistical analysis to interpret the effect of selected variables and their interactions. However, during the course of the preliminary experiments it became evident that some of the selected factors, such as pH and calcium, had a minor influence on the mass transfer. In some of the experiments the changes in the weight of cheese during dialysis were small. This made it difficult to infer from the data on mass transfer as the change in weight could also have occurred partly due to the experimental errors. The loss of weight from material inside the tubing due to evaporation during weighing and inaccuracies involved in wiping the tubing dry increased the source of experimental error. Further the test method became complicated due to rapid loss of low molecular weight protein breakdown material during the initial stages of dialysis. Plans to carry out a statistical designed experiment were therefore abandoned.

10.3 Experimental

10.3.1 Material: Feta cheeses and exudates of about 2-3 months of age were used, unless specified otherwise. Dialysis membranes, size 20 DM (Union Carbide,

¹ Water purified by reverse osmosis and Milli-Q treatment (Millipore Corporation, Bedford, MA).

Chicago) of pores in the molecular mass range of 12,000 - 20,000 daltons (Stewart, 1977), were used. Milli-Q water was used for the preparation of SES. Exudate obtained from the cheese was analysed for calcium (complexometric method), lactose (enzymatic colorimetric), lactate (enzymatic colorimetric), NaCl (potentiometric titration), total nitrogen (Kjeldahl), total solids and pH. SES was made with composition similar to that of the exudate.

The approximate composition of Feta used for the dialysis studies was: 26.5% fat, 16.2% protein, 51.1% moisture, 4.3% NaCl, 91 mM/kg calcium and pH 4.67.

The approximate composition of exudate from 2 - 3 month old Feta cheese was:

Calcium	:	110 mM/kg
Lactose	:	50 mM/kg
Lactates	:	210 mM/kg
NaCl	:	9.0%
Total nitrogen	:	0.455%
Total solids	:	16.0%
pH	:	4.55

10.3.2 Preparation of simulated external solution

The SES was prepared using the following.

	(%)	(mM/kg)
PEG ²	: 3.4	1.7
NaCl	: 9.0	1540.0
Lactose	: 1.8	50.0
Calcium lactate	: 3.2	110.0
Lactic acid (88%)	: 1.0	97.7
Water	: 81.6	45333.0

The pH of the SES was adjusted to 4.55 by adding 50% NaOH.

10.3.3 Cheese in dialysis tubing: Dialysis tubes, each of 20 cm length, were soaked in water for 2 h and tied at one end. About 10 g of grated Feta cheese was packed into the weighed tube with the help of a funnel and a spatula. The weight of the

² Details of optimisation of the concentration of PEG in the SES has been provided later on in this Chapter.

tube with cheese was recorded. The cheese inside the tube was compacted by squeezing. The length of the filled cheese in the tube was adjusted to 5.5 cm. The open end of the tube was tied. This was put in a beaker containing 2-3 kg SES for dialysis at room temperature (20 °C). The SES was continuously stirred on a magnetic stirrer. The weights of the dialysis tubings with cheese were recorded after selected time intervals. Each dialysis tubing was wiped dry using tissue paper before weighing. Usually 5-6 dialysis tubings with cheese were put into the SES at the same time for dialysis. One dialysis tubing was drawn at each specified time-interval for weighing. After dialysis, the contents in the tubing were analysed for total solids, calcium (complexometric method) and NaCl (potentiometric titration). The details of the analytical methods are outlined in Appendix 4.1.

10.4 Results

The results are described in two parts. The first part refers to optimisation of the test method and the second part describes the influence of selected factors on exudation.

10.4.1 Optimisation of assay procedure

The objective was to optimise the composition of SES so that cheese would be close to equilibrium with the SES when dialysis commenced, except for the factor under study.

Material for dialysis: 'Feta cheese', 'exudate from Feta' and 'mixture of cheese and exudate' were dialysed in SES to determine the appropriate material for experimentation. Concentrations of NaCl in simulated external solutions were varied (10, 20 & 25%). The dialysis time taken to reach equilibrium between NaCl contents of the dialysed material and SES was about: one hour for exudate; 3-4 h for mixture of cheese and exudate; and longer (≈ 12 h) for cheese. Equilibrium was attained rather too soon with the use of exudate. The gross composition of exudate is expected to have changed with the attainment of equilibrium. In such a short time it was difficult to predict the trend in change of weight of exudate or the mixture of cheese and exudate. Cheese was therefore selected as the material to be dialysed.

Variability: The standard deviations for the percent mass transfers from cheese during dialysis ranged between 0.1 - 0.3% when measured at varying time-intervals. The pooled standard deviation was 0.2%. The reproducibility of the test method was satisfactory.

Loss of nitrogenous material through dialysis membrane: When dialysis extended beyond 24 h the cheese in the dialysis tubing lost weight irrespective of the initial concentrations of PEG, NaCl and Ca^{2+} in the SES. This can be explained as being due to gradual diffusion of lower molecular weight protein breakdown material from the cheese into the SES. PEG, used as the substitute for proteins/peptides in exudate, is of uniform high molecular weight and does not pass through the dialysis membrane. This creates an imbalance in osmotic pressure across the dialysis membrane and consequently a continuous loss of water from cheese. The gross composition of cheese is therefore affected over a long period of dialysis.

The effect of increase in time of dialysis and age of cheese on the loss of total nitrogen from cheese during dialysis was investigated (Fig. 10.1). It appears that most of the protein breakdown material of lower molecular weight that can pass through the dialysis membrane does so rapidly. Increased loss of protein breakdown material from 26 week old cheese after 2 h of dialysis may be attributed to the presence of large amounts of low molecular weight protein breakdown material. Duration of dialysis up to 4 hours for cheeses of about 9 weeks old was chosen as standard time for subsequent trials. It is assumed that the loss of low molecular protein breakdown material, being less than an equivalent of 5% of the total nitrogen in the cheese, would not significantly alter the trend of mass transfer.

Influence of temperature: The effect of temperature of dialysis on mass transfer is shown in Table 10.1. The variations in the weight of cheeses at the two temperatures are small. It is apparent that temperature would not markedly affect the trend in 'mass transfer' in 4 h of dialysis. For convenience, the temperature of 20°C was chosen as the standard for dialysis.

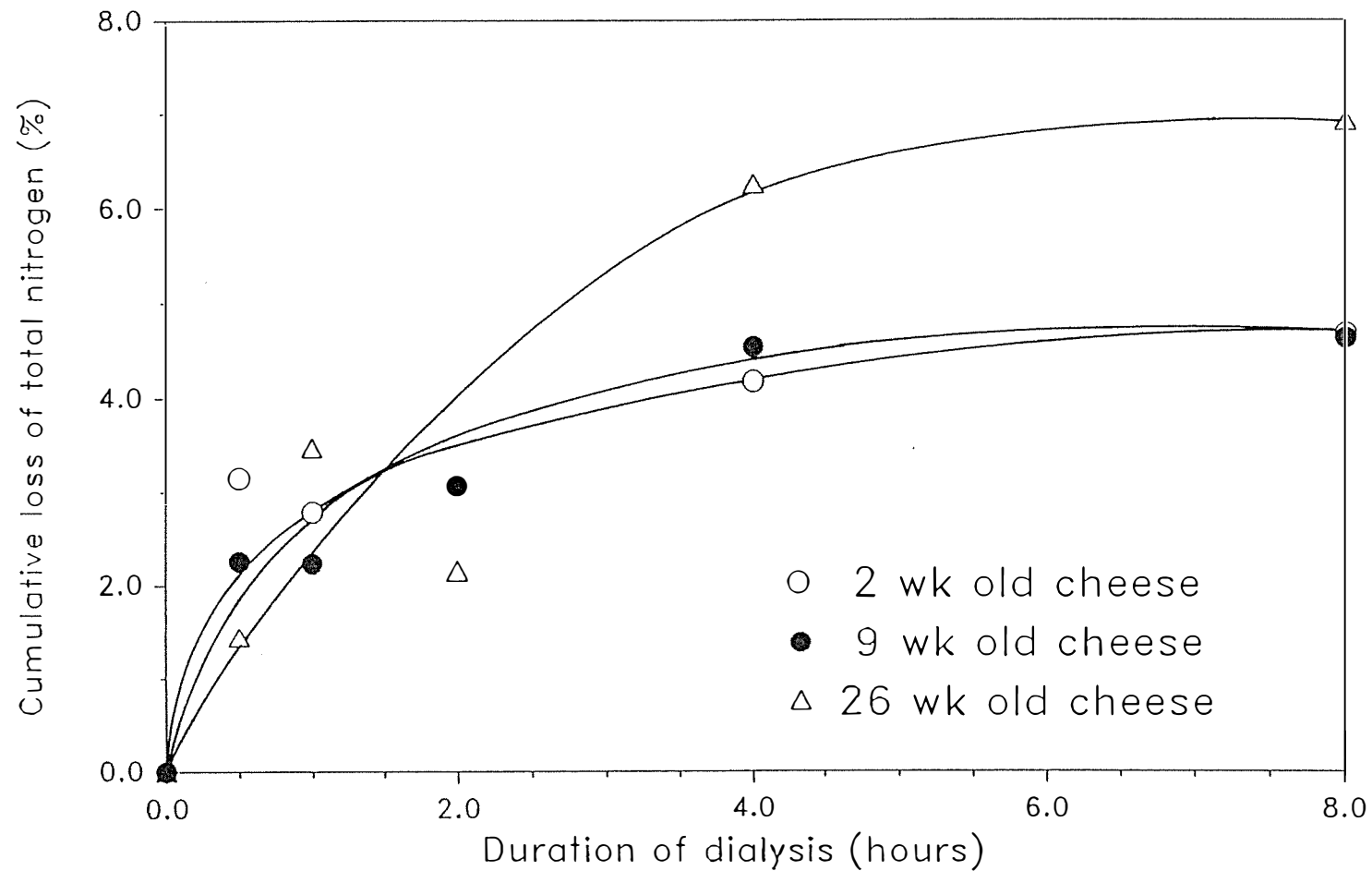


Fig. 10.1 Loss of total nitrogen from Feta cheeses of different age during dialysis.

Table 10.1 Effect of temperature on mass transfer from cheese (16 wk old) during dialysis

Dialysis time (hours)	Cumulative change in weight ^a of cheese (%)	
	Dialysis at 20°C	Dialysis at 4°C
0.5	- 0.35	- 0.52
1.0	+ 0.17	- 0.32
2.0	+ 0.36	- 0.11
4.0	+ 0.33	+ 0.23
6.0	+ 0.61	+ 0.54
7.5	+ 0.54	+ 0.54

^a + denotes gain of weight, - denotes loss of weight.

Age of the cheese: The effect of age on the mass transfer from dialysed cheese is shown in Table 10.2. Cheeses of less than 14 weeks age were not greatly affected by mass transfer during dialysis. Accordingly, about 2-3 month old cheese was considered satisfactory for conducting the experiment. Increased mass transfer from very old Feta may be attributed to the presence of large amounts of low molecular weight protein breakdown material that occurs as a result of increased proteolysis.

Table 10.2 Effect of age of cheese on the mass transfer from Feta cheese during dialysis

Duration of dialysis (h)	Cumulative change in weight ^a of cheese (%)		
	4 wk cheese	14 wk cheese	77 wk cheese
0.5	+ 0.19	+ 0.00	+ 0.64
1.0	+ 0.38	+ 0.10	+ 1.66
1.5	+ 0.28	+ 0.31	+ 1.91
2.0	0.00	+ 0.21	+ 2.42
2.5	- 0.47	+ 0.21	+ 2.93
3.5	- 1.04	+ 0.21	+ 2.93
4.5	- 1.71	- 0.31	+ 2.93
6.5	- 2.37	- 1.03	+ 2.81

^a + denotes gain of weight, - denotes loss of weight.

10.4.2 Influence of selected factors on mass transfer

Influence of proteolysis: Proteolysis causes an increase in the amount of low molecular weight protein breakdown material in cheese. The effect of this material

on the mass transfer was determined by dialysing 3 month old Feta cheese (and exudate) in exudate from 18 month old Feta cheese (Table 10.3). The exudate was used as the external solution to reduce the experimental error involved in using SES. Persistent loss of weight from both exudate and cheese was detected. The loss of weight may be attributed to the increased osmotic pressure exerted by the large amount of low molecular weight protein breakdown material in exudate (external solution).

Table 10.3 Effect of low molecular weight protein breakdown material on mass transfer from cheese and exudate: estimated by dialysing cheese^a (3 mo) and exudate^b (3 mo) in exudate^c from older Feta cheese (18 mo).

Duration of dialysis (h)	Cumulative change in weight ^d of cheese in dialysis tubing (%)	Cumulative change in weight ^d of exudate in dialysis tubing (%)
0.5	- 0.21	- 1.59
1.0	- 0.42	- 3.49
2.0	- 0.95	- 6.03
3.0	- 1.37	- 7.93
18.0	- 3.89	- 14.6
26.0	- 4.84	- 14.5

^a Composition of cheese: 5.8% NaCl, 85.7 mM/kg Ca²⁺, 50.8% moisture;

^b Composition of exudate: 9.5% NaCl, 117.5 mM/kg Ca²⁺, 18.3% TS;

^c Composition of old exudate: 6.67 % NaCl, 147 mM/kg Ca²⁺, 18.2% TS;

^d + denotes gain of weight, - denotes loss of weight.

Influence of protein breakdown material: Cheese dialysed in SES without PEG always recorded a gain in weight. This was probably due to the higher osmotic pressure exerted by the protein breakdown material in the cheese. High molecular weight PEG was therefore used in SES as a substitute for protein and protein breakdown products. Effect of variation in the amount of PEG in SES on the mass transfer from cheese are shown in Table 10.4. The standard amount of PEG used in SES was chosen as 3.4%.

Table 10.4 Effect of variation in the concentration of PEG in SES on the mass transfer from Feta cheese (14 wk old) during dialysis

Dialysis time (h)	Cumulative change in weight ^a of cheese (%)				
	0 % PEG	3.3% PEG	3.6% PEG	3.9% PEG	6.0%PEG
0.5	+ 2.26	0.00	- 0.57	- 0.37	- 0.01
1.0	+ 3.34	+ 0.10	- 0.38	- 0.73	- 0.40
1.5	n.d.	+ 0.31	- 0.28	- 0.85	n.d.
2.0	+ 4.71	+ 0.21	- 0.28	- 1.10	- 0.96
2.5	n.d.	+ 0.21	- 0.19	- 1.10	n.d.
3.5	+ 6.08	+ 0.21	- 0.28	- 1.58	- 2.08
4.5	n.d.	- 0.31	- 0.57	- 2.20	n.d.
6.5	n.d.	- 1.03	- 0.57	- 2.56	n.d.

^a + denotes gain of weight, - denotes loss of weight; n.d. denotes not determined.

Influence of NaCl: The effect of NaCl concentration in SES on the mass transfer from cheese is shown in Table 10.5. An increase in weight of cheese was detected with reduced NaCl concentration in SES and vice versa.

Table 10.5 Effect of variation in the NaCl content in SES on the mass transfer from cheese (12 wk old) during dialysis

Duration of dialysis (h)	Cumulative change in weight ^a of cheese (%)		
	0% NaCl	9.0% NaCl	20% NaCl
0.5	+ 2.51	+ 0.23	- 1.96
1.0	+ 3.93	+ 0.57	- 3.08
1.5	+ 5.24	+ 1.37	- 3.74
2.0	+ 6.33	+ 1.48	- 4.58
3.0	+ 7.64	+ 1.94	- 5.33
6.0	+ 10.26	+ 3.08	- 6.54
7.0	+ 10.70	+ 3.31	- 6.73

^a + denotes gain of weight, - denotes loss of weight.

Influence of pH: The effect of variation in the pH of SES on the mass transfer from cheese during dialysis is shown in Table 10.6. The limiting values of pH, 4.3 & 4.8, were chosen based on the extreme values of pH expected during storage of Feta cheese. Even though there is a trend in the pattern of mass transfer with respect to pH, the changes in weight are too small to be of practical significance.

Table 10.6 Effect of variation in pH of SES^a on the mass transfer from cheese (15 wk old) during dialysis

Dialysis time (h)	Cumulative change in weight ^b of cheese ^a (%)		
	Low pH (4.3)	Control (4.54)	High pH (4.8)
0.5	- 0.89	- 0.17	- 0.13
1.0	- 0.40	+ 0.17	+ 0.12
2.0	+ 0.05	+ 0.36	+ 0.39
4.0	+ 0.14	+ 0.33	+ 0.70
6.0	+ 0.54	+ 0.61	+ 0.80
7.5	+ 0.63	+ 0.54	+ 1.08

^a pH of cheeses and the respective simulated external solutions had not changed after dialysis; ^b + denotes gain of weight, - denotes loss of weight.

In the above trial the pH of the cheese did not change during dialysis. It is possible that the apparent differences in mass transfer were not related to pH. Therefore, another trial was carried out where the pH of cheeses was changed before dialysis. This was done by adding the same volume of lactic acid or 50% NaOH or water to the cheese to produce low pH, high pH and control cheeses, respectively. The pH of SES was kept constant. Dialysis of these cheeses in SES also did not show any specific trend or substantial mass transfer (Table 10.7). It was therefore concluded that a variation of pH within the normal pH range of Feta cheese had little influence on the mass transfer.

Table 10.7 Effect of variation in pH of cheeses^a on the mass transfer from cheeses (7 wk old) during dialysis in SES of constant pH

Dialysis time (h)	Cumulative change in weight ^b of cheese ^a (%)		
	Low pH (4.15)	Control (4.45)	High pH (4.68)
0.5	- 0.40	- 0.49	- 0.16
1.0	- 0.62	- 0.64	- 0.67
2.0	- 1.02	- 1.27	- 0.95
3.0	- 1.20	- 1.86	- 1.12
4.0	- 1.45	- 1.96	- 1.22

^a pH of cheeses and the respective SES had not changed after dialysis. Loss of weight in all cheeses was probably due to use of younger cheese (7 weeks) and addition of some water for pH adjustment;

^b + denotes gain of weight, - denotes loss of weight.

In both the above trials the pH of simulated external solutions did not change due to dialysis. It was therefore not necessary to buffer the SES.

Effect of calcium: The effect of calcium concentration on mass transfer from cheese was studied by changing the calcium levels in the SES (Table 10.8). The levels (80 mM/kg for low, 110 mM/kg for control, 140 mM/kg for high) were chosen on the basis of maximum variation expected in normal Feta cheese. The mass transfer from cheese due to variation of calcium in SES was small. The cheese gained weight with the low level of calcium in SES and vice-versa. It is however uncertain whether the weight change was due to the calcium concentration or due to differences in the osmotic pressure. The calcium contents in the cheeses after dialysis were 82, 96 and 110 mM/kg for low, control and high levels, respectively. To further investigate the effect, the calcium contents of cheeses were altered to different levels (98, 118 & 140 mM/kg) by addition of calculated amounts of 3 M CaCl_2 solution or water, and the modified cheeses were dialysed in SES of constant calcium (Table 10.9). The magnitude of mass transfers were again small. A trend opposite to that observed earlier was detected. It is worth noting here that in all cases there was a loss of weight. The loss of weight in control cheese was apparently due to experimental error in standardising a suitable composition for SES. Once again the changes could have occurred due to osmotic pressure differences. As the weight changes in both instances were small, it was concluded that calcium concentration of cheese did not have a noticeable effect on the mass transfer from cheese during dialysis.

Table 10.8 Effect of variation in calcium of SES^a on the mass transfer from cheese (16 wk old) during dialysis

Dialysis time (h)	Cumulative change in weight ^b of cheese ^a (%)		
	Low Ca^{2+} SES	Control SES	High Ca^{2+} SES
0.5	+ 0.54	+ 0.58	- 0.22
1.0	+ 0.32	+ 0.54	- 0.32
2.0	+ 0.77	+ 0.45	- 0.41
4.0	+ 1.25	+ 0.38	- 0.22
8.0	+ 1.86	+ 0.92	+ 0.20

^a Calcium of cheeses and the respective SES had not equilibrated after dialysis;

^b + denotes gain of weight, - denotes loss of weight;

SES = Simulated external solution.

Table 10.9 Effect of variation in calcium of cheese on the mass transfer from cheeses (7 wk old) during dialysis in SES of constant calcium

Dialysis time (h)	Cumulative change in weight ^a of cheese (%)		
	Ca ²⁺ in cheese = 98 mM/kg	Ca ²⁺ in cheese = 118 mM/kg	Ca ²⁺ in cheese = 140 mM/kg
0.5	- 0.49	- 0.44	- 0.28
1.0	- 0.64	- 0.88	- 0.51
2.0	- 1.27	- 1.37	- 0.95
3.0	- 1.86	- 1.67	- 1.56
4.0	- 1.96	- 2.64	- 1.56

^a + denotes gain of weight, - denotes loss of weight.

10.5 Discussion

The study was aimed to develop a suitable test method and then to use the method as a model for investigating the effect of selected factors on exudation. However, the test method that was developed did not prove to be very accurate. Contrary to earlier expectations, the protein/peptides in exudate made a significant contribution to the mass transfers. PEG could substitute only for the large molecular weight protein breakdown material. Ideally a membrane impervious to all material except water was required. The dialysis tubing could not fulfil this. Rapid loss of low molecular weight protein breakdown material from cheese during dialysis increased the experimental error. The loss of nitrogenous material in the first four hours of dialysis represented an equivalent of $\approx 0.6\%$ of the total weight of cheese. While studying the effects of selected factors on exudation, instances where the loss of weight is around this figure, the mass transfer could not solely be attributed to the experimental variation. Nevertheless, the study provided some very useful information. The results of this investigation are to be treated only as an indication of the trend.

Cheese lost weight when dialysed with exudate from older Feta. Thus a mass transfer took place from the region of high molecular weight protein breakdown material towards the region of low molecular weight protein breakdown material. It is proposed that a similar process goes on within cheese during storage resulting

in increase in the amount of exudate. Moisture in Feta cheese may be grouped into three types: moisture bound to the protein; moisture held in the capillaries of the protein gel; and the moisture in the form of exudate or the moisture that is loosely associated to the proteins, such as in crevices, voids and surface of the cheese. These moistures co-exist in a state of dynamic equilibrium. The moisture that remains bound to the protein is not available for exudation. However, with the hydrolysis of protein, some of the bound water may become available for exudation. As proteolysis progresses, the protein breakdown material become soluble, and together with the water bound to the ionic groups of peptide bonds, form part of the exudate (Chapter 9). Some moisture is released from part of the protein matrix (casein gel structure) that weakens or disintegrates with proteolysis. Thus, the amounts of protein breakdown material increase in the exudate as a consequence of proteolysis. This creates an unequal distribution of protein breakdown material between the exudate and moisture held in the intact part of protein matrix. The exudate which has a larger amount of low molecular weight protein breakdown material draws out moisture from the protein matrix to attain equilibrium. This process is similar to that observed during dialysis of young cheese in older exudate.

Another important implication from the findings of dialysis of younger cheese in older exudate is that the exudates are unlikely to be reabsorbed back into the intact protein matrix because of the higher osmotic pressure exerted by the low molecular weight protein breakdown material. Thus once exudation commences, it may be impossible to stop or reverse the process.

An increase in NaCl content in SES during dialysis produced considerable loss of mass from cheese in dialysis tubing, and vice versa. A similar situation occurs in cheese during brining when the brine is about 20% NaCl and the cheese has no NaCl. After brining, the outer layers of cheese would be expected to have higher percentages of NaCl while the inner layers may have very little NaCl. This difference in NaCl concentration would persist until equilibrium in NaCl distribution is attained throughout the cheese. There would thus be a difference of osmotic pressure throughout the cheese. A process similar to that of mass

transfer with the dialysis trial would occur: the moisture from the inner layers of cheese would flow out to the outer layers, and come out as exudate because the moisture is not reabsorbed by the outer layers (Chapter 11). It follows from the effect of NaCl concentration that cheeses dry salted on the surface would lose an increased amount of exudate due to a higher NaCl concentration gradient.

Proteolysis would be faster in the inner layers of cheese due to a reduced NaCl concentration. This will further increase the amount of exudate. A combined effect of increased proteolysis and salt gradient appear to provide the ideal conditions for the occurrence of exudation.

Exudation does not seem to be appreciably affected by the calcium content in cheese. A decrease in pH of cheese (4.8 to 4.3) during storage did not have any discernible effect on the exudation. The effect of residual lactose in reducing cheese pH may therefore have a minor influence on exudation. The lowering of cheese pH due to the residual lactose may however influence proteolysis and indirectly affect exudation.

10.6 Summary and conclusion

A test method was devised, based on the principles of osmosis and diffusion, to study the effect of selected factors on the movement of exudate. Feta cheese was dialysed in selected non-equilibrium solutions. The change in weight of the cheese during dialysis was used as an estimate of the effect of a variable on exudation. The test method provided only an indication of the trends in exudation as experimental errors were expected to be high.

It is concluded that exudation is influenced by the gradient in the salt concentration and the protein breakdown material. The effects of change in pH and calcium in cheese on exudation were minor.

CHAPTER 11

SALT DIFFUSION IN FETA CHEESE AND ITS EFFECT ON EXUDATION

11.1 Introduction

The uptake of NaCl and the concurrent loss of moisture during brining of cheese is an impeded mutual diffusion process (Geurts *et al.*, 1974b). Osmotic equilibrium is reported to play a significant role in transport of water (Guinee & Fox, 1986a), particularly in brine-stored Feta-type cheeses (Mansour & Alais, 1972).

In the case of brine salted cheeses, such as Feta, the exterior layers of cheese initially have a high NaCl content and low moisture. It is suggested that during storage diffusion of NaCl from the region of higher concentration (outer layers of Feta) to the region of lower concentration (inner layers of Feta) is accompanied by displacement of substantial amount of moisture in the opposite direction (Chapter 10). The moisture displaced from the inner layers would be retained in the cheese if the displaced moisture is absorbed in the protein matrix at the outer layers due to the fall in NaCl concentration. However, if the moisture is not reabsorbed it would exude from the cheese, along with some soluble material, as exudate.

The amount of exudate is expected to be related to the concentration gradients of NaCl and water, existing within the block of cheese at the time of packing. It is of interest to know the time taken for the equilibration of the concentration gradients of NaCl and water in the cheese. The scope of this investigation was to determine:

- the gradient in concentration of NaCl within a block of Feta cheese after brining,
- the time taken until substantial equilibrium of NaCl concentration in the entire block of cheese is attained, and
- the pattern of moisture distribution in different layers of cheese until equilibrium of salt-in-moisture (S/M) concentration was attained.

11.2 Experimental

Feta cheese was manufactured using the method outlined in Chapter 6 (Fig 6.1 and Appendix 6.2). Blocks of Feta cheese were drawn for analysis at selected storage intervals. The approximate dimensions of a typical block of cheese were 10 X 10 X 5 cm. A slice of 2.5 cm thickness was cut from each of the four 5 X 10 cm faces of the block leaving a 5 cm long cube of cheese (Fig. 11.1). Using a special cutting device the cubic middle portion of the cheese was sliced horizontally into 10 equal parts. The pairs of corresponding layers from the top and bottom of the cube were combined and mixed. Thus the central portion of the cheese was subdivided into 5 portions. These layers have been referred as outer to inner layers in cheese. Each of these five portions weighed about 30 g. These five portions, the initial cut-out portions (four sides) and the exudate from Feta were weighed; and the cheese portions separately grated. The cheese portions and the exudate were analysed for pH, moisture and NaCl (potentiometric titration). Details of analytical methods are provided in Chapter 4 and Appendix 4.1. The NaCl and moisture of the entire block of cheese was calculated from the known weights, NaCl and moisture contents of these portions. All the analyses were carried out with at least two lots of cheese.

11.3 Results & discussion

Table 11.1 shows a typical example of NaCl and moisture distribution in various layers of Feta cheese at selected periods of storage. Fig. 11.2, Fig. 11.3 and Fig. 11.4 show the distribution of NaCl, moisture and salt-in-moisture, respectively, in different layers of cheese.

The pH values of different layers of Feta cheese after brining were similar. The cut-out portions were the segments with highest NaCl and S/M concentration following brining. As expected, the S/M and NaCl concentrations were higher at the outer layers and decreased gradually towards the inner layer. These concentration gradients decreased with time. The outer layers were always the region of lowest moisture content. The moisture content of cheese increased towards the inner layers. The moisture content of cut-out portions was mostly close to the average moisture in cheese. The initial gradient in moisture concentration of cheese also decreased with time. During storage, the moisture content in all the layers either decreased or remained steady, but never showed an increase.

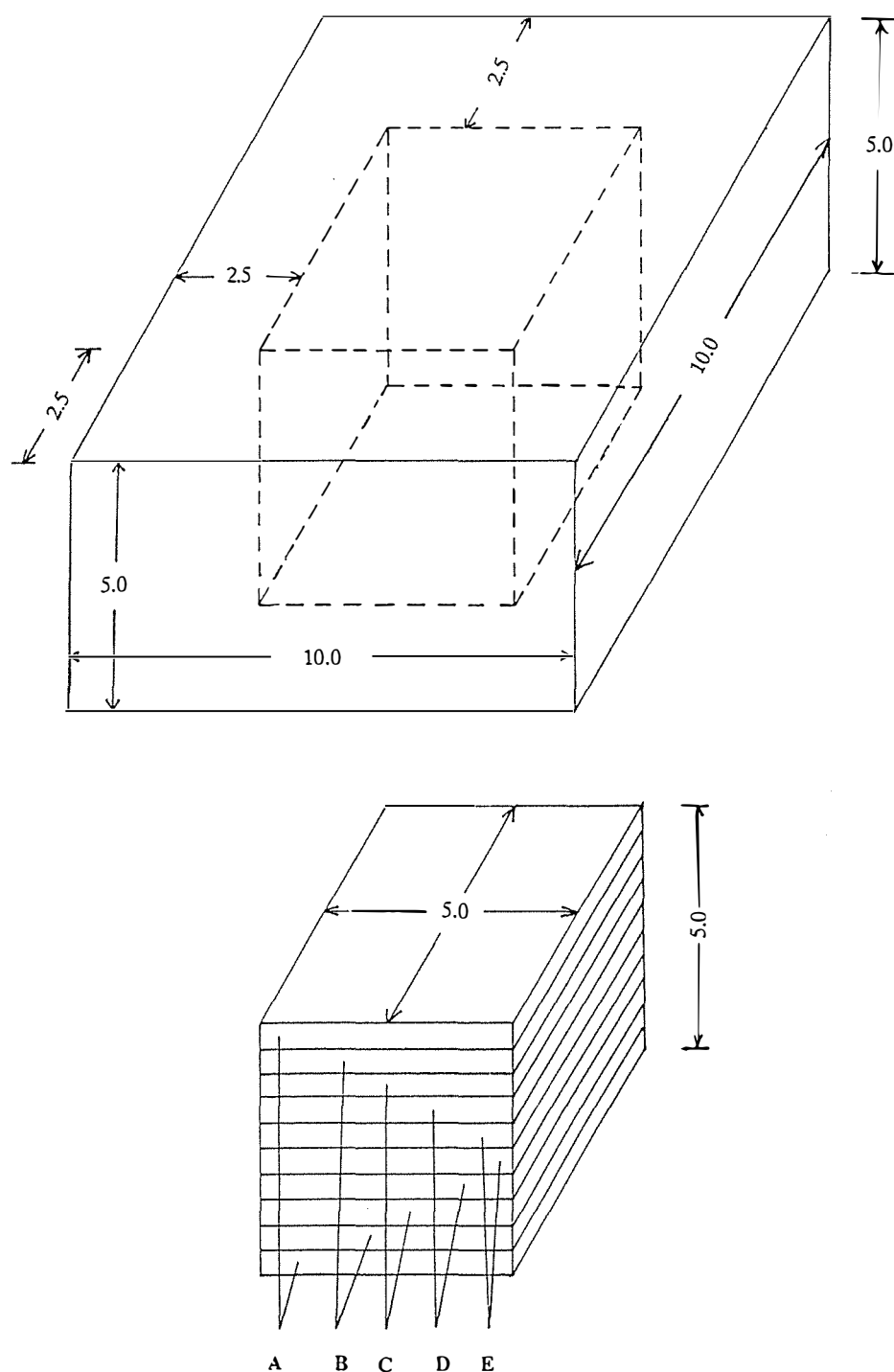


Fig. 11.1 Cutting a block of Feta cheese into different layers. All the measurements are approximate estimates and are reported in cm.

Top: The continuous lines represent the outline of the block of Feta cheese (10 X 10 X 5 cm). A slice of 2.5 cm thickness was cut from each of the four 5 X 10 cm faces of the block leaving a 5 cm long cube of cheese. The dotted lines show the outline of the middle portion of the block of Feta.

Bottom: Middle portion of the block of Feta showing the sliced layers. The pairs of corresponding layers from the top and bottom of the cube were combined and mixed. A: Outer layer; B: Second layer from outside; C: Third layer from outside; D: Fourth layer from outside; E: Inner layer.

Table 11.1 NaCl and moisture distribution in various layers of Feta cheese at selected periods of storage

Layer	After brining ¹			One week old		
	NaCl	Moisture	S/M	NaCl	Moisture	S/M
	%	%	%	%	%	%
1st (outer)	3.27	50.06	6.53	3.85	48.03	8.02
2nd	2.11	53.87	3.92	3.94	51.44	7.66
3rd	0.85	55.48	1.53	3.86	51.88	7.44
4th	0.38	56.20	0.68	3.77	51.34	7.34
5th (Inner)	0.19	56.81	0.33	3.67	51.51	7.12
Cut-outs ²	4.65	51.67	9.00	4.31	51.20	8.42
Overall ³	3.71	52.52	7.06	3.86	51.99	7.42
Exudate	n.a.	n.a.	n.a.	7.52	86.17	8.73
Layer	Two weeks old			Three weeks old		
	NaCl	Moisture	S/M	NaCl	Moisture	S/M
	%	%	%	%	%	%
1st (Outer)	3.48	47.75	7.29	3.42	46.97	7.28
2nd	3.52	49.97	7.04	3.57	49.57	7.20
3rd	3.56	50.46	7.06	3.58	50.10	7.15
4th	3.59	50.59	7.10	3.62	49.98	7.24
5th (Inner)	3.58	50.50	7.09	3.59	50.97	7.04
Cut-outs ²	3.73	50.60	7.37	3.60	50.22	7.17
Overall ³	3.75	51.25	7.32	3.67	50.94	7.20
Exudate	6.27	84.92	7.38	6.13	85.57	7.16

¹ The pH of the portions from outer to inner layers of cheese were 4.69, 4.69, 4.71, 4.72 and 4.71, respectively;

² 'Cut-outs' refers to the vertical segments, each of 2.5 cm thickness, cut from the cross-section(4 sides) of the cheese;

³ 'Over-all' refers to estimates of weighted average of all the portions of cheese and exudate; n.a. = not applicable.

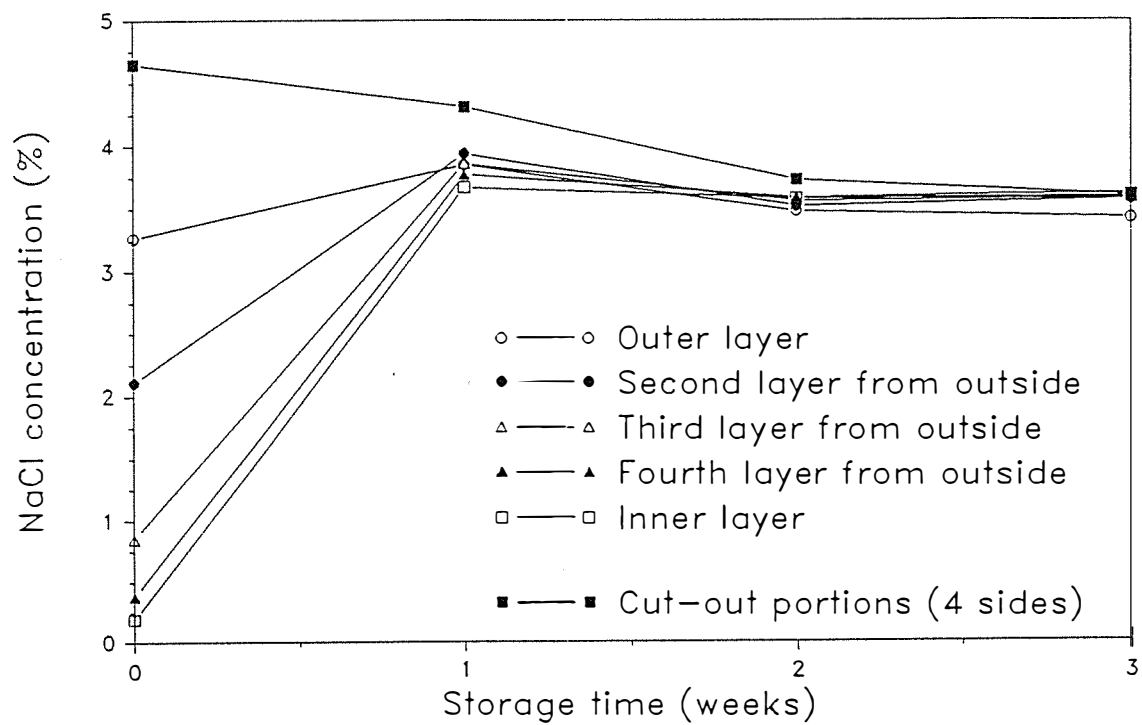


Fig. 11.2 Pattern of NaCl distribution in various layers of Feta cheese during storage.

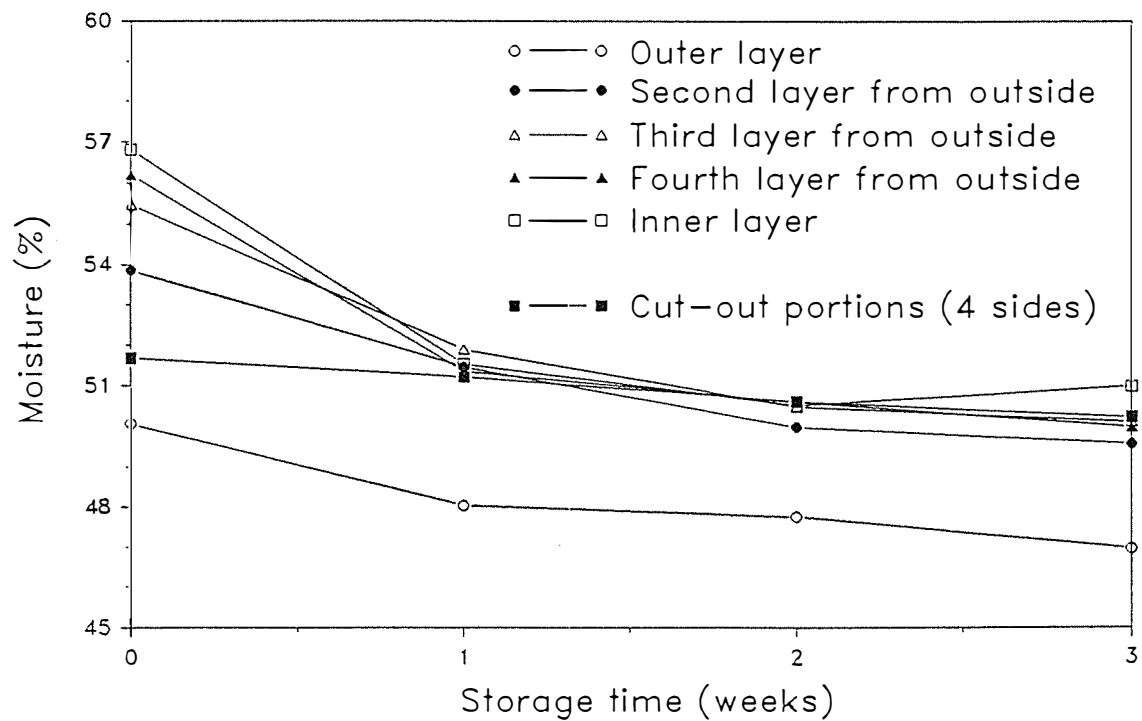


Fig. 11.3 Pattern of moisture distribution in various layers of Feta cheese during storage.

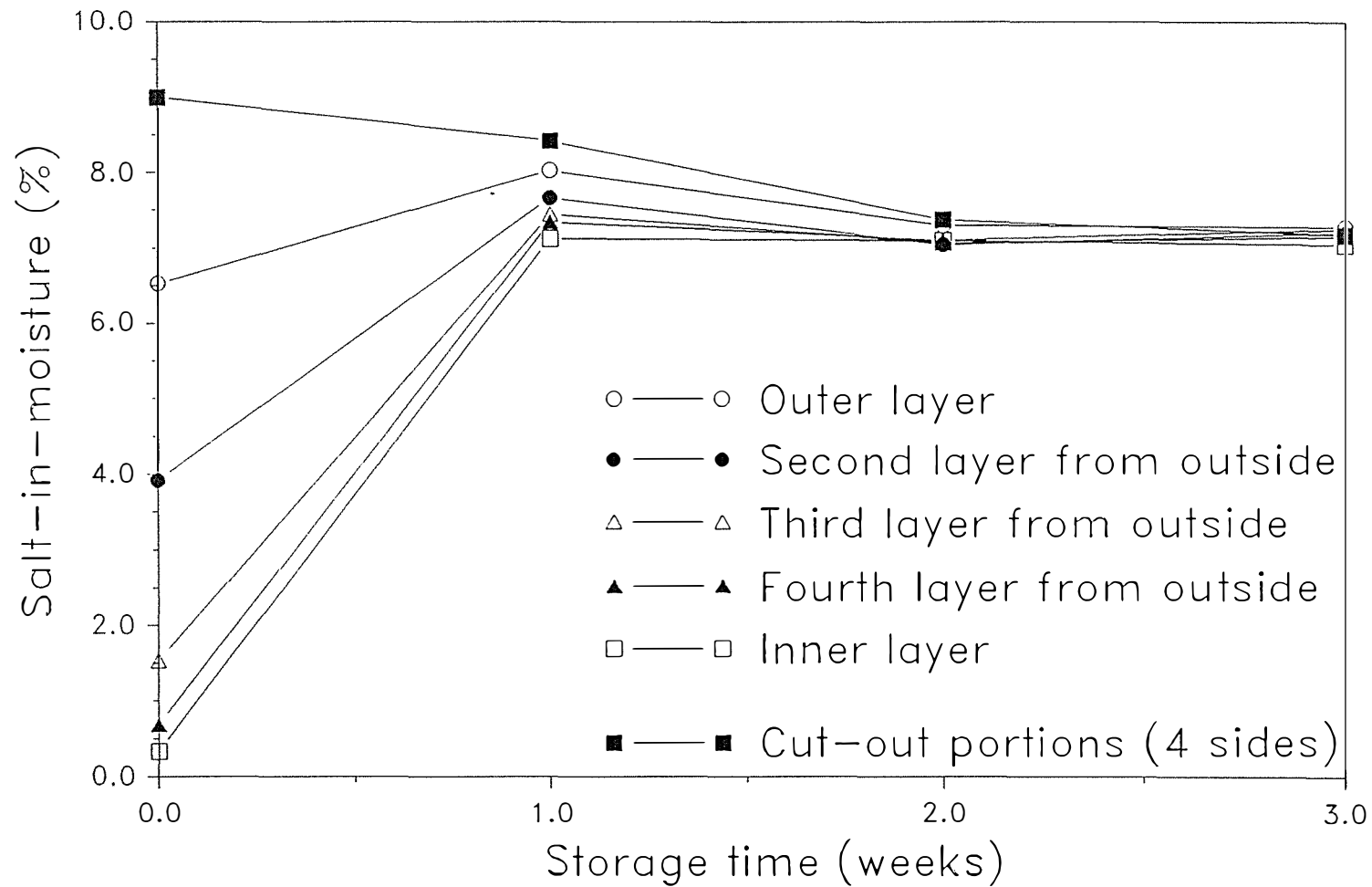


Fig. 11.4 Pattern of distribution of salt-in-moisture in various layers of Feta cheese during storage.

Substantial equilibrium of NaCl distribution was attained in about two weeks after brining. By the third or fourth week equilibration of NaCl was nearly total in the whole of the cheese and the exudate. It follows from this that the concentration gradient of NaCl in cheese can affect exudation only in the first few weeks after brining, *i.e.* until such time the concentration gradient exists. As exudation continues beyond this period it is evident that, in addition to NaCl concentration gradient, exudation is influenced by other factors.

The time taken for attaining NaCl concentration equilibration was less than the 40 days stated by Georgakis (1973). This may be attributed to the size and shape of the cheese block, diffusion coefficient and method of salting (dry salting on surface/method of brine-storing). By influencing NaCl diffusion in cheese these factors are expected to indirectly affect the exudation.

With an increase in storage time there was an increase in the uptake of NaCl by the inner layers of cheese from the outer layers. This was simultaneously accompanied by loss of moisture from the inner layers of cheese. The loss of moisture, however, does not appear to have been matched by an uptake of moisture in the outer layers. In fact, none of the layers of cheese showed an increase in the moisture content at any stage of storage (Fig. 11.2). This shows that the protein gel in Feta cheese is not in a position to take up moisture, once it is affected by high NaCl concentration. The moisture displaced from the inner layers of cheese as a consequence of NaCl diffusion is released as the exudate.

The inability of the protein gel to take up moisture may be explained as follows: Cheese probably contains more moisture than the equilibrium amount but is slow to lose this excess. High NaCl brings about a loss of the excess moisture which is not taken up when the NaCl concentration falls. Equilibrium is a dynamic state rather than one of very slow or no change. The test for equilibrium is to perturb the system and see if it returns to its original state when the perturbation is removed. If it does not return then it is not in equilibrium. In the present case the perturbation is caused by high NaCl.

Factors that are likely to be associated with the failure of the protein gel in cheese to take up moisture are ongoing proteolysis, solubilisation of part of casein by NaCl (McDowall & Whelan, 1938), reduction in the water-holding capacity of proteins due to interaction of paracasein with NaCl (Hardy & Steinberg, 1984), and closeness of the pH of cheese to the iso-electric point of casein (Ruegg & Blanc, 1976). Another possible factor could be the effect of NaCl *per se* on the water-binding ability of proteins, via its effect on electrostatic interactions (Kinsella & Fox, 1987). Monovalent electrolytes at around 2 M decrease bound water because at this concentration ions compete with protein groups for water, suppress the electrical double layer surrounding the macromolecules, change protein conformation and thereby diminish protein hydration. The average concentration of NaCl in the moisture content of Feta cheese is around 1.5 M. This concentration is even greater at the outer layers of cheese following brining. The high NaCl concentration could have reduced the water-binding ability of caseins.

The gradients of NaCl and moisture concentration within a block of cheese would affect microbial activity and hydrolysis of casein. It is therefore reasonable to expect a variation in proteolysis at different layers of cheese until NaCl equilibration is achieved. The proteolysis pattern for whole Feta cheese showed considerable proteolysis of α_{s1} -casein after brining, rapid proteolysis by the end of four weeks, and a rather slow and steady proteolysis thereafter (Chapter 6). One of the factors contributing to this typical proteolytic trend could be the gradient in NaCl concentration in different layers of Feta cheese in the first few weeks.

11.4 Conclusion

The pH values of various layers of Feta cheese after brining are similar. Initially there is less NaCl but more water in the interior of cheese. The NaCl and water concentration gradients tend to even out rather quickly, *i.e.* in about two weeks after brining of cheese. Exudation is expected to be influenced by this NaCl concentration gradient (Chapter 10). Moisture released from the interior of the cheese as a result of NaCl diffusion is not taken up by the outer layers even though the concentration of NaCl decreases. This moisture along with the solubles are released as exudate.

CHAPTER 12

OVERALL DISCUSSION

Exudation of whey from Cream cheese was affected by the method of manufacture (Chapter 5). Cream cheese showed no signs of proteolysis during storage. In the case of Feta cheese manufacturing variables, except for homogenisation, did not have a pronounced effect on exudation (Chapter 6). Proteolysis in the ripening Feta cheese was the predominant factor affecting exudation (Chapter 9). Cream cheese made by the hot-pack method, and Feta cheese made from recombined milk by the traditional method, proved to be suitable examples for study of exudation of unripened and ripened varieties, respectively.

An overview of the factors affecting exudation and their possible causes is described below:

Protein to fat ratio in the cheesemilk: Variation of the protein to fat ratio in cheesemilk had the desired effect on the composition of Cream and Feta cheeses.

The effect of protein to fat ratio on exudation varied depending on whether or not the cheese was ripened. For Cream cheese an increase in the protein to fat ratio resulted in a decrease in the amount of exudate. For Feta cheese the effect was the opposite. This difference could be explained by the effect of proteolysis on the water-holding ability of the protein matrix. In Cream cheese there is no proteolysis and the water-holding ability of the protein gel is not greatly reduced during storage. The effect of proteolysis on exudation from Feta cheese is described later in this Chapter.

Effect of fat content: Within the selected limits of variation of P/F ratio, fat content did not affect exudation from Cream cheese. However, an increase in the fat content reduced the amount of exudate from Feta cheese of comparable moisture in non-fat substance (MNFS). In Feta cheese fat appears to have mechanically hindered the flow of exudate.

Effect of MNFS in cheese: The amount of exudate increased with an increase in MNFS in both the types of cheese. This was in agreement with the proposed hypothesis that, for a given set of conditions, the non-fat substance in cheese has the ability to hold a certain maximum amount of moisture, and that a variation in the moisture in the non-fat substance (MNFS) above this limit would result in a proportional variation of the available moisture for exudation.

Homogenisation: Cream cheese made from cheesemilk homogenised at a higher pressure showed a decrease in the amount of exudate (Chapter 5). This reduction is attributed to the increase in the fat globule surface area and coating of the fat globules with casein.

In Feta cheese homogenisation of cream was effective in decreasing syneresis of whey during manufacture and subsequent exudation during storage (Chapter 8). Further, a variation in the 'homogenisation pressure' had little or no effect on exudation (Chapter 6). This suggests that the effect of homogenisation on reducing the amount of exudate is more due to change in the surface coat of the fat globules than to the size reduction. The effect of homogenisation in reducing the size and increasing the number of fat globules, on exudation, needs to be further examined. The type of material adsorbed to the fat globule surface had a significant influence on exudation from cheese and syneresis of whey during cheese manufacture (Chapter 8). The mechanism by which the material adsorbed to the fat globule affects the exudation and syneresis needs further study. While experimental evidence is lacking, it is suggested that the material adsorbed to fat globule surface may also affect the diffusion of NaCl in cheese during brining. Standardisation of a method for quantitative measurement of protein adsorbed to the fat globule surface would be useful.

Handling of Feta cheese (Chapter 6): Feta cheese subjected to thermal shocks during storage showed an increase in the amount of exudate. However, turning and piling of Feta cheese blocks did not have a significant effect on exudation. Vacuum packaging of cheese reduced the extent of exudation considerably. It is suggested

that the moisture is held by the capillary forces in the protein network. Vacuum packaging seals the ends of the capillaries and impedes exudation.

pH and mineral balance: Exudation is common in cheeses that have a pH close to the iso-electric point of casein. This may be attributed to the micellar structure of casein being affected by the low pH (Rose, 1968), and the minimal water sorption of casein in the pH range from 4.0 to 5.0 (Ruegg & Blanc, 1976; Kinsella & Fox, 1987). As expected, the amount of exudate increased with the lowering of pH of Cream cheese (Chapter 5). In Feta cheese, a noticeable change in amount of exudate was not detected over the normal pH range of 4.8 to 4.3 (Chapter 10). Residual lactose, which causes a lowering of cheese pH during storage by conversion to lactic acid, was therefore considered not to be significantly contributing to exudation. The effect of variation of calcium content, usually related to the pH changes, on the exudation of whey from Feta cheese was small (Chapter 10). It is likely that the colloidal calcium phosphate is completely solubilised at the low pH of ≈ 4.6 (Pyne & McGann, 1960) and not associated with the casein in cheese, and that the micellar casein structure is affected because of the absence of colloidal calcium (Rose, 1968). Calcium therefore does not affect the process of exudation.

Whey proteins: Partial heat-denaturation of whey protein through heat treatments such as pasteurisation of cheesemilk, cooking of curd and heat-processing of curd proved to be an effective means of controlling exudation in Cream cheese (Chapter 5). This effect is attributed to complex formation between β -lactoglobulin and κ -casein that prevents fusion of casein micelles and consequently exudation, a process similar to that occurring in yoghurt (Brooker, 1987). In Feta cheese, incorporation of heat-denatured whey protein did not affect exudation and resulted in a substantial increase of cheese yield (Chapter 7). Presence of heat-denatured whey protein did not affect proteolysis. It is suggested that incorporation of heat-denatured whey proteins may prove effective in retarding the extent of exudation in other cheeses, such as Cheshire, where exudation is less severe. Heat-denaturation of whey protein in cheesemilk has the potential for reducing the extent of exudation in cheese and needs to be investigated further.

NaCl in cheese: Salts, via their effects on electrostatic interactions, markedly affect water-binding by protein. At low concentrations of NaCl (0.1 - 0.15 M) the amount of water bound to protein increases (Kinsella & Fox, 1987). However, at NaCl concentrations of around 2 M and water activity in the range 0.75 - 0.95, the water bound to protein decreases (Hardy & Steinsberg, 1984). At this concentration ions compete with protein groups for water, suppress the electrical double layer surrounding the macromolecule, change protein conformation and thereby diminish protein hydration (Kinsella & Fox, 1987). The concentration of NaCl in the moisture content of Feta cheese is about 1.5 M ($\approx$ 9.0% S/M). This concentration is even higher in the outer layers of a cheese block following brining. It is possible that high NaCl concentration in Feta cheese may be an inherent cause of exudation. This aspect needs to be investigated.

NaCl gradient in Feta cheese (Chapter 11): Concentration gradients of NaCl and moisture exist between the outer and inner layers of Feta cheese following brining. These concentrations equilibrate over the next 2-3 weeks with NaCl from the exterior diffusing inwards and moisture from the centre migrating outwards. Although the concentration of NaCl decreases in the outer layers during this equilibrium, the curd is unable to re-absorb the moisture diffusing from the centre. This moisture is consequently lost from the surface as exudate. This suggests that the level of moisture present in Feta cheese after brining is higher than what the protein matrix can hold and that the concentration gradients of NaCl and moisture help in the release of the excess moisture.

Proteolysis (Chapter 9): A variation in proteolytic rate was brought about by varying residual rennet levels in the Feta cheese. An increase in the rate of exudation was concomitant with an increase in the rate of proteolysis. Residual rennet in cheese can therefore influence exudation. The effect of proteolysis on exudation is probably due to disintegration of the casein network in cheese and the release of free moisture held in the capillaries.

Proteolysis appears to be one of the primary factors influencing exudation. The option of controlling proteolysis in cheese to reduce the extent of exudation is

however limited by the need to have some proteolysis for desired flavour and texture. Coagulants and starter microorganisms influence proteolysis and may therefore be expected to influence exudation. This aspect needs further examination.

Storage time and temperature (Chapters 5 & 6): The amount of exudate increased with an increase in storage time and storage temperature for both the types of cheese. The effect may be partly due to the thermodynamic effect of a decrease in water sorption of milk proteins with increasing temperatures (Kinsella & Fox, 1987). Increase in the amount of exudate from Feta cheese with an increase in storage time and storage temperature could be explained by the initial NaCl concentration gradient and the ongoing proteolysis.

Exudation could not be correlated to the changes in water activity and the amount of unfreezable water in Feta cheese during storage. The water activity depended on the salt-in-water concentration in the cheese. Differential Scanning Calorimetry studies showed that the amount of unfreezable water in Feta cheese was markedly higher than in exudate. This difference was apparently due to a large proportion of the water being bound to the casein gels in cheese.

The results of this investigation are consistent with the following hypothesis:

"The protein in cheese binds the moisture. Moisture is held in the three-dimensional casein network of the cheese by physical entrapment, capillary and electrostatic forces. The ability of the network to hold moisture is influenced by amino acid composition, surface polarity or charge, conformation and topography of the protein, pH, ion species and temperature (Kinsella & Fox, 1987). Fat acts as a mechanical barrier to the flow of moisture within the protein matrix. Presence of fat, which is surrounded by water loosely-bound to the casein matrix (Kimber *et al.*, 1974), disturbs the continuity of the casein network and creates regions of weak structure. When the fat globule surface is coated by casein, as happens with the newly-created fat globule surface during homogenisation, the casein-coated fat globules behave as casein and form a part of the casein network.

The slow diffusion of NaCl into the cheese during and after brining causes the moisture held in the casein network to exude as the protein gel does not reabsorb the moisture. Proteolysis leads to several changes in cheese. The casein network is weakened and tends to disintegrate. The moisture held in the capillaries of the casein network is released. Proteolysis products bind some of the moisture due to the increase in the electrical charged terminal groups. Some of the protein breakdown material becomes soluble and forms a part of the exudate. Excess moisture containing the protein breakdown material and the other solubles is released as exudate."

Conclusion: In Cream cheese partial heat-denaturation of whey protein can be used to control exudation. In Feta cheese homogenisation of cream and vacuum packaging of cheese are effective means of reducing the extent of exudation. Other factors influencing exudation from Feta cheese are protein to fat ratio, material adsorbed to fat globules, NaCl gradient, thermal shocks during storage and proteolysis.

APPENDICES

Appendix 4.1

STANDARD ANALYTICAL (CHEMICAL) METHODS

(a) Chemical methods for analysis of milk, cream, whey and exudate

Particulars	Method	Reference	Principle of the method
Fat, protein, lactose and total solids.	Milko-Scan 133 B	A/S N. Foss Electric (Denmark)	The Milko-Scan 130 series is a semi-automatic, microprocessor controlled instrument for the determination of fat, protein and lactose in milk and other dairy products. Its basic operation is similar to that of an infra-red spectrophotometer. It involves an infra-red beam which is focused to pass through the sample and strike a detector. The energy detected is then amplified and, through microprocessors, converted to a read-out. Measurement of the components is based on their infra-red energy absorption at specific wavelengths. The analytical results are expressed as percentages of milk (w/v).
WPNI	Amido-black dye binding	Sanderson (1970)	Casein and denatured whey protein are precipitated from the reconstituted milk powder using sodium chloride and filtered. The undenatured whey protein in the filtrate is precipitated using amido-black dye, and the excess dye determined spectrophotometrically.

Total solids	Gravimetric	NZDDM 1.12.a, IDF 21 : 1962	A weighed sample is dried in an oven for 5 h at 103°C.
Fat	Rose-Gottlieb	NZDDM 1.4.1a, IDF 1A : 1969	Fat is extracted from an ammoniacal solution of the sample with diethyl ether and petroleum ether, the solvents evaporated and the residue weighed.
Total nitrogen (TN) and protein	Kjeldahl	NZDDM 1.11.1a, IDF 20 : 1962	A weighed sample is catalytically digested with sulphuric acid, converting the organic nitrogen into ammoniacal nitrogen. The ammonia is released by the addition of sodium hydroxide, distilled and absorbed in boric acid and then titrated. This provides the estimate for TN. The protein content was obtained by multiplying the TN by 6.38.
Calcium	Complexometric method	Pearce (1977), NZDDM 1.2.1a	A sodium hydroxide solution of the sample is titrated with a standard EDTA solution in the presence of Patton and Reed's indicator.
NaCl	Potentiometric titration	IDF 88: 1979	The sample is suspended in dilute nitric acid solution and titrated potentiometrically for chloride with standard silver nitrate solution using a measuring electrode system for determination of the end-point. A Mettler DL 40 RC Memo Titrator was used.
pH			The pH was directly read using a pH meter (PHM 80 Portable pH meter, Radiometer, Copenhagen).

Peptides	High performance liquid chromatography (HPLC)	Swergold and Rubin (1983)	HPLC separates peptides according to their molecular size (optimum range 500-10,000 molecular weight) using a gel permeation column (by size exclusion chromatography). The peptides can be quantitatively measured. The equipment used was a Shimadzu LC 6A HPLC with auto-injector. It contained TSK G2000SW 60 cm with TSK Guard column. Elution solvent consisted of 36% Fat UV-grade acetonitrile, 0.1% Trifluoroacetic acid in Milli-Q water. The flow rate was 0.5 ml/min. The absorbance was detected at 205 nm. 1-2 mg of sample was weighed and dissolved in 10 ml elution solvent. Both solvent and sample were filtered through 0.45 μ m pore size PTFE filters before use.
Fat globule size	Spectro-turbidimetric method	Walstra (1965, 1968)	This is based on the light-scattering effect by milk fat globules. A small alkaline solution is used to dilute the milk/cream and the absorbance of the emulsion is measured in a spectrophotometer at several wavelengths; and with other data such as concentration and refractive indices, a specific turbidity spectrum is calculated and plotted. This is compared with theoretical spectra that are computed for assumed globule-size distributions to determine the fat globule size. A computer programme (Kevin N. Pearce, NZDRI, unpublished) was used for the calculations.

(b) Chemical methods for analysis of curd and cheese

Particulars	Method	Reference	Principle of the method
Moisture	(i) Gravimetric (ii) Microwave	NZDDM 4.4.3.0	Drying in oven at 105°C for 16 hours. A microwave moisture analyser (Photo Volt Corporation, New York, model Apollo mark-12) was used.
Fat	(i) Babcock (ii) Schmidt-Bonzynski-Ratzlaff	(i) NZDDM 4.1.3a, APHA (1978) (ii) NZDDM, 4.1.1a IDF-5A (1969)	(i) Use of sulphuric acid breaks down any membranous material, solubilises the cheese and enables the liberated fat to rise to the surface. (ii) Fat is extracted from a hydrochloric acid digest of the sample with diethyl ether and petroleum ether, the solvents evaporated, and the residue weighed.
Calcium	Complexometric method	Pearce (1977) NZDDM 4.4.8.1	Grated cheese is dissolved in weak HCl and diluted with water. NaOH is added and titrated against EDTA. Calcium ion is determined by complexometric titration using Patton and Reed's indicator.
pH		NZDDM 4.5.1a	The pH of curd or grated cheese was directly read using the EMF between a glass electrode and a reference electrode in a pH meter (PHM 82 standard pH meter, Radiometer, Copenhagen).

Total nitrogen and protein	Semi-micro Kjeldahl	NZDDM 1.11.19, IDF 20:1962	The principle is similar as that described for milk. 1.0-1.5 g sample was used for analysis. The protein content was obtained by multiplying the TN by a factor 6.38.
Casein proteins	Urea-polyacrylamide gel electrophoresis (Urea-PAGE)	Creamer (1991) IDF : 1991 (In press)	Casein proteins are separated electrophoretically on the basis of molecular size and net electric charge in a polyacrylamide mini-gel in the presence of high concentration of urea. A Biorad mini Protean II apparatus was used for running the gels. Concentration of the stained protein bands was measured using a densitometer.
Casein proteins and Whey proteins	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)	Creamer (1991) IDF : 1991 (In press)	The ability of sodium dodecyl sulphate (SDS), an amphiphillic detergent, in forming SDS-protein complex involving the monomer protein is used to electrophoretically separate the caseins and major whey proteins, mainly on the basis of molecular size, in a polyacrylamide mini-gel. A Biorad mini Protean II apparatus was used for running the gels. Concentration of the stained protein bands was measured using a densitometer.

NaCl	Potentiometric titration	IDF 88 : 1979	The principle is similar as described for milk. About 0.5 g of Feta cheese or 1.5-2.0 g Cream cheese sample was taken for analysis.
Water activity	Novasina water activity meter	Novasina A-G, Zurich, Switzerland	The humidity sensor measures the relative humidity based on conductivity changes in a hygroscopic electrolyte. The instrument is composed of a measuring station (type EEJA - 3/BAG) and a temperature controlled chamber (type: AW - Box).
Residual rennet (in cheese and whey)	Enzymatic method	Singh and Creamer (1990)	The method is based on the time-dependent decrease in coagulation time of a milk coagulation system caused by a low level of an additional enzyme added to the system. Apparatus for measuring milk clotting times consisted of glass bottles rotated by an electric motor connected to a shaft rotating at 7 rpm at an angle of 30° to the horizontal.
Glucose	Enzymatic colorimetric method (Peridochrome glucose)	Trinder (1969)	Glucose is oxidised by glucose-oxidase to form D-glucono- δ -lactone and hydrogen peroxide. The hydrogen peroxide reacts with a chromophore (4-aminophenazone + phenol) in the presence of peroxide to form a red dye. The intensity of the colour developed, measured at 510 nm, is proportional to the glucose content.

Lactose	Enzymatic colorimetric	Trinder (1969)	β -galactosidase and $MgCl_2$ are used to hydrolyse lactose to glucose and galactose. Glucose content is then estimated using the normal assay. Free glucose already present in the sample is accounted by estimating glucose in the absence of β -galactosidase.
L(+)-Lactate	Enzymatic colorimetric	Gutmann & Wahlefeld (1974)	L(+)-LDH catalyses the reaction of L(+)-lactate and NAD^+ to form NADH and pyruvate. This is not a favourable reaction thermodynamically, hence the reaction must be carried out at high pH and in the presence of hydrazine. Hydrazine reacts chemically with pyruvate, prevents pyruvate from taking part in the reaction and thus allows all the L(+)-lactate to be measured. NADH is measured by the increase in absorbance at 340 nm.
D(-)-Lactate	Enzymatic colorimetric	Gawehn & Bergmeyer (1974)	The principle is the same as for L(+)-Lactate except the use of D(-)LDH instead of L(+)LDH. (LDH stands for Lactate dehydrogenase).

Citrate	Enzymatic colorimetric	Dagley (1974)	Citrate is hydrolysed in the presence of citrate lyase to Oxaloacetate (OA) and acetate. Some of the oxaloacetate formed is decarboxylated by OA-decarboxylase to pyruvate. The pyruvate and oxaloacetate react with NADH in the presence of Lactate dehydrogenase and malate dehydrogenase respectively to form lactate, malate and NAD^+ . The decrease of the NADH concentration, as measured by the change in absorbance at 340 nm is proportional and stoichiometric to the concentration of citrate.
Acetate	Enzymatic colorimetric	Bergmeyer & Mollering (1974)	Acetate is phosphorylated by Adenosine Triphosphate in the presence of acetate kinase to form acetyl-phosphate. Acetyl-phosphate reacts with coenzyme-A (CoA) in the presence of phosphotransacetylase to form Acetyl-CoA. Acetyl-CoA reacts with oxaloacetate in the presence of citrate synthase to form citrate and CoA. The required oxaloacetate is obtained by the conversion of added malate in the presence of malate dehydrogenase and NAD^+ . This reduction of NAD^+ to NADH, which is a quantitative measure of oxaloacetate formed, is thus indirectly related to acetate though not linearly proportional. The change in NADH is measured by the change in absorbance at 340 nm.

Appendix 4.2 Equations used to express the exudation of whey from Feta cheese

- (a) Exudate in g/kg cheese = $(\text{Initial weight of cheese in g} - \text{final weight of cheese in g}) \times 1000 / \text{initial weight of cheese in g}$
- (b) Exudate in g/kg moisture in cheese = $(\text{Initial weight of cheese in g} - \text{final weight of cheese in g}) \times 1000 \times 100 / (\text{initial weight of cheese in g} \times \% \text{ initial moisture in cheese})$
- (c) Final MNFS in cheese, % = $[(\text{Initial weight of cheese in g} \times \% \text{ initial moisture} / 100) - \{(\text{initial weight of cheese in g} - \text{final weight of cheese in g}) \times \% \text{ moisture in exudate} / 100\}] \times 100 / \{\text{final weight of cheese in g} - (\% \text{ initial fat in cheese} \times \text{initial weight of cheese in g} / 100)\}$
- (d) % reduction in MNFS = $(\text{Initial \% MNFS in cheese} - \text{final \% MNFS in cheese}) \times 100 / \text{initial \% MNFS in cheese}$

Some of the values in the above equations have been calculated as follows:

Initial moisture in cheese =

$[\{\text{Weight of cheese (without exudate) at four weeks in g} \times \text{moisture \% in cheese at four weeks} / 100\} + \{(\text{weight of exudate from cheese at four weeks in g}) \times (100 - \% \text{ total solids in exudate}) / 100\}] \times 100 / \{\text{initial weight of cheese in g}\}.$

Initial fat % in cheese = $(\text{Weight of cheese at four weeks in g} \times \text{fat \% in cheese at four weeks}) / \text{Initial weight of cheese in g}$

Initial MNFS % in cheese = $(\text{Initial moisture \% in cheese} \times 100) / (100 - \text{initial fat \% in cheese})$

Note: In the above equations *initial* refers to the state of cheese before exudation *i.e.* the cheese immediately after brining. Likewise, *final* refers to the cheese after exudation and therefore applies to cheese after the respective storage interval.

Appendix 4.3 QUESTIONNAIRE USED TO EVALUATE FETA CHEESE

Name: EVALUATION OF FETA CHEESE Date:

Please evaluate the Feta cheese samples for the following attributes in line with the scale given below. Desirable Feta cheese is expected to have scores close to 3 for acidity, saltiness and mouthfeel; and close to 1 for oxidised, bitterness, structure and overall acceptability.

Flavour : Acidity : Low 1 2 3 4 5 High
 Saltiness : Low 1 2 3 4 5 High
 Oxidised : None 1 2 3 4 5 High
 Bitterness : None 1 2 3 4 5 High

Texture : Mouthfeel : Too smooth 1 2 3 4 5 Crumbly
 Structure : Sliceable 1 2 3 4 5 Brittle

Overall acceptability: Very good 1 2 3 4 5 Unacceptable

Attributes		Sample No.					
		1	2	3	4	5	6
Flavour	Acidity						
	Saltiness						
	Bitterness						
	Oxidised						
Texture	Mouthfeel						
	Structure						
Overall acceptability							

Comments:

Note: The attribute 'overall acceptability' for the cheese was normally not expected to be given a score of 1. A good quality of cheese would have an overall acceptability score of 2, while an average and acceptable quality of cheese was expected to score 3 out of 5 (not quoted in the questionnaire).

Appendix 4.4 QUESTIONNAIRE USED TO EVALUATE CREAM CHEESE

Name: EVALUATION OF CREAM CHEESE Date:

Please evaluate the Cream cheese samples for the following attributes in line with the scale given below.

Flavour : Pleasant 1 2 3 4 5 Unpleasant
 Body : Too soft/weak 1 2 3 4 5 Over-firm/hard
 Texture : Too smooth 1 2 3 4 5 Lacks smoothness
 Overall
 acceptability: Very good 1 2 3 4 5 Unacceptable

Attributes	Samples							
	1	2	3	4	5	6	7	8
Flavour								
Body								
Texture								
Overall acceptability								

Comments:

Note: If the flavour, body or texture is found undesirable, the defect may be specified. Some of the common defects are listed below.

Flavour defects: flat, sour, yeasty, rancid, salty etc.

Body and texture defects: grainy, gritty, lumpy, sandy, sticky, coarse etc.

Appendix 5.1

(a) Brief description of equipment and accessories used during manufacture of Cream cheese

1. Cream Separator: APV make, model MSD 50-01-076, 3,000 L/h capacity self-desludging separator.
2. Rectangular vats: 100 L jacketed stainless steel vats with variable speed mechanical agitators.
3. Homogeniser: APV Manton Goulin make, Model K 3 with a pumping capacity of 500 L/h.
4. Plate heat exchanger (PHE): Alfa-laval make, Model No A 3 HRB, with heating, holding and cooling sections. It is designed for a flow capacity of 500 L/h and a corresponding residence time of 15 s in the holding section.
5. Positive pump: Waukesha D25, positive lobe pump with a variable speed drive and a flow capacity of 2,000 L/h. This was used to supply hot water to the PHE.
6. Cheese vat (NZDRI designed, drawing No 6164): A 100 L capacity jacketed stainless steel circular vat with provisions for indirect heating by steam or by a 1 KW electric element. The jacket is connected to a Dan Foss thermostat to automatically regulate the electric heater to the set temperature during incubation of milk and has an in-built provision to record the temperature of the contents inside the vat. The vat is also connected with a steam inlet at the bottom to heat the water in the jacketed portion for indirect heating of the vat contents. The lid on top of the vat has openings, one to inoculate starter and another to act as an air vent. These openings were plugged with non-adsorbent cotton wool during the operation. The inner chamber of the vat used for incubation of milk is also connected with a drain outlet at the bottom end to remove the contents.

7. Sieves: Kadova sieves, 10 kg capacity inner plastic liners for Gouda cheese hoops.
8. Dry blender: Crypto Peerless blender, Model EB 20B with a variable 3-speed agitator.
9. Processing kettle (NZDRI designed): A 4.0 kg capacity processing kettle jacketed for indirect heating was used. Some of the attached accessories include a variable speed agitator, an inlet for steam, an outlet valve for steam, pressure gauge to measure steam pressure, and a temperature recorder for measuring the temperature of the contents in the kettle.
10. Cups for packing: Supplied by Plastic Wholesalers (Waikato), Te Rapa, Hamilton, New Zealand (Code No 420 for pots and 421 for lids). These are honey pots, 125 ml capacity, natural polyethylene pots with white caps.

(b) Procedure for homogenising and pasteurising standardised milk: The homogeniser and plate heat exchanger (with heating, holding and cooling sections) were set up in-line to facilitate the continuous and enclosed operations of homogenisation followed by high-temperature-short-time pasteurisation. Milk was indirectly heated in the plate heat exchanger by passing hot water at 93 - 95 °C from a 400 L vat through a speed-adjustable positive pump. Likewise, milk was indirectly cooled (after holding) by passing tap water through the plates (16-18 °C). The temperature of milk in the plate heat exchanger in the heating and cooling sections was controlled by regulating the flow of hot or cold water in the respective sections. The flow of milk through the plate heat exchanger was regulated by the homogeniser pump. In-place-cleaning was done before and after the 'homogenisation & pasteurisation' operation by the usual cleaning regime. To ensure the destruction of bacteriophage and to provide a near-sterile environment the unit was sanitized by chlorine (100 ppm) and then circulated with hot water at 75 - 80 °C for half-an-hour before milk processing began. The holding vat for milk, cheese vat, cans and other containers used during the process were chlorine washed and steamed.

Appendix 5.2 Manufacturing process for Cream cheese

Bulk whole milk was supplied by Tui Milk Products Limited. The raw milk was clarified and separated in a cream separator at 55 °C. Cream was then pasteurised by high-temperature-short-time pasteurisation. Skim milk was cooled and stored at 4 °C until used. About 100 kg of standardized milk of desired protein to fat ratio was prepared by adding a calculated amount of pasteurised cream to skim milk.

The standardized milk was pumped into a double-jacketed, rectangular stainless steel vat, continuously stirred by a mechanical agitator and heated to 60-65 °C. The milk was then homogenized, pasteurised, cooled to about 22 °C and collected in a sanitized cheese vat. The first 20 - 30 L of milk was discarded to ensure that pasteurising conditions were uniform. Usually about 70 L of milk were pumped into the cheese vat and held for about an hour to ensure that the temperature was stable and uniform. The temperature of milk in the cheese vat was thermostatically controlled at 22 °C.

Starter was inoculated into the milk in the cheese vat at the rate of 0.1 - 0.2% (NZDRI strain, *Lactococcus lactis* subsp. *cremoris* strains) and incubated for 15-16 h until the desired pH was attained. The curd was then cooked to the desired temperature. The temperature of the continuously stirred curd was raised at the rate of 1 °C per min by indirect steam-heating in the jacket. Once the desired lower cooking temperature (60 °C) was attained, approximately half of the curd was immediately removed and filtered through sieves placed on top of buckets. The remaining curd in the cheese vat was heated to the higher cooking temperature (75 °C) and filtered. Although both lots of curd were processed separately from this point onwards, the procedure was identical.

During initial stages of draining the curd in the sieve was turned 5-6 times at hourly intervals to facilitate better drainage. After that it was left to drain overnight. After draining, the curd was blended in a dry blender for one minute at the lowest

speed, then sampled and tested for moisture, pH and fat. The moisture of curd and whey were determined in a microwave moisture analyser.

To produce 4 kg of the final product calculated amounts of curd, whey (or water), cheese salt (1% in the final product) and potassium sorbate (0.02% in the final product) were weighed into the processing kettle. The kettle was closed and the contents mixed by gradually increasing the speed of the agitator to 100 rpm. The cold-mixing was continued up to 1 min from the start of the operation. The contents were then indirectly heated with steam at a pressure of 20 psi to a temperature of 80°C. The steam supply was stopped when the temperature reached 75°C as the heat retained in the jacket was usually enough to take the product up to 80°C. The total heating time was typically 3 min. The contents were further held with continuous stirring until a total of 10 minutes had elapsed from the start of heating. During the final minute of stirring the speed of the agitator was reduced. After 10 min of stirring the agitator was turned off, the lid opened and the contents poured quickly into polyethylene cups. The cups were sealed, and turned upside-down so that the contents rested on the lid. These containers were then stored in the upside-down position at the specified storage temperatures. Samples were then drawn at appropriate intervals for analysis, grading and storage studies.

As air pockets in the Cream cheese tended to become filled with exudate during storage care was required when filling the cups to ensure that no air was entrapped within the body of Cream cheese.

Appendix 5.3

Calculations for the amount of water to be added to or removed from curd during processing for adjustment of moisture in Cream cheese

Total quantity of mix used in the kettle	= 4.0 kg
Required moisture in Cream cheese (for calculations only)	= 55.0%
Total quantity of moisture required in the processing mix	= 2.2 kg
Quantity of salt to be added (1% in cheese) in mix	= 0.04 kg
Quantity of potassium sorbate to be added (0.02% in cheese)	= 0.0008 kg
Total quantity of curd and water = $4.0 - (0.040 + 0.0008)$	= 3.9592 kg
Let the amount of curd used in the mix	= C kg
Moisture in the curd	= M %

(i) Amount of water required to be added

Let the amount of water required to be added to the mix = W kg

$$C + W = 3.9592 \quad \text{Equation (i)}$$

$$(C \times M/100) + W = 2.2 \quad \text{Equation (ii)}$$

M was determined by analysis. Using the two above equations the quantity of curd (C) and the quantity of water (W) to be added were calculated.

(ii) Amount of water to be removed from curd

Let the amount of water to be removed from curd = w kg

$$C - w = 3.9592 \quad \text{Equation (iii)}$$

$$(C \times M/100) - w = 2.2 \quad \text{Equation (iv)}$$

M was determined by analysis. Using the two above equations the quantity of curd (C) and the quantity of water (w) to be removed were calculated.

Note : The amount of moisture that evaporated during processing in the open kettle was about 150 g in 5 min. This amount did not vary much with variation in batch size. When there was a need for excess amount of moisture to be removed, a proportionately reduced amount of mix was taken for processing. However, it was not easy to have an accurate control over this process. This increased the source of experimental error.

Appendix 5.4 Composition of standardised milks used for cheese manufacture with respect to the selected manufacturing variables

Source & levels of variation	Fat (%)		Protein (%)		TS (%)	
	LSM	F	LSM	F	LSM	F
A		333 ***		45.7 **		65.8 ***
High	9.36		2.83		17.32	
Low	11.51		2.51		19.08	
B		1.04		0.64		0.14
High	10.37		2.69		18.24	
Low	10.49		2.65		18.16	
C		11.01*		8.57*		5.74
High	10.24		2.60		17.94	
Low	10.63		2.74		18.46	
D		4.34		0.07		4.02
High	10.31		2.66		17.98	
Low	10.55		2.68		18.41	
E (High & Low)	10.43		2.67		18.2	
S.D.	0.235		0.094		0.434	

A = P/F ratio; B = Homogenisation pressure; C= Pasteurisation temperature; D = Curd pH at cooking; E = Cooking temperature;

LSM = Least-square mean; F = F ratio;
S.D. = Standard deviation of raw data;

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;
F values without asterisks denote 'not significant'.

Appendix 5.5

(a) Statistical technique used for the test of significance of the manufacturing variables

Analysis of variance (ANOVA) was performed on the exudation data (g/kg cheese moisture) using the statistical package SAS (1985). The distribution of data on exudation was not normal, as only 30.5% of the total number of experimental cheeses had exudation during storage. Among the cheeses that had exudation, the variation in the quantity was very large, *e.g.* some had as much as 200 g/kg cheese moisture. A study of the residuals indicated that the data contravened the assumptions of the general linear model in that the residuals were non-normally distributed. Attempts to induce normality in the residuals, and thereby in the response variables (*i.e.* amount of exudate), were made using a variety of transformation techniques (*viz.* arcsin, square root, and natural logarithm). None of these resulted in a residual distribution that satisfied the assumptions of ANOVA. Further attempts to induce normality involved transforming the response variables, *i.e.* expressing the exudation data in other forms. However, these procedures also failed because the distribution of the transformed data was always skewed. Because of the above limitations in the construction of an ANOVA model, Chi-squared analysis was used to test the significance of variation.

ANOVA was used to obtain least square mean values (LSM). The data estimated by missing value technique appeared unrealistic. The LSM values were therefore calculated from the actual data.

Chi-square tests were performed by partitioning the data on the amount of exudate (g/kg cheese moisture) into the following sub-groups: 'none', 'slight/moderate (range 1-20 g/kg)', and 'excess' (> 20 g/kg). The sub-groups were chosen such that they satisfied the assumption for the Chi-squared test which states that the expected counts should be always greater than 5. Majority of the cheeses which did not have exudation were classified into one group (*i.e.* none). Of the samples that had exudation, most of them exuded in moderate quantities which were grouped

as 'slight/moderate'. The cut-off point at 20 g/kg was chosen arbitrarily to represent slight or moderate exudation. The cheeses that had extensive exudation were categorised under 'excess'.

(b) Example showing application of Chi-squared test of significance

The data in this instance refers to exudation of whey at storage temperature of 5°C (Chapter 4, Section One) for the two levels of P/F ratio. The observed data on exudation (g/kg cheese moisture) is grouped into three categories as 'none', 'slight/moderate' (range 1 - 20) and 'excess'(> 21) and tabulated as below.

Levels of P/F ratio	Number of incidences of exudation			
	Nil	Slight/Moderate	Excess	Total
Low	68 (75)	22 (14)	6 (7)	96
High	82 (75)	6 (14)	8 (7)	96
Total	150	28	14	192

Note: The values outside parentheses denote the actual number of incidences of the event and the values inside the parentheses denote the expected number of incidences of the event.

Null hypothesis: The values in the rows are independent of the values in the columns. Here it implies that the incidences of exudation is independent of the P/F ratio.

Assumption: The above hypothesis is based on the assumption that the expected counts should be always greater than 5.

Calculation of Chi square (X^2)

$$X^2 = [(68 - 75)^2 / 75] + [(22 - 14)^2 / 14] + \dots = 10.735$$

Degrees of freedom = (No of rows - 1) X (no of columns -1) = (3-1) (2-1) = 2

Observation: Calculated value of X^2 is greater than that the tabulated value of 9.21 (at 1% level of significance with 2 degrees of freedom).

Conclusion: The hypothesis of P/F ratio being independent of the incidences of exudation is rejected at 1% level (**) of significance .

Appendix 5.6 Calculations for adjustment of curd to a constant MNFS

Total quantity of mix used in the kettle = 4.0 kg

Required MNFS in Cream cheese curd (for calculations only) = 83.5%

Quantity of salt to be added (1% in cheese) in mix = 0.04 kg

Quantity of potassium sorbate to be added (0.02% in cheese) = 0.0008 kg

Total quantity of curd and water = $4.0 - (0.040 + 0.0008) = 3.9592$ kg

(i) Adjustment by addition of whey prior to processing

Moisture in the Cream cheese curd = M %

Fat in Cream cheese curd = F %

Let the amount of water required to be added to the mix = W kg

Let the amount of curd to be used in the mix = C kg

$$C + W = 3.9592$$

Mass equation (i)

$$[(C \times M/100) + W] / [3.9592 - (C \times F/100)] = 0.835$$

MNFS equation (ii)

Using the two above equations the unknown values of quantity of curd (C) and the quantity of water (W) were determined. The quantity of whey with W kg water was calculated and added to the mix. The contribution of fat and SNF from this amount of whey to the mix was too small and hence neglected.

(ii) Adjustment by evaporation of moisture from curd

Let the moisture in the Cream cheese curd be = M %

Let the fat in Cream cheese curd be = F %

$$\% \text{ MNFS in the curd} = 100 M / (100 - F)$$

Let it be assumed that the amount of curd to be used in the mix = C kg

$$\text{Quantity moisture in C kg curd} = (C \times M/100) \text{ kg}$$

$$\text{Quantity fat in C kg curd} = (C \times F/100) \text{ kg}$$

Let the moisture that needs to be removed for attaining 83.5% MNFS = m kg

Quantity of curd in the mix after removal of moisture =

$$(C - m) = 3.9592$$

Mass equation (i)

MNFS in (C - m) kg curd =

$$[C \times M/100 - m] 100 / [C - m - (C \times F/100)] = 83.5$$

MNFS equation (ii)

Using the above two equations the unknown quantities of moisture to be removed (m kg) and curd to be used (C kg) are estimated.

Note : The amount of moisture that evaporated during processing in the open kettle was about 150 g in 5 min. This amount did not vary much with variation in batch size. When there was a need for excess amount of moisture to be removed, a proportionately reduced amount of mix was taken for processing. However, it was not easy to have an accurate control over this process. This increased the source of experimental error.

Appendix 5.7 Composition of standardised milk with respect to the manufacturing variables

Source of variation	Fat (%)		Protein (%)		Lactose (%)		TS (%)		Ca ²⁺ (mM/g)	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A		57.88**		9.31		14.95*		23.98*		0.32
High	10.28		3.09		4.66		18.43		28.2	
Low	12.89		2.89		4.38		20.54		29.1	
B		0.21		1.20		0.62		0.46		0.11
High	11.51		2.96		4.49		19.34		28.4	
Low	11.66		3.03		4.55		19.63		28.9	
C		2.26		1.03		0.73		1.79		0.64
High	11.84		3.03		4.49		19.78		28.1	
Low	11.33		2.96		4.55		19.20		29.3	
D		0.29		0.09		0.99		0.02		0.19
High	11.68		2.99		4.48		19.52		28.3	
Low	11.49		3.01		4.56		19.46		29.0	
E		0.00		0.00		0.00		0.00		0.00
High	11.58		2.99		4.52		19.49		28.7	
Low	11.58		2.99		4.52		19.49		28.7	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature; D = Curd pH at cooking; E = Cooking temperature;

LSM = Least-square mean; F = F ratio;

* $p < 0.05$; ** $p < 0.01$; F values without asterisks denote 'not significant'.

Appendix 5.8 Effect of manufacturing variables on the composition of whey and fines lost in whey

Source of variation	Fat (%)		Protein (%)		Lactose (%)		TS (%)		Ca ²⁺ (mM/kg)		Fines lost (%)	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A		2.37		4.00		3.54		5.47		0.74		4.94
High	0.52		0.84		4.22		5.99		34.5		3.24	
Low	0.43		0.80		4.06		5.69		33.4		5.53	
B		10.00		15.50*		1.27		8.36		2.43		0.97
High	0.38		0.79		4.09		5.66		34.9		4.89	
Low	0.57		0.86		4.19		6.03		32.9		3.87	
C		0.29		14.50*		0.03		0.47		9.79		0.27
High	0.46		0.79		4.14		5.80		35.9		4.65	
Low	0.49		0.86		4.13		5.89		31.9		4.11	
D		0.06		10.00		2.09		2.66		13.91		0.18
High	0.48		0.85		4.19		5.95		31.5		4.60	
Low	0.47		0.79		4.07		5.74		36.3		4.16	
E		1.56		255.6***		12.71*		0.36		0.07		31.91*
High	0.49		0.77		4.15		5.83		34.1		5.78	
Low	0.45		0.87		4.12		5.86		33.8		2.98	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature; D = Curd pH at cooking; E = Cooking temperature;

LSM = Least-square mean; F = F ratio;

* p < 0.05; *** p < 0.001; F values without asterisks denote 'not significant'.

Appendix 5.9 Effect of manufacturing variables on the composition of curd

Source of variation	Moisture (%)		Fat (%)		MNFS (%)		FDM (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
A		15.00*		0.16		13.24*		8.43
High	53.67		33.94		81.16		73.19	
Low	55.41		33.77		83.59		75.56	
B		142.77**		135.89**		7.83		7.46
High	57.23		31.36		83.31		73.26	
Low	51.86		36.34		81.44		75.49	
C		43.74**		35.47**		4.16		0.96
High	56.03		32.58		83.06		73.97	
Low	53.06		35.13		81.69		74.77	
D		91.91**		81.47**		6.47		3.41
High	52.39		35.78		81.53		75.13	
Low	56.69		31.92		83.23		73.62	
E		0.00		0.08		2.03		2.31
High	54.56		33.95		82.54		74.63	
Low	54.53		33.75		82.21		74.11	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature; D = Curd pH at cooking; E = Cooking temperature;

LSM = Least-square mean; F = F ratio;

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;

F values without asterisks denote 'not significant'.

Appendix 5.10 Effect of manufacturing variables on the mean scores of sensory parameters of cheeses

Source of variation	Flavour		Body		Texture		Overall acceptability	
	LSM	F	LSM	F	LSM	F	LSM	F
A		4.96		2.49		1.32		0.34
High	2.76		2.61		2.73		2.74	
Low	3.07		2.25		2.34		2.83	
B		7.07		3.19		3.23		2.34
High	2.73		2.64		2.84		2.67	
Low	3.10		2.23		2.23		2.90	
C		4.88		2.49		0.31		11.93*
High	2.76		2.61		2.63		2.53	
Low	3.07		2.25		2.44		3.04	
D		3.41		0.16		1.46		2.04
High	2.78		2.48		2.33		2.68	
Low	3.04		2.39		2.74		2.89	
E		7.52		17.09*		0.17		4.66
High	2.76		2.73		2.57		2.57	
Low	3.06		2.13		2.49		3.00	

A = P/F ratio; B = Homogenisation pressure; C = Pasteurisation temperature;
D = Curd pH at cooking; E = Cooking temperature;

LSM = Least square mean; F = F ratio;

* $p < 0.05$; F values without asterisks denote 'not significant'.

Appendix 6.1 Equipment and accessories used for manufacture of Feta cheese

1. Reconstitution unit: This included a funnel for introducing milk powder, a powder liquid reconstitution pump (Alfa CM open impeller, centrifugal pump) and a rectangular holding vat.
2. Homogeniser: Manton Goulin homogeniser, APV Ltd., UK, Model K 3 with a puppet valve and a pump capacity of 500 L/h.
3. Cheese Vats: 100 L jacketed stainless steel vats with variable speed agitators.
4. Hoops: Rectangular stainless steel 'Perfora' hoops with perforations.
5. Heat sealer: Gray Pak make, standard Model 220/240 - 1, with provision for controlled vacuum packaging.
6. Plastic pouches: Wrightvac Pouches Code No C-40, supplied by Courtaulds Packaging, Fielding, New Zealand. These are high barrier, heat sealable, non-shrink pouches.

Appendix 6.2 Manufacturing process for Feta cheese

Reconstituted Skim Milk (RSM) was prepared by mixing low heat skim milk powder (LHSMP) and tap water at 40 °C in the ratio of 1:8.5 in the reconstitution unit. The RSM was circulated for 10 min to ensure proper reconstitution.

Cream of about 25% fat was prepared by heating a mixture of fresh frozen milkfat for recombining (FFMR) and RSM (1:3 ratio) to 60 °C, and homogenising the mixture at single stage, 6890 kPa (1,000 psi) or as specified. Cream prepared in this manner is referred as **manufactured cream** to distinguish it from fresh cream.

RSM and 'manufactured cream' were mixed in suitable proportions to obtain a protein to fat (P/F) ratio of 0.73 (or as specified) in the standardised milk.

The standardised milk (or cheesemilk) was taken into the cheese vats for the manufacturing process. Usually 35 to 40 kg of milk was used for each vat. The temperature of cheesemilk was adjusted to 34 °C by circulating hot or cold water through the jacket. 0.02% CaCl₂ (w/w) was added to the cheesemilk. Starter was inoculated into milk (2.0% *Lactococcus lactis* subsp. *cremoris* strains - NZDRI strains 584/2128, 0.1% *Streptococcus thermophilus* and 0.1% *Lactobacillus casei*) and the milk was then primed for 30 min (or as specified). Calf rennet (from New Zealand Cooperative Rennet Company, Eltham, 59 RU/ml) was added at the rate of 16 ml/100 kg of milk. The milk was allowed to set for 45 min (or as specified). The curd was cut (standard three cuts) after 45 min using 12 mm cheese knives. After a dwell time of 15 min, the resultant curd and whey were subjected to gentle manual stirring for about 30 s after every 10 min so that the curd remained homogeneous in the whey. When the curd attained the desired pH (usually 6.2), it was scooped into hoops and allowed to drain overnight (16-17 h) at room temperature (20 °C). In the first few hours of draining, hoops with the curd were turned three to four times. After overnight draining the blocks of curd (cheese before brining) were taken out of the hoops, weighed, cut into two similar blocks (this was done to have enough number of blocks for analysis and storage studies) and salted in 20-21% (w/v) brine at 11-13 °C for 22 h. After brining the blocks were drained on paper towels for 10 min, packed into plastic bags, and vacuum sealed. Feta cheese was stored at 10 °C. Samples were drawn at specified intervals for analysis, grading and storage studies.

Appendix 6.3 Effect of manufacturing variables on the composition of whey.

Treatment & Levels	Whey at draining					Entire Whey
	Fat (%)	Protein (%)	Lactose (%)	TS (%)	Ca ²⁺ (mM/kg)	Ca ²⁺ (mM/kg)
	LSM F	LSM F	LSM F	LSM F	LSM F	LSM F
A						
First	0.081	0.795	5.04	6.33	14.6	22.6
Second	0.079	0.825	5.25	6.58	13.4	19.7
B						
High	0.084	0.789	4.99	6.27	14.8	21.7
Low	0.076	0.831	5.31	6.64	13.2	20.6
C						
High	0.078	0.808	5.18	6.49	13.4	20.5
Low	0.081	0.813	5.12	6.42	14.7	21.8
D						
High	0.068	0.801	5.11	6.39	14.2	20.5
Low	0.091	0.819	5.19	6.51	13.8	21.8
E						
High	0.084	0.825	5.32	6.64	13.0	20.8
Low	0.076	0.795	4.97	6.27	15.0	21.5
Control	0.12	0.855	5.43	6.81	13.0	20.5

A = Replicates; B = P/F ratio of milk; C = Homogenisation pressure for 'manufactured cream';

D = Priming time; E = Curd pH at draining;

LSM = Least-square mean; F = F ratio;

* p < 0.05; ** p < 0.01; *** p < 0.001; F values without asterisks denote 'not significant'.

Appendix 6.4 Effect of selected manufacturing variables on composition of Feta cheese after six months of storage at 10°C

Treatment & level	Moisture (%)		Fat (%)		MNFS (%)		FDM (%)		pH	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
A		0.33		3.03		0.37		4.44		2.55
First	48.25		26.97		66.13		52.06		4.54	
Second	48.55		26.16		65.79		50.59		4.51	
B		12.8**		179***		30.5***		217***		1.26
High	49.34		23.44		64.42		46.21		4.53	
Low	47.46		29.69		67.50		56.44		4.51	
C		18.03**		19.5**		4.25		7.34*		1.01
High	49.51		25.53		66.54		50.39		4.53	
Low	47.29		27.59		65.39		52.26		4.51	
D		6.57*		7.16*		1.7		2.25		0.28
High	47.73		27.19		65.59		51.85		4.53	
Low	49.07		25.94		66.32		50.80		4.52	
E		1.66		4.03		0.02		2.76		1.54
High	48.74		26.09		65.99		50.75		4.51	
Low	48.06		27.03		65.92		51.90		4.54	
S.D.	1.05		0.93		1.12		1.39		0.04	

A = Replicate; B = P/F ratio; C = Homogenisation pressure; D = Priming time; E = Curd pH at draining; LSM = Least square mean; F = F ratio; S.D. = Standard deviation of raw data, *** p < 0.00; ** p < 0.01; * p < 0.05; 'F' values without any asterisks are 'not significant'.

Appendix 6.5 Effect of manufacturing variables on the mean scores of sensory parameters of cheese.

Treatment & Levels	FLAVOUR				TEXTURE		
	Acidity	Saltiness	Bitterness	Oxidised	Mouthfeel	Structure	Overall acceptability
	LSM F	LSM F	LSM F	LSM F	LSM F	LSM F	LSM F
A	67.07***	1.60	1.66	1.76	0.00	6.43*	0.08
First	2.53	3.32	1.51	1.41	3.01	3.23	2.85
Second	2.92	3.19	1.69	1.56	3.02	2.99	2.82
B	1.86	7.05*	1.24	0.26	10.34*	4.79	2.01
High	2.76	3.39	1.68	1.46	3.24	3.21	2.93
Low	2.69	3.12	1.52	1.52	2.81	3.01	2.74
C	2.01	1.19	0.66	0.77	0.55	1.85	0.08
High	2.69	3.31	1.66	1.54	3.07	3.05	2.85
Low	2.76	3.20	1.54	1.44	2.97	3.17	2.81
D	3.98	0.06	0.53	0.00	1.20	1.08	0.09
High	2.77	3.27	1.55	1.49	3.09	3.06	2.81
Low	2.68	3.24	1.65	1.48	2.95	3.16	2.86
E	6.62*	10.25*	0.18	1.59	1.24	8.31*	0.93
High	2.66	3.09	1.57	1.56	2.95	2.98	2.77
Low	2.78	3.42	1.63	1.41	3.09	3.25	2.90
Control	3.06	3.16	2.35	1.5	2.63	2.88	2.69

A = Replicates; B = P/F ratio of milk; C = Homogenisation pressure for ' manufactured cream';

D = Priming time; E = Curd pH at draining; S = Storage time;

LSM = Least-square mean; F = F ratio;

* p < 0.05; *** p < 0.001; F values without asterisks denote 'not significant'.

Appendix 7.1 An example showing calculations for the preparation of cheesemilk

(i) Quantity of slurry containing heat-denatured whey protein to be added

Rate of addition of whey protein (dry matter) = 9.0 g/kg milk

Protein concentration of slurry = 12.0%^a

Total quantity of milk taken = 35.25 kg (control)

Quantity of slurry to be added = $[35.25 (9/1000) (100/12)]$
= 2.644 kg

(ii) Quantity of fat (i.e. FFMR) to be added for adjustment^b of protein to fat (P/F) ratio

Protein to fat ratio required in cheesemilk = 0.74

Quantity of additional protein incorporated

= 2.644 (12/100) = 0.317 kg

Quantity of additional fat required = $0.317/0.74 = 0.428$ kg

(iii) Details for preparation of cheesemilk

Quantity of reconstituted skim milk (RSM) and manufactured cream to be taken in cheesevat (control) = 35.250 kg

Less FFMR used for control (5.1% fat in milk) = 1.800 kg^c

RSM used for control

(3.520 kg SMP + 29.930 kg water) = 33.450 kg

Less provision for addition of acid/alkali = 2.000 kg

Quantity RSM (concentrated) to be used = 31.450 kg^d

Let the quantity of dilute alkali/acid used = 1.000 kg

Quantity of make-up water added after addition of alkali
= (2 - 1) = 1.000 kg

Procedure: Initially RSM was prepared. Then manufactured cream was prepared using FFMR and RSM (1:3 ratio). If required, slurry with heat-denatured whey protein was homogenised with FFMR and RSM. 'Manufactured cream', RSM, and slurry were taken into the cheesevat. pH of the cheesemilk was adjusted to 6.55 using 0.1% NaOH or 0.035% HCl and then make-up water (2 kg - quantity of acid/alkali) was added to the cheesevat. 0.02% CaCl₂ was added to the cheesemilk. The milk was ready for cheese manufacture.

^a For calculations the protein content in slurry was assumed to be 80% of the total solids.

^b This was done only when P/F ratio needed adjustment.

^c Where fat was to be adjusted in proportion to the whey protein added, an increased quantity of FFMR was taken.

^d 3.520 kg SMP and 27.730 kg water.

Appendix 7.2 Composition of cheesemilk^a with respect to the process treatments

Variation	P/F ratio		Fat (%)		TS (%)		Protein (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	0.75		4.90		14.26		3.66	
Added whey protein (W)		15.58*		0.85		8.46*		75.1***
W 2	0.83		5.23		14.89		4.29	
W 1	0.77		5.15		14.49		3.94	
Fat (F)		72.67***		77.31***		26.18**		0.67
F 2	0.73		5.60		15.05		4.10	
F 1	0.87		4.78		14.33		4.13	
Homogenisation (H)		0.7		0.72		0.95		1.79
H 1	0.80		5.24		14.82		4.16	
H 2	0.79		5.22		14.66		4.06	
H 3	0.81		5.11		14.59		4.13	
S.D.	0.027		0.163		0.243		0.069	
Source of variation			Calcium (mM/kg)					
			LSM			F		
Control			36.1					
Added whey protein (W)			0.27					
W 2			35.2					
W 1			35.7					
Fat (F)			0.31					
F 2			35.1					
F 1			35.7					
Homogenisation (H)			0.11					
H 1			35.1					
H 2			35.5					
H 3			35.7					
S.D.			1.71					

^a Includes slurry containing heat-denatured whey protein.

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;
 W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;
 F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein; F 1 = Fat level not adjusted; H 1 = Denatured whey protein added without homogenisation; H 2 = Denatured whey protein homogenised separately; H 3 = Denatured whey protein homogenised in combination with manufactured cream; Control = Cheese prepared without any added whey protein;
 LSM = Least-square mean; F = F ratio; S.D. = Standard deviation of raw data;
 * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; F ratios without asterisks denote 'not significant'.

Appendix 7.3 Effect of process treatments on the composition of whey

Variation	Fat (%)		TS (%)		Protein (%)		Ca ²⁺ (mM/kg)	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	0.067		6.80		0.97		21.0	
Added whey protein (W)		26.18**		0.93		2.74		3.33
W 2	0.088		6.77		1.02		20.5	
W 1	0.068		6.80		0.99		22.1	
Fat (F)		11.6*		4.25		0.22		8.14*
F 2	0.085		6.82		1.01		22.6	
F 1	0.072		6.75		1.00		20.1	
Homogenisation (H)		8.91*		2.82		1.36		1.77
H 1	0.088		6.82		1.02		21.0	
H 2	0.068		6.72		1.01		20.5	
H 3	0.080		6.80		0.99		22.4	
S.D.	0.0067		0.063		0.031		1.52	

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk;

W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein;

F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;

H 2 = Denatured whey protein homogenised separately;

H 3 = Denatured whey protein homogenised in combination with manufactured cream;

Control = Cheese prepared without any added whey protein;

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;

F ratios without asterisks denote 'not significant'.

Appendix 7.4 Effect of process treatments on the composition of cheese before brining

Variation	Fat (%)		Ca ²⁺ (mM/Kg)		Protein (%)	
	LSM	F	LSM	F	LSM	F
Control	26.50		107.1		16.33	
Added whey protein (W)		24.12**		0.73		0.60
W 2	24.28		94.7		16.33	
W 1	26.02		98.6		16.72	
Fat (F)		48.84**		6.53		2.85
F 2	26.38		90.8		16.14	
F 1	23.92		102.5		16.91	
Homogenisation (H)		11.82*		0.79		0.30
H 1	26.18		93.6		16.40	
H 2	25.20		100.5		16.40	
H 3	24.08		95.9		16.78	
S.D.	0.611		7.9		0.77	

Variation	Moisture (%)		pH		FDM (%)		MNFS (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	53.17		4.77		56.60		72.32	
Added whey protein (W)		6.05		0.23		15.29*		1.90
W 2	55.40		4.77		54.40		73.18	
W 1	53.33		4.76		55.69		72.07	
Fat (F)		3.78		2.10		109.95***		0.07
F 2	53.55		4.75		56.79		72.73	
F 1	55.18		4.78		53.31		72.52	
Homogenisation (H)		1.41		0.13		19.96**		0.15
H 1	53.65		4.76		56.40		72.67	
H 2	54.13		4.77		54.92		72.34	
H 3	55.33		4.76		53.84		72.87	
S.D.	1.455		0.042		0.575		1.385	

W 2 = Denatured whey protein (DM) added at the rate of 9.0 g/kg milk; W 1 = Denatured whey protein (DM) added at the rate of 4.5 g/kg milk; F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein; F 1 = Fat level not adjusted; H 1 = Denatured whey protein added without homogenisation; H 2 = Denatured whey protein homogenised separately; H 3 = Denatured whey protein homogenised in combination with manufactured cream; Control = Cheese prepared without added whey protein; LSM = Least-square mean; F = F ratio; S.D. = Standard deviation of raw data; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; F ratios without asterisks denote 'not significant'.

Appendix 7.5 Effect of process treatments on the composition of cheese at four weeks

Variation	Moisture(%)		Fat (%)		NaCl (%)		pH	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	48.34		27.43		4.67		4.63	
Added whey protein (W)		7.69*		9.64*		1.25		0.31
W 2	51.04		24.90		4.63		4.65	
W 1	48.59		26.50		4.49		4.64	
Fat (F)		3.08		24.81**		7.54		0.03
F 2	49.04		26.98		4.39		4.65	
F 1	50.59		24.42		4.73		4.65	
Homogenisation (H)		2.12		6.04		1.80		0.24
H 1	49.00		26.65		4.52		4.66	
H 2	49.37		25.95		4.44		4.64	
H 3	51.08		24.50		4.72		4.65	
S.D.	1.526		0.893		0.217		0.031	
Variation	Protein (%)		FDM (%)		MNFS (%)		S/M (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	16.35		53.09		66.60		9.67	
Added whey protein (W)		1.43		3.40		5.95		1.03
W 2	16.45		50.80		67.98		9.07	
W 1	16.97		51.51		66.09		9.24	
Fat (F)		3.18		86.8***		0.10		5.61
F 2	16.32		52.95		67.16		8.95	
F 1	17.10		49.37		66.92		9.36	
Homogenisation (H)		0.57		10.01*		0.65		0.84
H 1	16.59		52.17		66.77		9.23	
H 2	17.03		51.24		66.18		9.00	
H 3	16.51		50.07		67.66		9.24	
S.D.	0.754		0.667		1.341		0.298	

W 2 = Denatured whey protein (DM) added at the rate of 9.0 g/kg milk; W 1 = Denatured whey protein (DM) added at the rate of 4.5 g/kg milk; F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein; F 1 = Fat level not adjusted; H 1 = Denatured whey protein added without homogenisation; H 2 = Denatured whey protein homogenised separately; H 3 = Denatured whey protein homogenised in combination with manufactured cream; Control = Cheese prepared without added whey protein; LSM = Least-square mean; F = F ratio; S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; F ratios without asterisks denote 'not significant'.

Appendix 7.6 Effect of process treatments on the composition of exudate from four week old cheese

Variation	TS (%)		Ca ²⁺ (mM/Kg)		NaCl (%)		Protein (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	15.56		139.3		8.15		2.86	
Added whey protein (W)		0.02		2.22		0.91		2.69
W 2	15.26		123.7		7.83		1.41	
W 1	15.22		135.3		8.01		2.73	
Fat (F)		9.39*		0.15		0.08		0.0
F 2	14.76		128.0		7.89		2.54	
F 1	15.72		131.0		7.95		2.60	
Homogenisation (H)		0.47		0.13		0.02		0.31
H 1	15.21		129.9		7.91		2.57	
H 2	15.08		131.8		7.89		2.67	
H 3	15.44		126.9		7.95		2.46	
S.D.	0.539		13.49		0.331		0.274	

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk; W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein; F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;
H 2 = Denatured whey protein homogenised separately;
H 3 = Denatured whey protein homogenised in combination with manufactured cream;

Control = Cheese prepared without any added whey protein;

LSM = Least-square mean; F = F ratio;
S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;
F ratios without asterisks denote 'not significant'.

Appendix 7.7 Effect of process treatments on the mean scores of sensory parameters of cheeses

Source of variation & levels	Flavour							
	Acidity		Saltiness		Bitterness		Oxidised	
	LSM	F	LSM	F	LSM	F	LSM	F
Control	2.74	57.8	3.1	0.75	1.5	2.45	1.5	3.17
Others	2.66	*	3.1		1.5		1.3	
Added whey protein (W)		9.0		1.92		30.3		2.15
W 2	2.8		3.1		1.4		1.3	
W 1	2.7		3.1		1.5		1.4	
Fat (F)		1.0		17.3		20.3*		0.4
F 2	2.7		3.0		1.4		1.3	
F 1	2.7		3.2		1.5		1.4	
Homogenisation (H)		111.0**		42.1*		4.0		0.03
H 1	2.8		3.1		1.5		1.3	
H 2	2.7		2.9		1.5		1.3	
H 3	2.8		3.3		1.5		1.3	
Storage time		1.54		4.77*		0.58		2.03
4 weeks	2.7		3.2		1.5		1.3	
18 weeks	2.8		2.9		1.5		1.4	
S.D.	0.26		0.23		0.14		0.20	

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk; W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein; F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;
H 2 = Denatured whey protein homogenised separately;
H 3 = Denatured whey protein homogenised in combination with manufactured cream;

Control = Cheese prepared without any added whey protein;
'Others' = Includes all trials (12 out of 15) in which denatured whey protein was incorporated;

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;

F ratios without asterisks denote 'not significant'.

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Appendix 7.7 continued

Source of variation and levels	Texture					
	Mouthfeel		Structure		Overall acceptability	
	LSM	F	LSM	F	LSM	F
Control	3.5	0.18	3.5	0.37	3.6	0.66
Others	3.2		3.2		3.6	
Added whey protein (W)		1.17		0.37		1.06
W 2	3.0		3.1		3.0	
W 1	3.5		3.3		3.4	
Fat (F)		0.00		0.27		0.11
F 2	3.2		3.2		3.2	
F 1	3.2		3.2		3.3	
Homogenisation (H)		0.16		0.01		0.06
H 1	3.4		3.3		3.3	
H 2	3.3		3.2		3.2	
H 3	3.0		3.1		3.1	
Storage time		1.39		1.9		0.57
4 weeks	3.2		3.1		3.2	
18 weeks	3.3		3.3		3.2	
S.D.	0.30		0.22		0.20	

W 2 = Incorporation of denatured whey protein (on DM basis) at the rate of 9.0 g/kg milk; W 1 = Incorporation of denatured whey protein (on DM basis) at the rate of 4.5 g/kg milk;

F 2 = Fat level in cheesemilk adjusted in proportion to added whey protein; F 1 = Fat level not adjusted;

H 1 = Denatured whey protein added without homogenisation;
H 2 = Denatured whey protein homogenised separately;
H 3 = Denatured whey protein homogenised in combination with manufactured cream;

Control = Cheese prepared without any added whey protein;
'Others' = Includes all trials (12 out of 15) in which denatured whey protein was incorporated;

LSM = Least-square mean; F = F ratio;
S.D. = Standard deviation of raw data;

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$;
F ratios without asterisks denote 'not significant'.

Appendix 7.8

- (a) Data on quantities of input and output material, the calculated values of mass balance, yields and recoveries of the milk solids for all the trials

N	CONTROL	QWP	PFR	HTYPE	MQTY	MTOT_FAT	MTOT_TS	MTOT_PTH	MTOT_CA	QWTY	WTOT_FAT	WTOT_TS	WTOT_PTH	WTOT_CA	QWTY	STOT_FAT
					(kg)	(kg)	(kg)	(kg)	(mm/kg)	(kg)	(kg)	(kg)	(kg)	(mm/kg)	(kg)	(kg)
1	1	0	0	0	35.000	1.5975	4.3860	1.25941	1305.5	28.965	0.0174	1.9493	0.282739	561.9	6.319	1.5808
2	0	-1	-1	2	36.415	1.7989	5.2146	1.41255	1281.3	29.380	0.0179	1.9631	0.290233	579.6	6.337	1.7776
3	0	1	-1	2	37.330	1.7175	5.2924	1.50984	1312.7	30.575	0.0184	2.0245	0.307259	579.7	7.401	1.7170
4	0	-1	1	1	36.550	2.0561	5.4572	1.43570	1323.1	29.345	0.0179	2.0354	0.287521	737.1	7.074	1.9948
5	0	-1	1	2	36.535	1.9636	5.3890	1.44212	1282.2	29.360	0.0179	2.0514	0.302906	686.7	7.053	1.9043
6	0	-1	1	3	36.550	1.9058	5.1821	1.43102	1275.4	29.730	0.0208	2.0276	0.290206	728.3	7.228	1.8648
7	1	0	0	0	35.000	1.7710	5.1100	1.30854	1281.0	29.035	0.0203	1.9947	0.268603	653.2	6.390	1.7316
8	0	1	-1	3	37.345	1.7731	5.4435	1.51157	1325.1	30.190	0.0181	2.0288	0.286992	621.9	7.550	1.5983
9	0	1	1	3	38.085	2.1480	5.3727	1.54985	1321.6	30.185	0.0332	2.0435	0.298499	682.1	8.180	2.0859
10	1	0	0	0	35.000	1.5765	4.9770	1.27504	1204.0	28.560	0.0200	1.9489	0.287076	601.3	6.530	1.7105
11	0	-1	-1	1	36.322	1.7689	5.2703	1.46224	1271.3	29.775	0.0238	2.0307	0.305843	565.7	6.740	1.7726
12	0	1	1	1	38.085	2.2013	5.9108	1.54499	1264.4	30.405	0.0365	2.0645	0.312314	605.0	7.325	2.1284
13	0	-1	-1	3	36.435	1.7817	5.2248	1.43517	1380.9	29.280	0.0234	2.0203	0.300758	644.1	7.445	1.5974
14	0	1	-1	1	37.371	1.7799	5.4383	1.55266	1367.1	30.195	0.0272	2.0714	0.321716	615.9	7.365	1.3089
15	0	1	1	2	38.330	2.3075	5.9833	1.50666	1418.2	29.355	0.0269	2.0451	0.325712	615.3	8.750	2.1525

N	CTOT_TS	CTOT_PTH	CTOT_CA	QWTY	STOT_FAT	STOT_TS	STOT_PTH	STOT_CA	QWTY	HL_QTY	HL_PRCNT	Y_M	Y_HLFMST
	(kg)	(kg)	(mm/kg)	(kg)	(kg)	(kg)	(kg)	(mm/kg)	(kg)	(kg)	(%)	(%)	(%)
1	2.9888	1.0401	736.1	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4902	1.37	18.05	17.079
2	3.2065	1.1602	731.5	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4722	1.27	18.77	17.511
3	3.2934	1.2654	751.2	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.5282	1.37	19.56	17.411
4	3.4096	1.1328	657.3	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.5052	1.35	19.30	18.506
5	3.4207	1.1879	691.1	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4962	1.33	19.25	18.574
6	3.3537	1.2451	650.5	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4662	1.25	19.72	18.301
7	3.0863	1.0844	586.2	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.3492	0.98	18.25	17.536
8	3.3507	1.3470	765.0	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.5792	1.51	20.32	17.301
9	3.7137	1.3360	705.9	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4942	1.27	21.47	19.302
10	2.9768	1.0363	646.4	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4842	1.35	18.94	17.010
11	3.1610	1.1610	649.7	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.5812	1.57	18.55	17.405
12	3.5464	1.2381	642.4	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.6292	1.62	20.54	19.149
13	3.2087	1.1827	798.1	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4842	1.30	20.43	17.513
14	3.4370	1.3046	808.5	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.5852	1.51	20.76	18.151
15	3.3150	1.2839	834.7	0.77	0.0006	0.0742	0.0292	26.9	0.0042	0.4992	1.28	22.32	19.906

N	FAT_BAL	CA_BAL	TS_BAL	PTH_BAL	MST_BAL	FR_M	FR_CW	CAR_M	CAR_CW	TSR_M	TSR_CW	PTNR_M	PTNR_CW	MSTR_M	MSTR_CW
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	100.00	97.42	99.557	654.97	98.49	98.98	98.97	55.24	56.71	60.25	60.52	30.71	78.52	10.30	10.97
2	99.77	100.18	97.746	637.46	98.90	98.78	99.00	55.39	55.79	60.52	62.02	30.48	80.54	11.38	11.50
3	101.00	99.35	99.093	612.18	98.56	99.93	98.93	56.07	56.44	61.36	61.93	77.20	80.46	12.35	12.53
4	97.36	103.33	98.440	618.60	98.59	96.99	99.11	48.73	47.15	61.64	62.61	77.33	79.75	11.49	11.64
5	97.36	105.25	100.162	646.47	98.43	96.94	99.06	52.79	50.16	62.51	62.51	30.74	79.58	11.37	11.55
6	98.71	105.37	102.375	670.31	98.17	97.31	98.39	49.94	47.17	63.30	62.32	35.26	31.09	12.04	12.24
7	98.39	102.41	98.010	645.30	99.20	97.74	98.33	52.47	51.23	59.53	60.74	31.06	30.14	10.30	10.38
8	96.77	102.57	97.495	635.39	98.57	95.74	98.94	56.58	55.15	60.72	62.28	32.10	32.43	13.07	13.24
9	98.62	102.93	96.310	621.08	99.08	97.08	98.43	52.34	50.35	62.44	64.50	79.57	31.73	13.57	13.59
10	103.18	101.40	97.516	647.38	98.34	101.99	98.34	52.51	51.78	58.93	60.43	79.45	78.30	11.39	12.03
11	101.52	93.62	97.140	627.49	98.56	100.17	98.57	50.04	53.45	59.14	60.38	77.34	79.15	11.27	11.42
12	98.31	96.60	95.421	590.33	98.93	96.66	98.31	49.74	51.49	60.92	63.35	73.95	79.35	12.71	12.36
13	96.55	102.44	98.581	537.71	98.71	95.24	98.53	56.59	55.33	60.55	61.36	79.59	79.72	13.27	13.45
14	103.12	102.18	99.925	616.95	98.25	101.39	98.52	57.99	56.75	62.34	62.39	77.57	30.21	13.36	13.60
15	94.42	100.31	96.740	627.79	99.09	93.25	98.76	57.76	57.57	62.97	65.10	78.49	79.76	14.93	15.07

Note: Full forms of the abbreviations are provided in the following page.

Abbreviations used in Appendix 7.8.a

N = Trial No;

Control = Includes trials without incorporation of denatured whey protein, designated as 1, 0 indicates 'others';

DWP = Level of incorporation of heat denatured whey protein :

0 is none (control), -1 is 4.5g/kg, 1 is 9.0 g/kg;

PFR = Protein to Fat ratio of milk: 0 is control, -1 is fat not adjusted in proportion to added Denatured Whey Protein (DWP),

1 is fat adjusted in proportion to added DWP;

Htype = Type of homogenisation: 0 is control, 1 is slurry containing DWP not homogenised, 2 is slurry containing DWP homogenised separately, 3 is slurry with DWP homogenised in combination with manufactured cream;

MQTY = Quantity of milk; MTOT_FAT = Total fat in milk;

MTOT_TS = Total TS in milk; MTOT_PTN = Total protein in milk;

MTOT_CA = Total Ca in milk; WQTY = Quantity of whey;

WTOT_FAT = Total fat in whey; WTOT_TS = Total TS in whey;

WTOT_PTN = Total protein in whey; WTOT_CA = Total calcium in whey;

CQTY = Quantity of cheese; CTOT_FAT = Total fat in cheese;

CTOT_TS = Total TS in cheese; CTOT_PTN = Total protein in cheese;

CTOT_CA = Total calcium in cheese; SQTY = Quantity of starter;

STOT_FAT = Total fat in starter; STOT_TS = Total TS in starter;

STOT_PTN = Total protein in starter; STOT_CA = Total calcium in starter;

RQTY = Quantity of rennet; HL_QTY = Handling loss of material expressed in terms of quantity;

HL_PRCNT = Handling loss of material as a percentage of milk used;

Y_TS = Yield of cheese expressed in terms of recovery of total solids;

Y_HLFM = Yield of cheese (constant moisture of 50%) expressed in terms of percentage of weight of milk;

FAT_BAL = Fat balance; CA_BAL = Calcium balance; TS_BAL = Total solids balance;

MST_BAL = Moisture balance;

FR_M = Recovery of fat in cheese expressed as a percentage of fat in milk and starter;

FR_CW = Recovery of fat in cheese expressed as a percentage of fat in cheese and whey;

CAR_M = Recovery of calcium in cheese expressed as a percentage of calcium in milk and starter;

CAR_CW = Recovery of calcium in cheese expressed as a percentage of calcium in cheese and whey;

TSR_M = Recovery of total solids in cheese expressed as a percentage of total solids in milk and starter;

TSR_CW = Recovery of total solids in cheese expressed as a percentage of total solids in cheese and whey;

PTNR_M = Recovery of protein in cheese expressed as a percentage of protein in milk and starter;

PTNR_CW = Recovery of protein in cheese expressed as a percentage of protein in cheese and whey;

MSTR_M = Recovery of moisture in cheese expressed as a percentage of moisture in milk, starter and rennet;

MSTR_CW = Recovery of moisture in cheese expressed as a percentage of moisture in cheese and whey.

Appendix 7.8 continued

(b) An example of mass balance calculation : mass balance of protein in trial no 2

Quantity of protein in milk

$$= \text{Quantity of milk} \times (\% \text{ protein} / 100) \\ = 36.415 \times 3.879 / 100 = 1.4125 \text{ kg}$$

Quantity of protein in starter

$$= \text{Quantity of starter} \times (\% \text{ protein} / 100) \\ = 0.770 \times 3.7897 / 100 = 0.0292 \text{ kg}$$

Quantity of protein in whey

$$= \text{Quantity of whey} \times (\% \text{ protein} / 100) \\ = 29.880 \times 0.9379 / 100 = 0.2802 \text{ kg}$$

Quantity of protein in cheese

$$= \text{Quantity of cheese} \times (\% \text{ protein} / 100) \\ = 6.837 \times 16.9708 / 100 = 1.1603 \text{ kg}$$

Protein balance (%) = 100 (Output of protein/Input of protein)

$$= \frac{[(\text{Protein in cheese} + \text{protein in whey}) \times 100]}{(\text{protein in milk} + \text{protein in starter})} \\ = (1.1603 + 0.2802) \times 100 / (1.4125 + 0.0292) \\ = 99.916 \%$$

Protein recovery on the basis of input (%)

$$= \frac{[(\text{Protein recovered in cheese} \times 100)]}{(\text{protein in milk} + \text{protein in starter})} \\ = (1.1603 \times 100) / (1.4125 + 0.0292) = 80.48 \%$$

Protein recovery on the basis of output (%)

$$= \frac{[(\text{Total protein recovered in cheese} \times 100)]}{(\text{protein in cheese} + \text{protein in whey})} \\ = (1.1603 \times 100) / (1.1603 + 0.2802) = 80.55 \%$$

Appendix 7.8 continued

(c) Effect of process treatments on the mass balance of selected milk constituents for each trial

Fat balance : Trial no 15 had the greatest loss of fat followed by trials 13 and 8. Other trials did not have much variation. In trial 15 increased fat level could be one of the causes for increased fat loss. This is because in other trials involving manufacture of cheese with higher fat level, there is also a lower fat recovery, though of a smaller magnitude. However, the common variable in trials 15, 13 & 8 was the 'type of homogenisation', *i.e.* homogenisation of manufactured cream in combination with the added heat-denatured whey protein. This might have caused the increased fat loss. It will be observed in the following Chapter (Chapter 8) that a greater amount of whey protein is firmly adsorbed to the fat globules when fat is homogenised in the presence of whey protein. It appears that this leads to coating of the fat globules with a water soluble protein and consequently failure of fat globules to be firmly embedded in the casein matrix, causing the increased fat loss through whey.

Calcium balance: The end-point (colour change) obtained during complexometric titration (method for estimation of calcium) is not very stable. This reduces the accuracy of the analytical method. The accuracy of the test-method has been reported to be ± 3.0 mM/kg cheese (Creamer *et al.*, 1985). Considering this variability, it was not surprising that some of the values were outside the limits. Overall, the calcium balance was interpreted as reasonable.

Protein balance: Increased loss of protein in trials 12, 4, 14 were noted. In all these trials the heat-denatured whey protein were incorporated without homogenisation. This is likely to have caused the increased loss. The increased levels of denatured whey protein and fat in these trials could also have added to the protein loss. Trial 3 also had an increased loss of protein and this may have been due to the increased amount of denatured whey protein incorporated. All other values of protein balance are reasonable.

TS balance: Increased loss of total solids in trials 12 & 15 may be related to the increased losses of protein and fat contents, respectively (already described).

Moisture balance: The data on moisture balance, ranged between 98.18 to 99.21%, appears very reasonable. As all the values are less than 100, it implies that errors mostly in terms of only losses have occurred. However, it is more likely that loss of moisture due to evaporation has had an over-riding effect on most other factors.

Conclusion: The overall mass balance was satisfactory and supports the findings, particularly with respect to the cheese yield.

Appendix 7.8 continued

- (d) Justification for the variations in the mass balances of milk components
 (i) Approximate estimates of loss or gain of weight of milk components due to experimental errors

Details of possible variation in weight of milk components due to some of the experimental errors

Sources of error	Variation in weight (g)	Effect on individual components			
		Fat (g)	TS (g)	Protein (g)	Ca ²⁺ (mM)
Variations due to weighing scale:					
- Weighing of FFMR	± 5	5.0	5.0	-	-
- Weighing of slurry	± 5	-	0.75	0.600	-
- Weighing of milk (Can+milk) 5+20	± 25	1.213	3.5	0.912	0.875
- Weighing of cheese Hoop & cheese(5+5)	± 10	2.50	5.0	1.646	1.166
- Weight of whey Can & whey (5+20)	± 25	0.015	1.585	0.255	0.485
Loss of material					
- Handling losses ¹	(-)	3.395	9.8	2.520	2.45
- Fines ²	(-)	5.0	10.0	3.292	2.33
Variation in composition of starter ³					
	(±)	-	19.25	0.670	0.674
Variation due to analyses ⁴					
- Milk	(±)	3.85 (0.011%)	14.70 (0.042%)	4.243 (0.002%)	12.95 (0.37%)
- Cheese	(±)	6.319 (0.1%)	12.006 (0.19%)	10.884 (0.027%)	6.32 (1.0%)
- Whey	(±)	5.8 (0.02%)	6.67 (0.023%)	11.841 (0.0024%)	10.73 (0.37%)
Total Variation ⁵					
(+)		24.697	68.461	31.051	33.200
(-)		-33.092	-88.261	-36.863	-37.98
Total quantity ⁶					
		1700.0	4960.0	1288.0	1334.0
Variation, %					
(+)		1.45	1.38	2.41	2.49
(-)		1.95	1.78	2.80	2.85

Note

- Details for moisture have not been calculated as it was expected to be inversely related to the TS.
- Details of footnotes are provided in the following page.

¹ The handling losses have been considered to be 70 g milk-equivalent. This estimate was calculated by assuming certain values as provided in the following break-up.

- sticking to sides of can - 15 g
- sticking to sides of knife - 5 g
- sticking to sides of vat and other parts - 50 g
- evaporation at manufacture and overnight draining (could lead to slight concentration of milk/whey)

² One source of loss of unaccounted fines was through whey. The composition of whey did not show these because whey was filtered prior to analysis. The other form of loss as fines occurred while turning the hoops. For calculation purposes it is taken as equivalent to 15 g cheese, though these could be much more depending on the quantity and method of incorporation of denatured whey protein.

³ Starters were not analysed on a regular basis for the composition. The possible variation has been taken as $\pm 2.5\%$ of the SNF content.

⁴ These values are computed from standard deviations of analytical data compiled (over 1 year) in Chemistry Lab of NZDRI.

⁵ Some of the assumptions made in arriving at these values are provided below. The actual values were always close to these figures.

	Quantity (kg)	Fat (%)	TS (%)	Protein (%)	Ca ²⁺ (mM/kg)
FFMR	1.700	100.0	100.0	-	-
Slurry	-	15.0	11.99	-	-
Milk	35.000	4.85	14.0	3.64	35.0
Whey	28.965	0.06	6.34	1.02	19.4
Cheese	6.319	25.0	50.0	16.46	116.5
Starter	0.770	-	9.75	3.83	36.0

⁶ Values obtained from trial no. 1 involving control cheese.

Appendix 7.8.d continued

(ii) Variations observed in the trials: Actual variations in the trials are about twice (Section 'a') of that estimated above. This may be attributed to some of the following sources of experimental error which could not be quantified but influenced the variability.

Variability in sampling: The accuracy of analytical results depends on the sample being representative. This, in turn, depends on homogeneity of the entire product. In this instance the cheesemilks were not very homogeneous because the incorporated heat-denatured whey proteins tended to sediment rapidly. This made initial sampling difficult. This difficulty was further experienced in redrawing another sample during analysis.

Loss of fines: Actual loss of fines are expected to be much more than the figures mentioned earlier. Increased losses were noticed in trials where fat level was adjusted in proportion to the added denatured whey proteins.

Block to block variation in cheese: A sample of cheese was drawn from only one block because the other blocks had to be used for storage studies. Thus, experimental error due to block to block variation could not be eliminated.

Quantity of milk: The operational losses are high when a small quantity of milk is handled.

(iii) Conclusion: It was concluded that the observed variations in mass balance values of the components of milk were within reasonable limits.

Appendix 7.9 Comparison of theoretical estimates of ratio of β -lactoglobulin to para- κ -casein (approximate estimates) with the observed ratios in cheeses incorporated with denatured whey protein

Proportion of whey protein retained in control cheese

Approximate values of protein composition in milk (Walstra & Jenness, 1984):

α_s -casein =	38.6%
β -casein =	28.4%
K-casein =	10.1%
β -lactoglobulin =	9.8%
α -lactalbumin =	3.7%

Protein in 1 kg milk (3.75% protein) = 37.5g

β -lactoglobulin in 1 kg milk = $(37.5 \times 0.098) = 3.68$ g

K-casein in 1 kg milk = $(37.5 \times 0.101) = 3.79$ g

Para- κ -casein in 1 kg milk = $3.79 (105 / 169)^1 = 2.35$ g

Observed ratio of β -lactoglobulin to para- κ -casein (from densitometer plots of SDS-PAGE) in control cheese = 0.45

To have this ratio, amount of β -lactoglobulin that needs to be retained in cheese from 1 kg of milk (assuming that all of para- κ -casein from milk is retained in cheese)

$$= 0.45 \times 2.35 = 1.06 \text{ g}$$

Thus, percentage of β -lactoglobulin retained in cheese =

$$(1.06 / 3.68) 100 = 28.8\%$$

Assuming that β -lactoglobulin in cheese is proportionate of the whey protein, the theoretical value of β -lactoglobulin retained in cheese = 28.8%

Total moisture retained in Feta cheese as a percentage of moisture in cheesemilk
 $\approx 12\%$

At least 12% of the native whey protein is expected to be retained with the moisture in cheese. In addition to this, whey protein that is in a denatured state

¹ Para- κ -casein constitutes 105 of the 169 amino acid residues in κ -casein.

would also be retained. Therefore, a figure of 28.8% of the original whey protein being retained in cheese appears reasonable.

Proportion of whey protein retained in cheese incorporated with heat-denatured whey protein

Assuming that the theoretical values calculated above were correct, the quantity of β -lactoglobulin in control cheese may be considered equal to 1.06 g, and the amount of para- κ -casein in control cheese equal to 2.35 g per kg of milk.

Amount of denatured whey protein incorporated per kg cheesemilk = 4.5 g

Amount of β -lactoglobulin in 4.5 g whey protein (using the earlier quoted percentages) = $[4.5 (9.8/13.5)] = 3.27\text{g}$

Percentage of added whey protein recovered in cheese (from mass balance data)
 $\approx 80\%$

Quantity of β -lactoglobulin that may be expected to have been recovered in cheese
 $= 3.27 (80 / 100) = 2.616\text{ g}$

Total amount of β -lactoglobulin in cheese

$$= 1.06 + 2.616 = 3.676\text{ g}$$

Theoretical ratio of β -lactoglobulin to para- κ -casein

$$= 3.676 / 2.35 = 1.564$$

Observed ratio of β -lactoglobulin to para- κ -casein (1.58) is thus close to the theoretically calculated value.

Similarly, when 9.0 g (DM basis) denatured whey protein was incorporated per kg cheesemilk, the calculated value for the ratio of β -lactoglobulin to para- κ -casein was found to be 2.68 which is again not very far from the observed value of 2.83.

Conclusion: It is concluded that (a) most of the added β -lactoglobulin, in the form of denatured whey protein, was present in cheese, and (b) there was no indication of decrease in the β -lactoglobulin in cheese after six months of storage.

Appendix 8.1 Effect of homogenisation and source of milk solids on the mean scores of sensory parameters of eight week old Feta cheeses

Source of variation	Acidity		Saltiness		Oxidised		Bitterness	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		6.0		2.35		1.05		0.0
1	2.8		3.2		1.5		1.5	
2	2.7		2.9		1.7		1.5	
Treatments		22.67**		1.54		4.70		6.40*
A(control)	3.2		2.8		2.2		1.7	
B	2.6**		3.0		1.3*		1.3*	
C	2.5***		2.9		1.6		1.3*	
D	2.8**		3.5		1.5*		1.6	
E	2.8**		3.2		1.5*		1.5	
S.D.	0.08		0.29		0.22		0.1	
C.V. (%)	2.83		9.4		13.56		6.76	

Source of variation	Structure		Mouthfeel		Overall acceptability	
	LSM	F	LSM	F	LSM	F
Replicates		6.92		2.42		0.0
1	3.0		3.3		3.0	
2	2.7		3.2		3.0	
Treatments		1.49		3.36		3.27
A (control)	3.0		3.1		3.4	
B	2.8		3.0		2.5	
C	2.9		3.6		3.2	
D	3.1		3.5		3.2	
E	2.8		3.2		2.8	
S.D.	0.18		0.18		0.30	
C.V. (%)	6.24		5.63		10.1	

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

F = F ratio; LSM = Least-square mean; S.D. = Standard deviation of raw data;

C.V. = Coefficient of variation;

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$;

F values without asterisks are 'not significant';

Individual LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 8.2 Composition of cheesemilks with respect to the experimental variations

Source of Fat (%) variation		Protein (%)		Lactose (%)		SNF (%)	
LSM	F	LSM	F	LSM	F	LSM	F
Replicates	4.07		3.39		1.53		1.55
1	4.38	3.25		4.89		8.85	
2	4.52	3.42		4.96		9.20	
Treatments	1.47		0.84		3.17		0.20
A(control)	4.37	3.29		4.93		8.92	
B	4.51	3.35		5.08		9.13	
C	4.50	3.30		4.88		8.88	
D	4.33	3.23		4.78		9.21	
E	4.54	3.34		4.96		8.99	
S.D.	0.11	0.08		0.087		0.441	
C.V. (%)	2.50	2.29		1.77		4.89	
Source of Ca ²⁺ variation (mM/kg)		FGS (μm)		Ca ²⁺ /SNF (g/100g)		P/F ratio (%)	
LSM	F	LSM	F	LSM	F	LSM	F
Replicates	11.86*		28.9**		2.43		0.31
1	32.8	1.41		1.49		0.744	
2	35.0	1.65		1.53		0.739	
Treatments	2.97		930.9***		3.51		1.04
A(control)	35.1	0.88		1.58		0.75	
B	35.3	0.88		1.55		0.74	
C	32.9	4.29***		1.49		0.73	
D	32.6	0.92		1.42		0.75	
E	33.7	0.66*		1.50		0.73	
S.D.	1.04	0.07		0.047		0.011	
C.V. (%)	3.06	4.69		3.08		1.52	

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

F = F ratio; LSM = Least-square mean;

S.D. = Standard deviation of raw data; C.V. = Coefficient of variation

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$;

F values without asterisks are 'not significant';

Individual LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 8.3 Effect of homogenisation and source of milk solids on the composition of whey

Source of variation	Fat (%)		Protein (%)		Lactose (%)		SNF (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		0.04		0.62		5.20		1.30
1	0.19		0.76		4.81		6.26	
2	0.19		0.77		4.95		6.37	
Treatments		11.06*		3.39		1.31		0.52
A(control)	0.15		0.73		4.84		6.27	
B	0.17		0.74		4.99		6.27	
C	0.33**		0.79		4.86		6.35	
D	0.14		0.78		4.78		6.26	
E	0.17		0.79		4.94		6.44	
S.D.	0.032			0.024		0.103		0.149
C.V. (%)	17.18		3.17		2.103		2.37	

Source of variation	Ca ²⁺ (mM/kg)	
	LSM	F
Replicates		5.84
1	19.5	
2	23.0	
Treatments		0.69
A (control)	19.6	
B	21.2	
C	21.9	
D	20.7	
E	23.2	
S.D.	2.28	
C.V. (%)	10.69	

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

F = F ratio; LSM = Least-square mean;

S.D. = Standard deviation of raw data;

C.V. = Coefficient of variation; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$;

F values without asterisks are 'not significant'; Individual LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 8.4 Effect of homogenisation and source of milk solids on the composition of Feta cheese (before brining)

Source of variation	Moisture (%)		pH		Ca ²⁺ (mM/kg)	
	LSM	F	LSM	F	LSM	F
Replicates		0.36		0.04		2.06
1	53.48		4.74		104.3	
2	53.16		4.73		113.1	
Treatments		17.27**		2.15		0.44
A (control)	55.23		4.75		112.3	
B	55.47		4.74		104.9	
C	49.16**		4.73		113.5	
D	53.26		4.77		103.1	
E	53.48		4.73		109.8	
S.D.	0.861		0.016		9.71	
C.V. (%)	1.62		0.34		8.94	

Appendix 8.5 Effect of homogenisation and source of milk solids on the composition of Feta cheese (after brining)

Source of variation	Moisture (%)		pH		Ca ²⁺ (mM/kg)	
	LSM	F	LSM	F	LSM	F
Replicates		0.08		6.05		0.06
1	49.9		4.66		103.6	
2	49.5		4.73		104.6	
Treatments		16.11*		0.25		1.59
A (control)	52.1		4.67		105.8	
B	51.7		4.70		97.8	
C	44.9**		4.69		111.1	
D	50.0		4.72		108.3	
E	49.9		4.71		97.7	
S.D.	0.96		0.042		6.87	
C.V. (%)	1.93		0.89		6.60	

A = Milk made from manufactured cream & reconstituted skim milk; B = Milk made from homogenised fresh cream & reconstituted skim milk; C = Milk made from fresh cream (unhomogenised) & skim milk; D = Milk made from manufactured cream and skim milk; E = Milk made from homogenised fresh cream and skim milk;
 F = F ratio; LSM = Least-square mean; S.D. = Standard deviation of raw data;
 C.V. = Coefficient of variation; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$;
 F values without asterisks are 'not significant'; Individual LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 8.6 Effect of homogenisation and source of milk solids on the composition of Feta cheese at three weeks

Source of variation	Fat (%)		Protein (%)		Moisture(%)		pH	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		13.27		0.31		0.22		2.67
1	26.9	*	17.0		48.62		4.70	
2	27.9		17.1		48.85		4.66	
Treatments		58.91***		28.42**		27.78**		0.47
A(control)	24.5		16.05		51.66		4.67	
B	25.7		16.30		50.89		4.71	
C	30.6***		18.85***		44.20***		4.67	
D	28.0**		16.97*		48.55*		4.70	
E	28.5***		16.94*		48.38*		4.67	
S.D.	0.44		0.291		0.782		0.035	
C.V. (%)	1.62		1.71		1.60		0.74	

Source of variation	NaCl (%)		S/M (%)		FDM (%)		MNFS (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		2.82		3.00		26.7**		6.05
1	4.19		8.6		52.3		66.5	
2	3.88		8.0		54.5		67.7	
Treatments		0.53		2.01		15.9*		12.49*
A(control)	3.83		7.4		50.6		68.4	
B	4.22		8.3		52.2		68.5	
C	4.01		9.1		54.7**		63.6**	
D	4.14		8.5		54.3**		67.4	
E	3.99		8.2		55.1**		67.6	
S.D.	0.288		0.59		0.68		0.79	
C.V. (%)	7.14		7.07		1.28		1.18	

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk; F = F ratio;

LSM = Least-square mean; S.D. = Standard deviation of raw data;

C.V. = Coefficient of variation;

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$;

F values without asterisks are 'not significant';

Individual LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 8.6 continued

Effect of homogenisation and source of milk solids on the composition of Feta cheese at three weeks

Source of variation	Ca ²⁺ (mM/kg)	
	LSM	F
Replicates		18.39*
1	102.1	
2	91.0	
Treatments		2.17
A (control)	98.7	
B	95.5	
C	102.8	
D	94.0	
E	91.9	
S.D.	4.09	
C.V. (%)	4.23	

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

F = F ratio; LSM = Least-square mean;

S.D. = Standard deviation of raw data;

C.V. = Coefficient of variation;

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$;

F values without asterisks are 'not significant';

Individual LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 8.7 Effect of homogenisation and source of milk solids on the composition of exudate from three weeks old Feta cheese

Source of variation	NaCl (%)		TS (%)		S/M (%)		Protein ¹ (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		10.3*		3.84*		10.1*		16.11*
1	7.49		15.10		8.8		2.46	
2	6.89		14.82		8.1		3.02	
Treatments		2.03		4.03		2.04		1.56
A (control)	6.68		14.60		7.8		2.77	
B	7.24		15.39		8.6		2.73	
C	7.44		15.20		8.8		2.63	
D	7.35		14.74		8.6		3.04	
E	7.26		14.88		8.5		2.52	
S.D.	0.298		0.231		0.37		0.22	
C.V. (%)	4.14		1.54		4.3		8.02	

Source of variation	Ca ²⁺ (mM/kg)	
	LSM	F
Replicates		0.86
1	147.7	
2	151.7	
Treatments		4.27
A (control)	135.1	
B	150.8	
C	161.2*	
D	155.8*	
E	145.5	
S.D.	6.86	
C.V. (%)	4.58	

¹ For convenience the total nitrogen in exudate is multiplied by the factor 6.38 to represent the protein content. This however includes proteins, peptides and amino acids.

A = Milk made from manufactured cream & reconstituted skim milk;

B = Milk made from homogenised fresh cream & reconstituted skim milk;

C = Milk made from fresh cream (unhomogenised) & skim milk;

D = Milk made from manufactured cream and skim milk;

E = Milk made from homogenised fresh cream and skim milk;

F = F ratio; LSM = Least-square mean; S.D. = Standard deviation of raw data;

C.V. = Coefficient of variation;

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$,

F values without asterisks are 'not significant';

Individual LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 8.8 Calculations for preparation of cheesemilk

Amount of cheesemilk to be taken in the vat =	35.25 kg
Less sample to be drawn =	0.25 kg
Net amount of cheesemilk taken in vat =	35.00 kg
Less FFMR present =	1.80 kg
Reconstituted skim milk in cheesemilk =	33.45 kg
Less SMP =	3.52 kg
Water in milk =	29.93 kg
Less water to be added with cream =	5.40 kg
Water to be added to make concentrated reconstituted skim milk (RSM) =	24.53 kg

Note

Concentrated RSM was prepared by blending 3.52 kg SMP in 24.53 kg water.

1.8 kg of FFMR was used for preparation of each batch of manufactured cream. Aqueous solutions of emulsifying agents were prepared by dissolving calculated amounts of emulsifier in 5.4 kg water at 60°C. A temporary emulsion of the mixture of the emulsifying agent, FFMR and water was created by mixing in the ultra-turrax homogeniser. This mixture of temporary emulsion was further homogenised to obtain manufactured cream. The manufactured cream was then added to calculated amounts of concentrated RSM to obtain cheesemilk.

In case of lecithin, it was dissolved in FFMR at 60°C. To this 5.4 kg of water was added. This was then blended by the ultra-turrax homogeniser.

While using SMP as the emulsifying agent, 4.530 kg concentrated RSM was made up to 5.4 kg (diluted to the desired concentration) with water, mixed with FFMR and homogenised. 4.53 kg water was added to the 'manufactured cream' and the rest of the total weight of cheesemilk was made up by adding concentrated RSM.

Appendix 8.9

(a) Effect of use of emulsifying agents on the mass balance of fat during cheesemaking

Source of variation	Weight of milk (kg)	Weight of whey (kg)		Weight of cheese (kg)	
	LSM	LSM	F	LSM	F
Replicate			1.12ns		0.99ns
1	35.053	28.439		6.915	
2	35.053	28.588		6.774	
Emulsifying agent			9.85*		8.15*
SMP (control)	35.000	28.438		6.890	
Sodium caseinate	35.039	28.288		6.975	
Sodium caseinate & lecithin	35.044	28.235		7.063	
Lecithin	35.108	28.957		6.586	
Tween-60	35.036	29.328*		6.037*	
WPC powder	35.090	27.835		7.516	
S.D.		0.243		0.246	

(b) Effect of use of emulsifying agents on the fat recovery based on input (milk) or output (cheese & whey)

Source of variation	Fat balance (%)		Fat recovery on input (%)		Fat recovery on output (%)	
	LSM	F	LSM	F	LSM	F
Replicate		0.07ns		0.35ns		4.39ns
1	99.6		93.1		93.6	
2	100.0		94.1		94.1	
Emulsifying agent		0.94ns		27.8**		1981***
SMP (control)	96.8		96.2		99.4	
Sodium caseinate	98.1		96.7		98.6	
Sodium caseinate & lecithin	99.6		98.2		98.6	
Lecithin	101.8		100.5		98.7	
Tween-60	101.2		73.2***		72.4***	
WPC powder	101.4		96.8		95.5***	
S.D.	2.96		2.71		0.34	

F = F ratio; L.S.M. = Least square mean;
 S.D. = Standard deviation of raw data; ns = not significant;
 * = $p \leq 0.05$; ** = ≤ 0.01 ; *** = ≤ 0.001 ; L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

Appendix 8.10 Effect of use of emulsifying agents on the mean sensory scores of eight week old Feta cheese

Source of variation	Acidity		Saltiness		Bitterness		Oxidised	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicate		4.24ns		1.03ns		2.35ns		1.4ns
1	2.6		3.4		1.6		1.2	
2	2.7		3.3		1.4		1.3	
Emulsifying agent	2.53ns		1.50ns		0.88ns		13.3*	
SMP (control)	2.6		3.7		1.6		1.1	
Sodium caseinate	2.6		3.2		1.3		1.1	
Sodium caseinate & lecithin	2.7		3.1		1.4		1.1	
Lecithin ¹	-		-		-		-	
Tween-60	2.8		3.4		1.5		1.6**	
WPC powder	2.5		3.2		1.6		1.1	
S.D.	0.09		0.28		0.21		0.08	
Source of variation	Mouth feel		Structure		Overall acceptability			
	LSM	F	LSM	F	LSM	F		
Replicate		2.29ns		0.72ns		1.19ns		
1	3.2		2.8		3.0			
2	3.0		2.7		2.9			
Emulsifying agent		7.45*		4.21ns		42.33**		
SMP (control)	3.1		2.6		2.8			
Sodium caseinate	3.0		2.7		2.5			
Sodium caseinate & lecithin	2.8		2.5		2.4*			
Lecithin ¹	2.4*		2.5		-			
Tween-60	4.0*		3.4*		3.7**			
WPC powder	3.0		2.7		3.1			
S.D.	0.27		0.24		0.12			

¹ Cheese made using lecithin was not evaluated for flavour characteristics and overall acceptability.

F = F ratio; L.S.M. = Least square mean;

S.D. = Standard deviation of raw data;

* = $p \leq 0.05$; ** = ≤ 0.01 ; *** = ≤ 0.001 ;

ns = not significant; L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

Appendix 8.11 Composition of cheesemilks for cheeses made with different emulsifying agents

Source of variation	Fat (%)		Protein (%)		Lactose(%)		SNF (%)		Calcium (mM/kg)	
	LSM	F	LSM	F	LSM	F	LSM	F	LSM	F
Replicate		0.64ns		16.03*		1.52ns		5.8ns		6.49ns
1	4.94		3.82		5.51		9.74		38.9	
2	4.98		3.90		5.56		9.87		37.9	
Emulsifying agent		1.42ns		24.41**		0.75ns		3.9ns		3.51ns
SMP (control)	4.84		3.78		5.51		9.69		37.5	
Sodium caseinate	4.94**		3.92		5.54		9.87		37.6	
Sodium caseinate & lecithin	4.96		3.93**		5.55		9.88		37.7	
Lecithin	5.05		3.72		5.49		9.63		39.6*	
Tween-60	5.00		3.79		5.61		9.80		39.1	
WPC powder	4.95		4.04***		5.53		9.97*		38.8	
S.D.	0.083		0.034		0.066		0.091		0.67	

F = F ratio; L.S.M. = Least square mean; S.D. = Standard deviation of raw data;
 * = $p \leq 0.05$; ** = ≤ 0.01 ; *** ≤ 0.001 ; ns = not significant;
 L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

Appendix 8.12 Effect of emulsifying agents on the composition of whey

Source of variation	Fat (%)		Protein (%)		Lactose (%)	
	LSM	F	LSM	F	LSM	F
Replicate		3.14ns		16.87**		2.99ns
1	0.39		0.92		5.09	
2	0.36		0.95		5.16	
Emulsifying agent	956.2***		42.75***		2.76ns	
SMP (control)	0.04		0.88		5.04	
Sodium caseinate	0.09		0.92*		5.17	
Sodium caseinate & lecithin	0.09		0.95**		5.27*	
Lecithin	0.08		0.91		5.07	
Tween-60	1.67***		0.91		5.08	
WPC powder	0.29***		1.05***		5.15	
S.D.	0.029		0.012		0.071	

Source of Variation	SNF (%)		Calcium (mM/kg)	
	LSM	F	LSM	F
Replicate		10.86*		0.62ns
1	6.62		25.1	
2	6.74		24.0	
Emulsifying agent		7.01*		0.96ns
SMP (control)	6.52		22.0	
Sodium caseinate	6.66		24.1	
Sodium caseinate & lecithin	6.77**		24.8	
Lecithin	6.59		24.7	
Tween-60	6.72*		24.4	
WPC powder	6.83**		27.4	
S.D.	0.062		2.49	

F = F ratio; L.S.M. = Least square mean;

S.D. = Standard deviation of raw data;

* = $p \leq 0.05$; ** = ≤ 0.01 ; *** ≤ 0.001 ; ns = not significant;

L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

Appendix 8.13 Effect of emulsifying agents on the composition of cheese (before brining)

Source of variation	Fat (%)		Moisture(%)		FDM (%)		MNFS (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicate		1.61ns		0.7ns		3.04ns		0.2ns
1	23.35		56.9		54.1		74.2	
2	24.17		56.0		54.9		73.9	
Emulsifying agent		6.13*		2.23ns		26.3**		1.9ns
SMP (control)	23.65		57.3		55.4		75.1	
Sodium caseinate	24.0		57.1		55.9		75.1	
Sodium caseinate & lecithin	24.15		56.1		54.9		73.9	
Lecithin	27.05*		52.9		57.4		72.5	
Tween-60	21.25		57.0		49.4***		72.3	
WPC powder	22.45		58.6		54.1		75.5	
S.D.	1.116		1.825		0.76		1.39	

Source of variation	pH		Calcium (mMoles/kg)	
	LSM	F	LSM	F
Replicate		2.15ns		0.58ns
1	4.79		106.5	
2	4.76		109.0	
Emulsifying agent		0.15ns		2.2ns
SMP (control)	4.79		106.2	
Sodium caseinate	4.76		106.4	
Sodium caseinate & lecithin	4.78		107.7	
Lecithin	4.78		107.5	
Tween-60	4.77		118.7	
WPC powder	4.77		100.3	
S.D.	0.039		5.70	

F = F ratio; L.S.M. = Least square mean;

S.D. = Standard deviation of raw data;

* = $p \leq 0.05$; ** = ≤ 0.01 ; *** ≤ 0.001 ; ns = not significant;

L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.

Appendix 8.14 Effect of emulsifying agents on composition of exudate from four week old Feta cheese

Source of variation	Calcium (mMoles/Kg)		TS (%)		Ca ²⁺ /TS (g/100g)	
	LSM	F	LSM	F	LSM	F
Replicates		7.37*		3.03		4.5ns
1	119.9		17.71		2.71	
2	109.3		17.54		2.50	
Emulsifying agent		2.17ns		8.98*		2.09ns
SMP (control)	126.8		17.55		2.89	
Sodium caseinate	111.2		17.58		2.54	
Sodium caseinate & lecithin	113.4		17.27		2.63	
Lecithin	118.8		17.60		2.71	
Tween-60	108.5*		18.33**		2.37*	
WPC powder	108.9*		17.44		2.50	
S.D.	6.78		0.172		0.177	

Source of variation	NaCl (%)		S/M (%)		Protein ¹ (%)	
	LSM	F	LSM	F	LSM	F
Replicate		1.16ns		1.26ns		1.65ns
1	10.03		12.19		2.03	
2	9.77		11.85		2.30	
Emulsifying agent		1.61ns		1.81ns		1.08ns
SMP (control)	9.94		12.06		1.97	
Sodium caseinate	9.62		11.67		2.18	
Sodium caseinate & lecithin	9.59		11.59		2.23	
Lecithin	9.68		11.75		2.72	
Tween-60	10.57		12.94		1.87	
WPC powder	10.01		12.11		2.03	
S.D.	0.412		0.523		0.404	

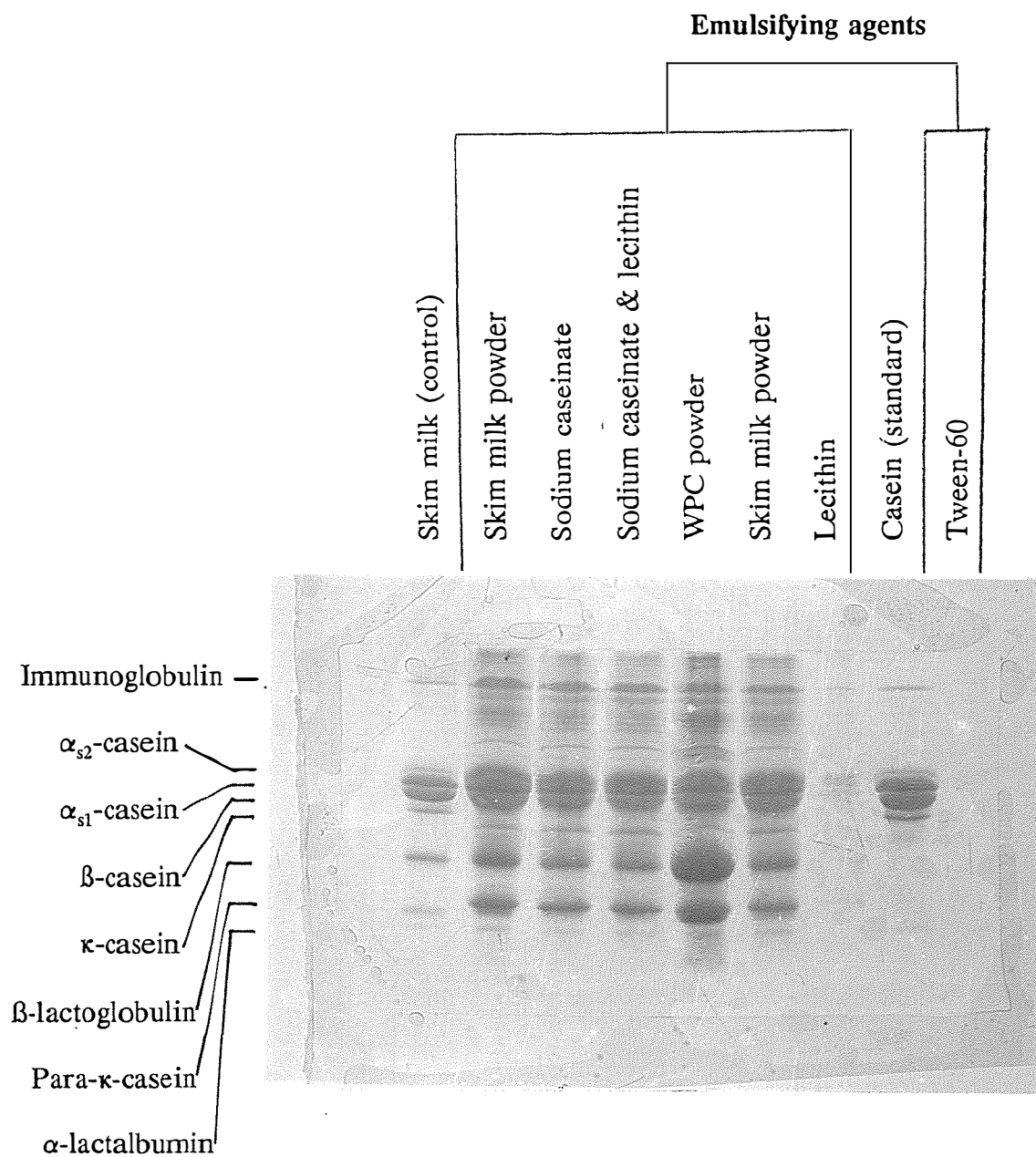
¹ For convenience the total nitrogen in exudate is multiplied by the factor 6.38 to represent the protein content. This however includes proteins, peptides and amino acids.

F = F ratio; L.S.M. = Least square mean;

S.D. = Standard deviation of raw data;

* = $p \leq 0.05$; ** = ≤ 0.01 ; ns = not significant;

L.S.M. values of individual emulsifying agents marked with asterisks show their significant variation in comparison to control.



Appendix 8.15 Proteins adsorbed to surface of fat globules in Feta cheeses made using different emulsifying agents (SDS-PAGE). Skim milk and standard casein have been used in the gel for identification of the protein bands in the experimental cheeses.

Appendix 8.16

Calculations to determine the distance between the fat globules in Feta cheese

Assumption: All the fat globules are spheres of same size and are equidistant from each other.

Let the size of the block of cheese be = 100 X 100 X 50 mm

Volume of the block of cheese = $5 \times 10^5 \text{ mm}^3 = 5 \times 10^{14} \text{ } \mu\text{m}^3$

Let the fat fraction in cheese = 27% (v/v)

Volume of milkfat in the block of cheese = $5 \times 10^{14} \times 0.27 \text{ } \mu\text{m}^3$

Let the diameter of the fat globule = 1 μm (Radius = 0.5 μm)

Volume of one fat globule =

$$(4 \times 22 \times 0.5 \times 0.5 \times 0.5) / (3 \times 7) = 0.5238 \text{ } \mu\text{m}^3$$

Total no. of fat globules in the cheese =

$$(5 \times 10^{14} \times 0.27) / 0.5238$$

Assuming that all the fat globules are spherical and equidistant from each other, the cheese can be subdivided into cubes equal to the number of fat globules.

Volume of each such cube in the cheese

$$= (5 \times 10^{14}) / [(5 \times 10^{14} \times 0.27) / 0.5238]$$

$$= 1.94 \text{ } \mu\text{m}^3$$

Each side of the cube = 1.247 μm

Distance between two fat globules = 1.247 - 1 = 0.247 μm

Calculations by the above method show that when the diameter of each fat globule is 5 μm the distance between two fat globules is 1.236 μm .

Appendix 9.1 Composition of milk for cheeses made with varying amounts of rennet

Variables & levels	Fat (%)		Protein (%)		Lactose (%)		SNF (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		0.19		0.89		0.37		0.49
1	4.53		3.35		5.05		9.09	
2	4.41		3.30		4.97		8.97	
Quantity of rennet		0.42		0.78		0.53		0.62
Low	4.49		3.37		5.09		9.16	
Control	4.37		3.29		4.93		8.92	
High	4.44		3.32		5.02		9.02	
S.D.	0.131		0.065		0.160		0.216	

Variables & levels	Ca ²⁺ (mM/kg)		FGS ^a (μm)		P/F ratio	
	LSM	F	LSM	F	LSM	F
Replicates		0.05		33.08*		0.29
1	36.4		0.813		0.752	
2	35.9		0.928		0.748	
Quantity of rennet		0.32		0.47		0.30
Low	36.4		0.863		0.750	
Control	35.1		0.885		0.753	
High	37.0		0.866		0.747	
S.D.	2.373		0.024		0.008	

^a Fat globule size expressed as mean diameter;

LSM = Least-square mean;

F = F ratio;

* p < 0.05;

F values without asterisks denote 'not significant';

S.D. = Standard deviation of raw data.

Appendix 9.2 Composition of whey as affected by the variation in the amount of rennet used

Variables & levels	Fat (%)		Protein (%)		Lactose (%)		SNF (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		0.43		1.05		0.22		112.0**
1	0.13		0.74		4.94		6.29	
2	0.14		0.71		4.88		6.19	
Quantity of rennet		0.19		0.11		0.38		32.14*
Low	0.14		0.73		4.99		6.27	
Control	0.15		0.73		4.84		6.27	
High	0.13		0.72		4.91		6.19*	
S.D.	0.025		0.032		0.166		0.011	

Variables & levels	Ca ²⁺ (mM/Kg)	
	LSM	F
Replicates		4.72
1	19.9	
2	20.7	
Quantity of rennet		4.75
Low	20.8	
Control	19.6	
High	20.7	
S.D.	0.432	

LSM = Least-square mean;

F = F ratio;

S.D. = Standard deviation of raw data;

* $p < 0.05$; ** $p < 0.01$;

F values without asterisks denote 'not significant'.

Appendix 9.3 Composition of Feta cheese (before brining) as affected by the variation in the amount of rennet used during cheesemaking

Variables & levels	Moisture (%)		pH		Ca ²⁺ (mM/kg)	
	LSM	F	LSM	F	LSM	F
Replicates		0.01		0.43		0.83
1	55.59		4.74		112.9	
2	55.48		4.75		110.4	
Quantity of rennet		0.19		1.29		0.25
Low	55.89		4.73		110.3	
Control	55.24		4.75		112.3	
High	55.48		4.76		112.3	
S.D.	1.071		0.019		3.36	

LSM = Least-square mean;

F = F ratio;

S.D. = Standard deviation of raw data;

F values without asterisks denote 'not significant'.

Appendix 9.4 Composition of Feta cheese (after brining) as affected by the variation in the amount of rennet used during cheesemaking

Variables & levels	Moisture (%) ^a		pH ^a		Ca ²⁺ (mM/kg)	
	LSM	F	LSM	F	LSM	F
Replicates		1.61		0.40		2.92
1	52.14		4.71		101.3	
2	52.2		4.71		103.3	
Quantity of rennet		127.9		1.90		0.11
Low	53.1		4.70		98.4	
Control	51.8		4.71		105.8	
High	51.6		4.72		102.9	
S.D.	0.095		3.82		6.35	

^a Raw data had missing values.

LSM = Least-square mean;

F = F ratio;

S.D. = Standard deviation of raw data;

F values without asterisks denote 'not significant'.

Appendix 9.5 Composition of Feta cheese (three weeks old)
as affected by the variation in the amount of
rennet used during cheesemaking

Variables & levels	Fat (%)		Protein (%)		Moisture(%)		pH	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		0.26		3.86		8.65		16.89#
1	25.63		16.16		50.74		4.72	
2	25.37		15.97		51.96		4.63	
Quantity of rennet		4.53		20.62*		4.56		2.51
Low	25.7		15.69		51.91		4.71	
Control	24.5		16.05#		51.66		4.67	
High	26.4		16.46#		50.49		4.65	
S.D.	0.64		0.119		0.504		0.025	

Variables & levels	NaCl (%)		S/M (%)		FDM (%)		MNFS (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		1.72		2.45		0.39		3.05
1	4.19		8.26		52.03		68.2	
2	3.90		7.50		52.80		69.6	
Quantity of rennet		1.59		1.10		2.26		1.44
Low	4.32		8.31		53.45		69.9	
Control	3.83		7.43		50.60		68.4	
High	3.99		7.91		53.21		68.5	
S.D.	0.277		0.597		1.48		0.96	

Variables & levels	Ca ²⁺ (mM/kg)	
	LSM	F
Replicates		8.94
1	99.0	
2	95.2	
Quantity of rennet		2.38
Low	95.4	
Control	98.7	
High	97.3	
S.D.	1.54	

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

= $p < 0.10$; * = < 0.05 ;

F values without asterisks denote 'not significant';

LSM values marked with asterisks show their significant
variation in comparison to control.

Appendix 9.6 Composition of exudate from Feta cheese (three weeks old) as affected by the variation in the amount of rennet used during cheesemaking

Variables & levels	NaCl (%)		TS (%)		S/M (%)		Protein ^a (%)	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		2.89		1.78		2.89		0.86
1	7.27		15.16		8.57		2.71	
2	6.66		14.60		7.80		2.82	
Quantity of rennet		0.97		2.34		0.81		67.94*
Low	7.28		14.53		8.52		2.04	
Control	6.68		14.60		7.82		2.61	
High	6.94		15.53		8.22		3.65*	
S.D.	0.435		0.515		0.55		0.14	

Variables & levels	Ca ²⁺ (mM/Kg)	
	LSM	F
Replicates		0.59
1	136.2	
2	137.0	
Quantity of rennet		15.19
Low	133.9	
Control	135.1	
High	140.8	
S.D.	1.33	

^a It includes whey proteins, peptides and amino acids. These values have been obtained by multiplying the total nitrogen content in exudate by a factor 6.38;

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

* $p < 0.05$;

F values without asterisks denote 'not significant';

LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 9.7 Effect of variation in the quantity of rennet used in cheesemaking on the sensory parameters of eight week old cheese

Variables & levels	Acidity		Saltiness		Oxidised		Bitterness	
	LSM	F	LSM	F	LSM	F	LSM	F
Replicates		12.0		3.69		0.08		0.57
1	3.0		3.2		1.7		1.7	
2	2.8		2.8		1.8		1.6	
Quantity of rennet		21.0*		1.56		2.71		1.0
Low	2.9		3.1		1.6		1.5	
Control	3.2		2.8		2.2		1.7	
High	2.7*		3.3		1.6		1.8	
S.D.	0.07		0.25		0.29		0.22	

Variables & levels	Structure		Mouthfeel		Overall acceptability	
	LSM	F	LSM	F	LSM	F
Replicates		1.0		3.0		3.57
1	3.2		3.4		3.3	
2	3.1		3.2		3.1	
Quantity of rennet		4.0		4.0		9.57
Low	3.2		3.5		3.0	
Control	3.0		3.1		3.4	
High	3.2		3.3		3.3	
S.D.	0.08		0.14		0.11	

LSM = Least-square mean; F = F ratio;

S.D. = Standard deviation of raw data;

* $p < 0.05$;

F values without asterisks denote 'not significant';

LSM values marked with asterisks show their significant variation in comparison to control.

Appendix 9.8 Approximate estimates for mass balance of rennet used in manufacture of Feta cheeses with variations in the quantity of rennet

Variables		Rennet activity (RU/kg)		Total rennet ¹ activity (RU)		Rennet activity recovered in cheese & whey (RU)	Rennet activity added to milk (RU)	Rennet activity recovered in cheese & whey (%)	Rennet activity recovered in cheese (%) ²
Replicates		-----		-----					
		Cheese	Whey	Cheese (7 kg)	Whey (33 kg)				
Low rennet (8ml/100Kg)	I	3.08	3.02	21.56	99.66	121.22	188.8	64.2	11.42
	II	4.48	2.97	31.36	98.01	129.37	188.8	68.5	16.61
Control (16ml/100Kg)	I	6.49	6.28	45.43	207.24	252.67	377.6	66.9	12.03
	II	8.29	5.59	58.03	184.47	242.5	377.6	64.2	15.37
High rennet (32ml/100Kg)	I	17.04	12.33	119.28	406.89	526.17	755.2	69.7	15.79
	II	18.51	11.98	129.57	395.34	524.91	755.2	69.5	17.16

¹ It is assumed that each trial yielded 7 kg cheese and 33 kg whey from a batch of 40 kg milk.

² This is to be multiplied by a correction factor (it is about 1.25 for Cheddar cheese) for the absolute values.

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