THE EFFECT OF pH SHIFT ON EARLY CHEESE MATURATION

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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1997

ABSTRACT

During the first few weeks of maturation, the texture of Cheddar-like cheeses changes substantially from an elastic material, often containing strongly oriented protein fibres from which some moisture can be expelled readily, to a uniform smooth-bodied cheese. In the past, proteolysis, particularly the cleavage of α_{s1}-casein, has been invoked as an important factor in the early changes in cheese texture. However, some of the textural changes that occur early in cheese maturation may be related to the redistribution of water within the cheese matrix. To examine this, a model cheese curd system was devised and explored.

Initially, cheese curd was prepared using starter and chymosin and the curd pH was controlled by varying the draining and salting pH values. The changes in water distribution, as measured by the quantity of centrifugal serum, seemed to be influenced by the cheese pH, but this could not be confirmed because of the continuing changes in cheese pH. Substitution of starter by dilute lactic acid to alter the pH value at setting provided a means of controlling the cheese pH during the 2-week periods of study. In some trials, glucono-δ-lactone (GDL) was used to reduce the pH of the cheese to the desired level after curd manufacture. This simulated the time-dependent pH change in a normal cheese such that the effects on the water-holding capacity, microstructure and rheological properties of the cheese could be studied. This model system of cheesemaking proved to be very effective in adjusting the pH of the cheese to the desired level. In the protocol finally adopted, milk was acidified with lactic acid and coagulated with Rennilase 46L®. After cheddaring, salting and light pressing, the curd was finely diced and mixed with GDL to give curd samples with comparable moisture contents and similar minimal casein proteolysis rates but different pH values.

The quantity of centrifugal serum decreased with a decrease in the set pH of cheese curds between pH 6.30 and pH 5.30. The maximum quantity was obtained from cheese curd set at pH 6.30 whereas no serum could be centrifuged from cheese curds set at pH 5.70 or lower. The quantity of centrifugal serum was essentially constant with time for

cheese curds of all set pH values. Lowering of the pH of cheese curds from the set pH by the addition of GDL also affected the quantity of centrifugal serum which decreased with a decrease in the adjusted pH value of the samples. The quantity of serum also decreased with time for all samples with adjusted pH values, the decrease being more rapid for samples with lower pH values.

The set pH was also found to influence the rheological characteristics of the cheese curds. Both the maximum force, from the large strain method developed for this project (Instron), and G′(stiffness), from the small strain method (Bohlin), showed a maximum for cheese curd with a pH of 5.90. The values for maximum force of cheese curds adjusted to lower pH values using GDL were in the same range as those of cheese curds of a similar set pH value. This suggested that the samples tended to attain a new rheological equilibrium with time that was consistent with the conditions of the lower pH value.

The microstructure of the cheese curds seemed to be determined by the pH at setting as changes in structure were apparent when cheese curds were made at different set pH values but not when the pH was altered from the set pH value using GDL. The changes resulting from the alteration in pH may have been too subtle for detection by the confocal microscopy technique used. Micrographs of cheese curds from which a serum phase could be centrifuged appeared to be less compact and had open spaces in the structure where water may have been present. Such areas were absent from the micrographs of cheese curd samples from which no centrifugal serum was obtained.

Overall, the present study showed that changes with time in the quantity of centrifugal serum, microstructure and some of the rheological properties in cheese curds took place even when proteolysis was at a minimum and when the pH was essentially constant. This suggests that physical changes in the cheese curd, such as redistribution of water within the cheese curd, are also important during the initial stages of ripening and probably contribute to the differences in rheological properties observed in young cheese.

ACKNOWLEDGEMENTS

I wish to express my deep sense of appreciation and gratitude to my chief supervisor Mr R. J. Bennett and co-supervisors Dr L. K. Creamer, Dr O. H. Campanella and Mr K. A. Johnston for the encouragement, enlightening discussions and the invaluable guidance received right through the duration of the research project and writing of the thesis. The last four years have been very rewarding for me because of the extensive knowledge and skills gained under their able guidance and has been enjoyable because of the warmth and support extended by all of them. My special thanks to Dr L. K. Creamer for his expert guidance during the research work and the preparation of the manuscript; Mr K. A. Johnston for facilitating the experimental work; and Mr R. J. Bennett and Dr O. H. Campanella for all their assistance in their areas of expertise.

I am extremely grateful to the New Zealand Dairy Research Institute (NZDRI) for providing the bulk of the facilities, materials, analytical support, etc. for the research work without which this project would not have been possible.

I wish to thank the following people for their help at different stages of the research work and the writing of the thesis:

* Mr C.G. Honoré for helpful discussions and keen interest in the research project.
* Dr P. J. Watkinson for guidance in the rheological analyses and helpful discussions.
* Mr A. B. McKenna, Dr Ranjan Sharma, Dr S. K. Lee, Mrs N. J. White, Mr C. C. Dodds, Ms S. M. Gibson, Mrs C. J. Coker, Mr R. Burr, Ms M. Y. Blair, Dr R. E. Rowland, Ms E. M. Nickless and Mr G. Radford for the help and guidance received in various specific analyses.
* Mr W. P. Fitzsimons, Mr G. B. Holdaway, Mr G. Taekema, Mr G. S. Boleyn and Mr M. J. Montgomerie for help during cheesemaking.
* The staff of the analytical chemistry Section, NZDRI for some of the analyses.
* Mr P. J. Le Ceve for the preparation of the photographs.
* Mr M. Dorsey for assistance with computer graphics.

I am grateful to the Ministry of Foreign Affairs and Trade, Government of New Zealand for the Commonwealth Scholarship awarded. I am also thankful to the Scholarships Officers, Mrs M. Gilbert at Massey, and Mrs C. King, Mrs L. Shepard and Mrs K. A. Manuera at the NZVCC for their constant support and encouragement.

I am specially thankful to Dr P. S. Kindstedt for making available some of his research findings before publication.

I am very grateful to all my friends at Massey and the NZDRI. I wish to express my sincere appreciation and indebtedness to all my supervisors, the staff at the Department of Food Technology, Massey University and the staff at NZDRI, and all my friends for being there for me at times of sorrow and joy. It has really been a pleasure getting to know all of them.

Finally, I am extremely grateful to everyone in my family for their continued moral support and encouragement. I could not have seen this through without them.

I wish to dedicate this thesis to the fond memories of my father who passed away during the course of this study.

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LIST OF ABBREVIATIONS

a.u.	Arbitrary units
A_w	Water activity
°C	Degree Celsius
cm	Centimetre
CLSM	Confocal laser scanning microscopy
CS	Curd system
EDTA	Ethylene diamine tetra-acetic acid
FDM	Fat in dry matter
Fig.	Figure
GDL	Glucono-δ-lactone
g	Gram
Hz	Hertz
h	Hours
HPLC	High performance liquid chromatography
kg	Kilogram
l	Litre
m	Metre
µg	Microgram
µl	Microlitre
µm	Micrometre
ml	Millilitre
mm	Millimetre
mmol	Millimoles
M	Molar concentration
mM	Millimolar concentration
min	minutes
MNFS	Moisture in non-fat substance
nm	Nanometre
NZDRI	New Zealand Dairy Research Institute

PAGE	Polyacrylamide gel electrophoresis
Pa	Pascals
P/F	Protein/fat
RU	Rennet units
rev/min	Revolutions per minute
s	Seconds
S/M	Salt/moisture
SEM	Scanning electron microscopy
SDS	Sodium dodecyl sulphate
TAHD	Texture analyser, heavy duty
TEM	Transmission electron microscopy
w/v	Weight/volume
w/w	Weight/weight
α	Alpha
β	Beta
γ	Gamma
δ	Delta
κ	Kappa
λ	Lambda

1.0 INTRODUCTION

Cheese is a product that undergoes several changes during the first few weeks after manufacture. An understanding of these changes and the factors contributing to them would enhance the ability to control the process and to obtain product of a better quality.

The Food and Agriculture Organisation defines cheese as "the fresh or matured product obtained by the drainage (of liquid) after the coagulation of milk, cream, skimmed or partly skimmed milk, buttermilk or a combination thereof" (Scott, 1981).

The above definition does not indicate the various stages the milk goes through before it is finally converted into cheese. The traditional manufacture of (Cheddar) cheese consists of: (a) coagulating milk, containing starter culture, with rennet; (b) cutting the resulting coagulum into small cubes; (c) heating and stirring the cubes with the concomitant production of a required amount of acid and whey; (d) whey removal; (e) fusing of the particles of curd into slabs by cheddaring; (f) milling the cheddared curd; (g) salting; (h) pressing; and (i) packaging and ripening. Each of these stages plays a role in determining the final composition and physical characteristics of the cheese made.

The major constituents of cheese are casein, fat and water. They contribute to the structure and texture of cheese. Casein forms an open mesh in which fat globules are entrapped. Water binds to the protein and fills the interstices, resulting in a viscoelastic matrix (Jack & Paterson, 1992). The basic structure of the casein network is laid down at the beginning of cheesemaking (Green *et al.*, 1981). This basic structure is modified during cheesemaking by the amount of acid produced, which alters the amount of calcium in the casein and thereby the degree of association of casein micelles in the network (Lawrence *et al.*, 1987). The texture of cheese depends upon its basic structure and the extent to which this basic structure has been modified (Lawrence *et al.*, 1983).

Most Cheddar-like cheese varieties are ripened for periods between 2 months and 2 years. During this period, the cheeses undergo numerous biochemical changes leading to the development of a characteristic texture, flavour and aroma. Proteolysis during cheese ripening is mainly responsible for the textural changes in cheese (Fox *et al.*, 1993).

Creamer & Olson (1982) reported that Cheddar cheese is held together by an extensive network of α_{s1}-casein molecules. Hydrolysis of α_{s1}-casein results in a major change in the texture of cheese (de Jong, 1976; Creamer & Olson, 1982). The texture changes markedly in the first 1-2 weeks of ripening as the hydrolysis by rennet of a small fraction of α_{s1}-casein to the peptide α_{s1}-I results in a general weakening of the casein network (de Jong, 1976; Creamer & Olson, 1982; Lawrence *et al.*, 1987). The relatively slow change in the 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, microbial enzymes, salt-in-moisture content, casein to moisture ratio, pH and storage temperature (Lawrence *et al.*, 1987; Farkye *et al.*, 1991; Fox *et al.*, 1994). As hydrolysis progresses, increasing amounts of water that were previously available for protein solvation become incorporated into the new amino and carboxyl groups of the peptide fractions, making the cheese harder and more brittle with age (Stanley & Emmons, 1977; Creamer & Olson, 1982).

During maturation the structural details become less clear with time (Dean *et al.*, 1959; Kalab, 1977; Stanley & Emmons, 1977). Kimber *et al.* (1974) observed that the boundaries between fat globules tended to disappear and that fat was surrounded by "debris" consisting probably of hydrolysed protein in cheese as it approached maturity. Kalab (1977) observed the presence of curd junctions formed as a result of cutting of the coagulum and milling of the cheddared curd in mild Cheddar cheese and that the number of these junctions was fewer in old cheese.

However, the changes taking place during the early stages of ripening are not as well understood. Some of the textural changes that occur early in cheese maturation may be

related to the redistribution of water within the cheese matrix. The amount of water present in Cheddar cheese is determined by the extent of heat treatment of the curd during cooking (scalding), agitation, decrease in pH, salting and syneresis during cheesemaking. In a mature cheese, the amount of water bound to the casein and the presence of free water influence the rigidity of the casein network, and the texture of the cheese (Prentice *et al.*, 1993).

There are very few reports on changes in the water phase and the cheese texture that occur during the initial period of ripening, especially the first 2 weeks. The early changes in Mozzarella cheese have been studied to a certain extent.

The Mozzarella cheese manufacturing process is similar to that of Cheddar cheese until the curd is milled. In the process employed in the United States, the cheddared curd is milled and stretched in hot water at about 60 °C for 6-10 min. The hot curd is then moulded into blocks and brine salted at 4 °C. Thermophilic starters and enzymes present in the curd influence the rate and extent of proteolysis during aging. In comparison, the manufacturing process followed in New Zealand uses a higher stretching temperature and dry salting of the cheese curd. The higher stretching temperature used inactivates most of the starter enzymes and chymosin with mainly the heat-resistant plasmin being active during the aging of the cheese.

Rapid and extensive proteolysis during the ripening period in Mozzarella cheese made in the United States has been reported (di Matteo *et al.*, 1982; Farkye *et al.*, 1991). However, Creamer (1976a) observed less proteolysis in Mozzarella cheese made in New Zealand compared with that in Cheddar and that proteolysis was dependent on chymosin and starter enzymes surviving the stretching temperatures. Proteolytic changes during aging are strongly influenced by manufacturing factors such as: choice of starter culture and coagulant; levels of indigenous proteinases; and the thermal conditions (*i.e.* time/temperature/pH) during cooking and stretching (Kindstedt, 1993). Kindstedt *et al.* (1991) reported nearly 70% decrease in intact α_{s1}-casein during aging of Mozzarella cheese made with chymosin, whereas intact β-casein showed only slight change.

Guo & Kindstedt (1994, 1995) examined age-related changes in the water phase of one type of Mozzarella cheese using the separation procedure of centrifuging the cheese at 12 500 ***g*** at 25 °C. They observed dramatic increases in water-holding capacity of the cheese during the first 2 weeks of aging, as evidenced by steep declines in the quantity of "expressible serum". Decreases in the quantity of expressible serum during storage were accompanied by increases in the protein content of the serum, particularly in the content of β-casein. Urea-polyacrylamide gel electrophoresis (PAGE) showed that unhydrolysed α_{s1}-, α_{s2}- and especially β-casein accounted for a large proportion of the protein in the expressible serum.

Clearly, an understanding of the effects of the early stages of cheesemaking would be of considerable benefit in estimating how to bring about desirable changes in the properties of mature cheese. The major objective of this study was to determine the effects of the cheesemaking parameters on the early stages of ripening. In order to do this, a model system was developed so as to provide a reliable means with which changes in the water distribution, cheese microstructure and some of the textural attributes could be studied. Consequently, changes in cheese composition and microstructure needed to be measured with time and correlated with one another and with changes in the cheesemaking parameters. As the project progressed, it was found that new tests and techniques had to be investigated or devised to measure some of the cheese characteristics.

2.0 REVIEW OF LITERATURE

The major aim of this study was to determine the effects of cheesemaking parameters on the early stages of cheese ripening and to gain an understanding of the normal process of cheese ripening. The major cheese classes of interest included Cheddar and Mozzarella. This limited review of the relevant literature concentrates on the factors that affect the early stages of maturation and some of the consequences for mature cheese. In addition, the background relevant to some of the electrophoresis, microscopy and rheological techniques used and the rationale for the selection of particular techniques are covered.

2.1 THE PROCESS OF CHEESE MANUFACTURE

Several stages of the cheesemaking process, including preparation of the milk for cheesemaking, coagulation, cooking, cheddaring, milling, salting, pressing and ripening, influence the structure and texture of cheese and also its water-holding properties. These are discussed in the various sections of this review. It is appropriate at this stage to briefly discuss the cheesemaking process for a better understanding of the changes that occur in cheese during the initial period of ripening. The information was gathered from several sources, including Scott (1986), Lawrence *et al.* (1993) and Kindstedt (1993).

The changes with time in water distribution of Cheddar, Cheshire and Mozzarella cheeses were studied during an initial trial. The manufacture of Cheddar cheese is described in detail (Section 2.1.1). The differences in the manufacture of Cheshire (Section 2.1.2) and Mozzarella (Section 2.1.3) cheeses from that of Cheddar are briefly outlined.

2.1.1 Cheddar cheese manufacture

The flow chart for the manufacture of Cheddar cheese by the process typically used at the New Zealand Dairy Research Institute (NZDRI) pilot plant is shown in Fig. 2.1.1. The various stages of manufacture are briefly described.

(1) Preparation of cheese milk: Raw milk of good bacteriological quality is usually pasteurised, a portion is separated and skim milk and whole milk are mixed to standardise to a typical protein to fat ratio of 0.8. The milk is pasteurised by heating at 72 °C for 15 s in order to destroy any pathogenic organisms. It also alters the numbers of other microorganisms and enzymes present in the milk. Standardisation is necessary to achieve the desired fat-in-dry matter (FDM) content of the cheese. Excess fat in the milk decreases moisture loss from the curd and increases the moisture in the non-fat substance (MNFS) and high fat cheeses tend to have a weak body. The standardised milk is cooled to 32°C and pumped into the cheese vat.

(2) Addition of starter culture: Mesophilic starters, which have their growth optimum around 30 °C, are used. The quantity of starter used, the strain types and the strain ratios determine the rate and extent of acid production during cheesemaking. The starter strains are selected on the basis of temperature sensitivity, phage resistance and acid-producing ability. For cheesemaking at the NZDRI, three strains of *Lactococcus lactis* subsp. *cremoris* (formerly *Streptococcus cremoris*) prepared as bulk starter are added to the cheese milk at a level of about 1.8% w/w.

(3) Setting: Setting refers to the conversion of milk into a semi-solid gel through the action of the added coagulating enzyme. Calf rennet (which contains the enzyme chymosin) is the coagulating agent traditionally used. The proportion of rennet added to the cheese milk at 32 °C should be the minimum necessary to give a firm gel in 30-40 min and is typically 10-12 ml/100 l milk. Modifications to achieve a similar firmness of the coagulum throughout the season may involve the addition of calcium chloride and/or an increase in the temperature of the milk at which the rennet is added.

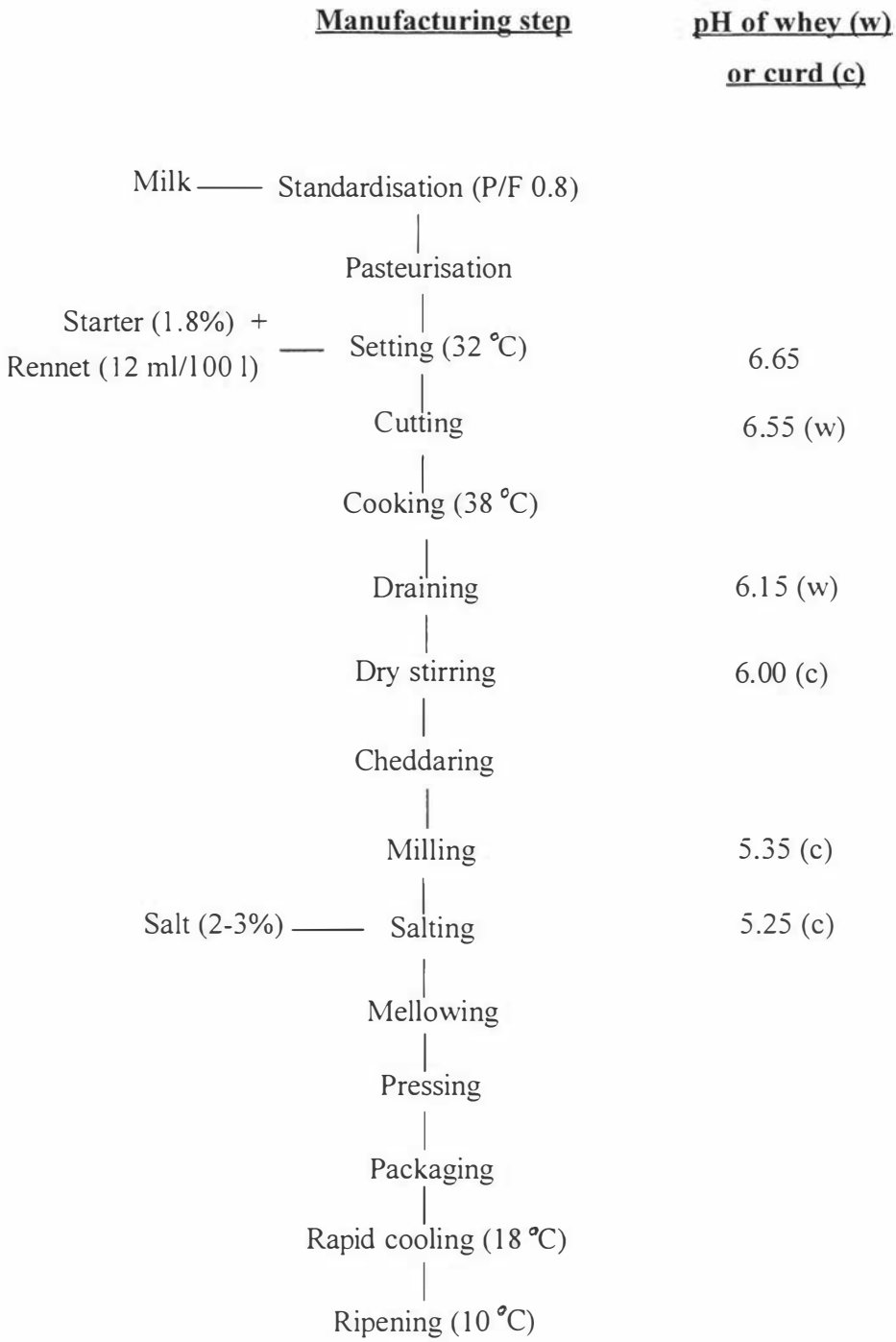

Fig. 2.1.1. *Flow chart for the manufacture of standard Cheddar cheese at the NZDRI pilot plant.*

Following the addition of rennet and thorough mixing, the milk is left undisturbed during coagulation. After about 40 min, the gel is sufficiently firm and ready to be cut.

(4) Cutting: The objective of cutting is to allow moisture loss or syneresis. By cutting the coagulum into small cubes of approximately 8 mm size, the surface to volume ratio is greatly increased facilitating easier moisture expulsion as the moisture has much less distance to travel to be expelled. The coagulum should be of the desired firmness at cutting. If it is weak or too firm, excess fines are generated which would be lost in the whey, reducing the yield. Damage to the curd also results in fat losses.

(5) Stirring/cooking: The mixture of curd and whey is stirred to prevent the curd particles matting or fusing together. The objective of cooking (in which the temperature is usually raised from 32 to 38 °C over a period of about 35 min) is to control acid development as well as to increase moisture expulsion. The combination of particle size, stirring rate and cooking regime controls the moisture content of the curd. The rate at which heat is applied is important. If the rate is too fast, "case hardening" or the development of a "skin" on the surface of the curd particles occurs preventing moisture expulsion.

The rate and the extent of acid development are dependent on the cooking temperature. The optimum growth temperature for starter bacteria is 29-30 °C. As the temperature increases during cooking, acid production by starter will be slowed down and depending on the temperature sensitivity of the strain, the numbers of viable starter will start to decrease. Cooking temperature thus helps to control both the rate of acid production in the vat and the number of organisms in the final cheese. The pH drop in the vat encourages syneresis, inhibits the growth of undesirable organisms and results in a concomitant loss of colloidal calcium phosphate from the casein submicelles. The pH also determines the proportions of residual calf rennet and plasmin in cheese. Cooking is continued until the desired whey pH of about 6.25 is reached. By the end of cooking, the curd particles are firmer, oval in shape and about one-third their initial size.

(6) Draining: Draining or "running" refers to the separation of the curd particles from whey. This is achieved by draining the whey through a screen to retain the curd. The whey pH at this stage is typically 6.15.

(7) Curd drying: The curd is dry stirred or agitated (by hand) for about 10 min to facilitate further removal of whey. The pH at the end of this stage is about 6.00.

(8) Cheddaring: This is essentially a holding stage to allow the curd to fuse or mat together. A series of operations consisting of packing, turning, piling and repiling the slabs of matted curd constitutes the cheddaring process. The influence of physical forces – pressure and flow – results in the development of a fibrous texture in the mass of curd. The pH continues to drop and is about 5.35 at the end of cheddaring.

(9) Milling: The milling operation consists of mechanically cutting the cheddared curd in small pieces. This increases the surface area to assist uniform salt distribution into the curd, and to encourage whey drainage. The cheddared blocks are cut into chips of about 15 mm × 15 mm × 150 mm in size. After milling, the curd is usually left for 2-3 min to allow for some loss of moisture so that the surface becomes wet and improves the adhesion of granular salt.

(10) Salting: Salt is added at 2-3% on a weight basis and thoroughly mixed with the milled curd. The salt crystals dissolve in the moisture on the surfaces of the milled curd particles and form a brine. Concentration differences result in a flow of water from within the curd and the subsequent release of some water, and the movement of salt in the opposite direction. The proportion of moisture in the curd, the pH of the curd and the amount of salt added all affect the final salt content of the curd, which influences the final pH and the overall flavour and texture of the cheese and controls the growth of microorganisms. Salt that is not absorbed by the curd separates as white whey or salt whey.

(11) Pressing: The pressing operation involves the compression of the curd particles into a block of cheese. In close-textured cheeses such as Cheddar, the curd is often vacuum pressed. This allows the curd particles to fuse together into a solid block of cheese. Free whey or moisture is also expelled during pressing. Traditionally, curd is filled into hoops and pressed overnight in a hydraulic press. In the modern block-forming system, the column of curd consolidates in a tower under vacuum and its own weight. The residence time in the tower is typically 30 min.

(12) Packaging: Individual 20 kg blocks of cheese are vacuum packaged in plastic bags that are designed to prevent moisture loss and act as an oxygen barrier. Removal of air and proper sealing of the bags are important to prevent mould growth. The cheese blocks are then placed in cardboard cartons which protect the plastic bag and also the cheese.

(13) Rapid cooling: Rapid cooling of the cheese from 30 to 18 °C within the next 24 h is critical in reducing the growth of non-starter lactic acid bacteria which can cause off-flavours in the cheese. During this process, the cheese also "firms" considerably, and the surface becomes smooth and shiny as the curd particles knit together.

(14) Ripening: The cartons of cheese are stacked on to pallets and transferred into ripening or curing rooms. The cheese is initially stored at 10 °C until the initial evaluation at about 30 days after manufacture. After this, the cheese goes into coolstores and is held at temperatures in the range of 2-10 °C for a period of 6 months or longer depending on the maturation required. Considerable changes in texture occur during ripening as a consequence of proteolysis. The rubbery texture of fresh cheese changes rapidly to a more brittle cheese. The biochemical changes during cheese ripening due to the activity of microorganisms and enzymes also lead to the development of the typical Cheddar cheese flavour.

2.1.2 Cheshire cheese manufacture

Cheshire cheese differs markedly from Cheddar as a consequence of the high level of acidity developed prior to the formation of the rennet coagulum. The manufacturing process for Cheshire cheese (Fig. 2.1.2) is essentially similar to that for Cheddar, with the following differences. It traditionally involves addition of starter at a higher level and a priming step, at the end of which a pH of about 6.20 is attained. The pH values at draining (pH 5.90) and salting (pH 5.05) are much lower than those for Cheddar. These result in a low pH (less than 4.9 at one day) and a low mineral content (less than 170 mM calcium/kg cheese) in Cheshire. The texture is therefore shorter and its flavour sharper than that of Cheddar.

2.1.3 Mozzarella cheese manufacture

The manufacture of low moisture Mozzarella cheese, as outlined in Fig. 2.1.3, is quite similar to that of Cheddar cheese until the curd is milled at pH 5.2, with some notable exceptions. Milk is standardised to a protein to fat ratio of 1.5. In the process traditionally followed in the United States, a mixed culture consisting of *Streptococcus salavarius* subsp. *thermophilus* (formerly *Streptococcus thermophilus*) and *Lactobacillus helveticus* prepared as bulk starter is used. These thermophilic bacteria thrive at the higher cooking and cheddaring temperatures (38-42 °C) used. The pH is about 6.10 at draining. After cheddaring to pH 5.2, the Mozzarella curd is milled and subjected to a kneading and stretching process in hot water at about 70 °C for 6-10 min. The hot plastic curd is moulded into 2.5-10 kg blocks, cooled to 4 °C and salted in chilled brine (4 °C). The cheese is stored for about 3 weeks before despatch.

In the New Zealand process, hot water at a temperature of 62-81 °C is used during the stretching process depending on the water to curd ratio, with the curd reaching a temperature of 58-64 °C. The Mozzarella curd is either dry salted or brine salted. Sometimes, a combination of dry and brine salting is used. Details of the manufacturing process have not been shown in the flow chart because of commercial sensitivity.

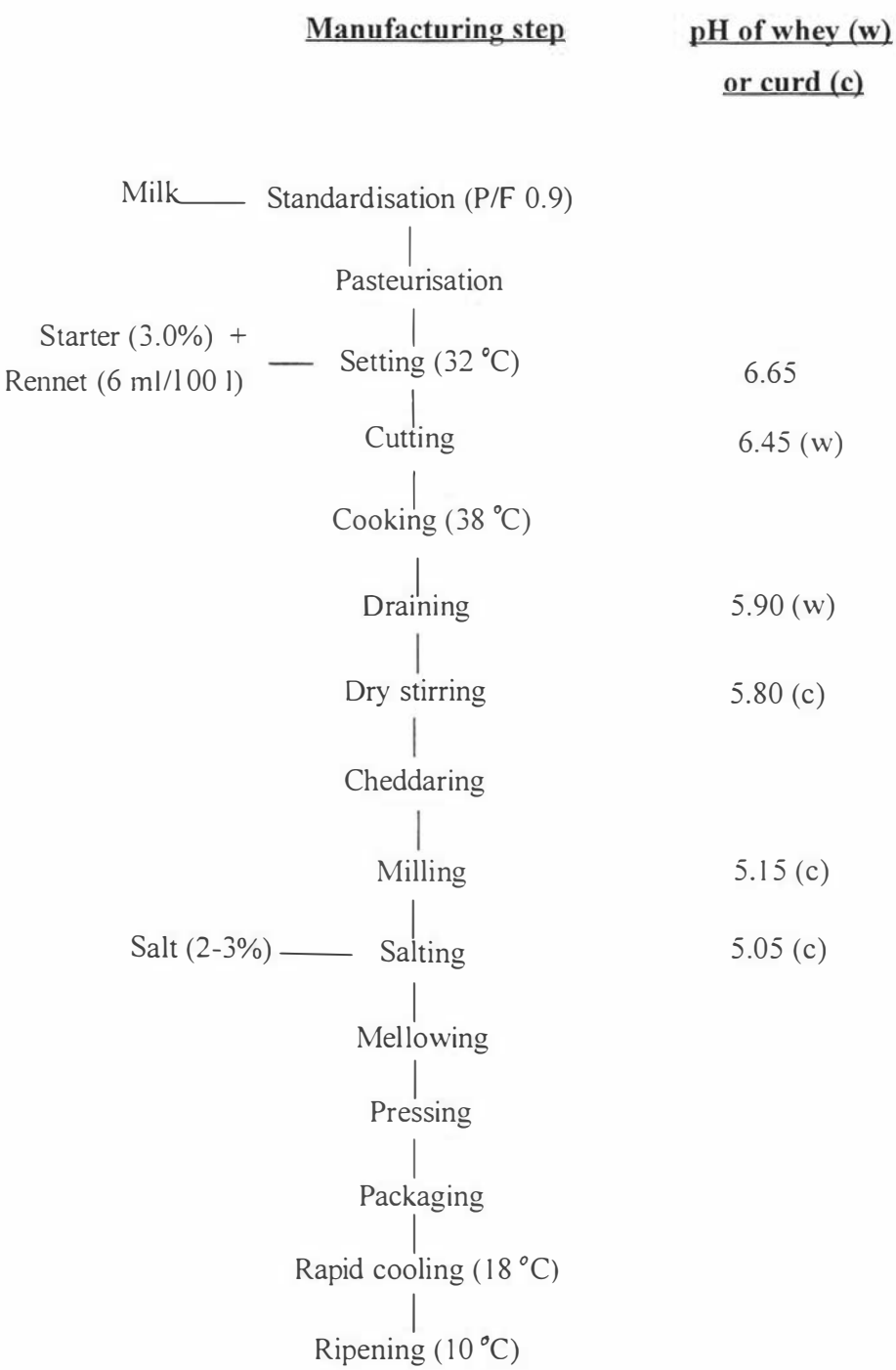

Fig. 2.1.2. *Flow chart for the manufacture of standard Cheshire cheese at the NZDRI pilot plant.*

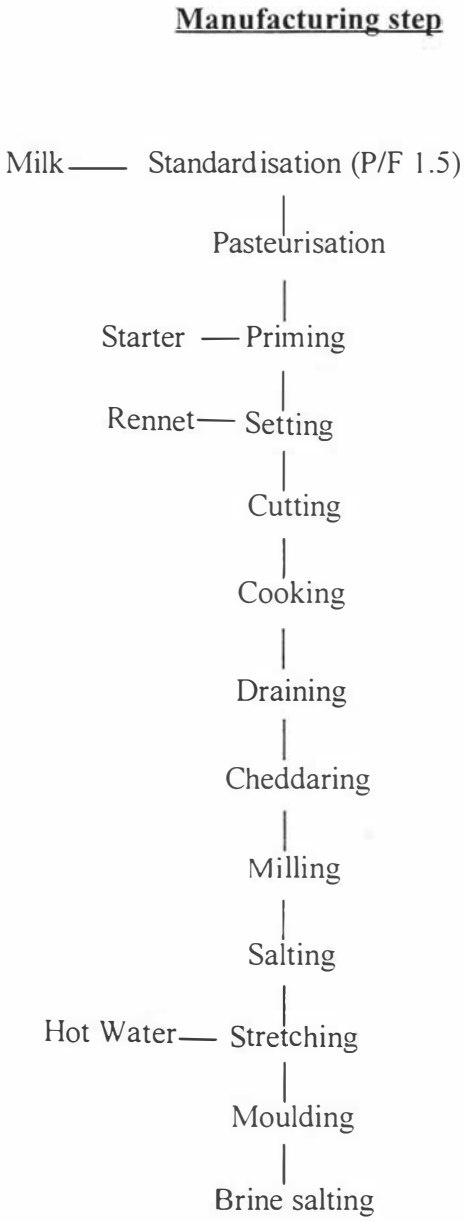

Fig. 2.1.3. *Flow chart for the manufacture of standard Mozzarella cheese at the NZDRI pilot plant.*

2.2 MILK COAGULATION AND CURD SYNERESIS

The addition of a coagulating enzyme, such as rennet, to milk results in the formation of a gel. The process continues as the gel becomes firm and whey is released from the gel due to syneresis. Syneresis is one of the factors that determines the moisture content of the curd. The processes of gel formation and syneresis are described.

2.2.1 The mechanism of gel formation

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 a predominantly hydrophilic outer layer. The submicelles are clustered into spherical aggregates with interstitial moisture and are most probably kept together by colloidal calcium phosphate (Walstra *et al.*, 1985).

The addition of an appropriate enzyme such as chymosin to milk leads to partial proteolysis of the κ-casein which is specifically cleaved at the bond Phe_{105}-Met_{106} yielding para-κ-casein, which is strongly hydrophobic, and a family of hydrophilic peptides (macropeptides) containing variable amounts of carbohydrates. The hydrolysis of κ-casein alters the charge distribution of the casein micelles (measured by the zeta potential) thereby reducing the intermicellar repulsive forces and the colloidal stability of the casein micelle system. In the presence of ionic calcium, the paracasein micelles (the casein and casein fractions that have been acted upon by the chymosin) interact to form chains, which eventually cross-link to form a gel matrix (Carlson *et al.*, 1986). The reaction rate increases with an increase in temperature as long as the enzyme is stable and is influenced by salts probably by promoting or reducing the binding between the enzyme and the substrate. A model proposed by Carlson *et al.* (1986) suggests 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 aggregation of casein micelles starts after about 90% of the κ-casein has been cleaved. The micelles form chains which then cross-link to form a network. Micelles are initially linked by bridges, which later on contract and bring the particles into contact and eventually cause partial fusion (Green *et al.*, 1978) resulting in a three-dimensional gel network. Thereafter, the gel continues to get firmer possibly by forming more linkages between micelles (Kimber *et al.*, 1974). The overall gel structure may be visualised as a casein sponge in which fat globules, bacteria and whey are entrapped.

2.2.2 Effect of pH on coagulum composition

At a higher pH, there is an increase in the negative charges on the casein molecules leading to charge repulsion and weakening of the bonds between molecules in the network (Jack & Paterson, 1992). Hydrophobic interactions, which impart stability to the matrix structure, are weakened by the absorption of water by the proteins to solvate the ionic charges. Precipitated casein may dissolve if sufficient water is present, but calcium ions bound tightly to the protein limit solubility. At pH 6.7, most of the casein and inorganic phosphate are a part of the casein micelles, but they become soluble when the pH is decreased. As the pH of milk is reduced, there is a concomitant loss of colloidal calcium phosphate from the casein micelles. At pH 5.3, all the "inorganic" phosphate is transferred to the serum, but about 14% calcium remains in the micelles associated with the "organic" phosphate of the caseins (van Hooydonk *et al.*, 1986). The remaining calcium dissolves at a lower pH. The solubilisation diminishes the interaction between the proteins, and this may cause swelling and dissociation of casein. On the other hand, electrostatic repulsion between the casein molecules will diminish due to a decrease in the negative charge within the protein (Walstra & Jenness, 1984). Collectively, these effects cause a maximum in the solubility of the proteins and in the voluminosity of the protein particles around pH 5.4-5.6 (Roefs *et al.*, 1985; van

Hooydonk *et al.*, 1986). The tendency of the protein aggregates to absorb water limits the amount of interstitial water present in high pH cheeses, whereas low pH cheeses are porous masses of caseins enclosing fat particles (Creamer & Olson, 1982).

2.2.3 Cheesemaking by chemical acidification

Acidulants such as hydrochloric, lactic or acetic acid and glucono-δ-lactone (GDL) have been used in cheesemaking (Sharma *et al.*, 1980; Hill *et al.*). GDL is an internal ester and when dissolved in water it hydrolyses to give gluconic acid. Gluconic acid tastes better than lactic, acetic or hydrochloric acid, but is more expensive.

$$C_6H_{12}O_7 \underset{\text{Hydrolysis}}{\overset{\text{Dehydration}}{\rightleftharpoons}} C_6H_{10}O_6$$

Gluconic acid Glucono-δ-lactone

The rate of hydrolysis is pH dependent. If organic or mineral acids are used to acidify milk prior to renneting, the rapid local increase in the acidity of the milk may cause the partial precipitation of the caseins, which is not a desirable effect. In contrast, GDL is transformed by hydrolysis into gluconic acid after dispersion of the lactone throughout the milk. The acidity is then obtained more slowly without protein precipitation and a homogeneous curd is formed. Acidification by GDL is therefore similar to that produced by lactic acid microflora, as it occurs *in situ* and is evenly distributed in the milk (Serpelloni *et al.*, 1990). The rate of hydrolysis of GDL and thus the speed of acidification also depend upon the temperature. The extent of the drop in pH depends upon the quantity of GDL added.

Direct acidification of milk to achieve optimum acidity has been successfully used for many years in the manufacture of some varieties of cheese (Sharma *et al.*, 1980; Hill *et al.*, 1982; Fernandez & Kosikowski, 1986; Modler, 1988) including Mozzarella (Section

2.3.2). Cheddar cheese is a difficult variety to manufacture even by biological acidification because body and textural attributes are almost as important as the flavour.

An early scientific study of direct acidification techniques was made on Cheddar cheese by Mabbitt *et al.* (1955). They studied several acidulation schedules and concluded that acids (hydrochloric or acetic) as sole acidulants gave unsatisfactory cheese. Partial acidification with acid to approximately pH 5.8 coupled with the use of GDL to reduce the pH to approximately 5.2 gave cheese of a more acceptable quality. Best quality curd was produced when the pH of the milk was reduced to 6.4 by the addition of 12% hydrochloric acid followed by GDL to reduce the pH to 6.0. More GDL was added to the curd at salting to decrease the pH to approximately 5.2. The body and texture of the cheese produced were reported to be normal although typical Cheddar cheese flavour failed to develop.

Breene *et al.* (1964a) developed a direct acidification process for the manufacture of Cheddar cheese. Milk was acidified to pH 5.4 or 5.6 with lactic acid and set with rennet. The gel was cut and cooked at 39-42 °C. Initially no starter was used but, because the cheese developed a variety of off-flavours, in later experiments 0.5, 1.0 or 2.0% lactic acid culture was added to the milk prior to addition of rennet in order to decrease the pH of the partially acidified curd to a final value of 5.2, control the growth of undesirable organisms and contribute to flavour development. Best results were obtained when milk containing 2% starter was set at pH 5.6 and cooked at 39 °C.

The pH of curd acidified by the method of Mabbitt *et al.* (1955) decreased more rapidly than that of curd produced in the traditional way using bacterial starter (Green & Foster, 1974; O'Keeffe *et al.*, 1975). This resulted in excessively rapid proteolysis during manufacture and early ripening. When assessed by changes in electrophoretic patterns, GDL cheese had undergone a level of proteolysis, after pressing, corresponding to that in normal Cheddar cheese after 2 months of ripening (O'Keeffe *et al.*, 1975). Excessive proteolysis was explained on the basis that the rapid decline in pH following addition of GDL to milk solubilised excessive amounts of colloidal calcium phosphate, rendering

the micellar caseins susceptible to proteolysis (Fox, 1970). Holmes *et al.* (1977) have shown that the proportion of added rennet (chymosin) retained in the Cheddar curd increases rapidly as the pH at setting decreases and this is likely to be an important contributing factor to excessive early proteolysis.

Creamer *et al.* (1985) investigated the effect of acidification of cheese milk to various extents with lactic or hydrochloric acid on the resultant Cheddar cheese. Chymosin retention by the acidified curd increased with increasing acidification of the milk resulting in a greater degree of proteolysis. However, proteolysis in cheese made with microbial rennet did not change with milk acidification because the retention of the enzyme was not influenced by the acidification, as shown by gel electrophoresis. Cheese made from acidified milk had lower concentrations of both calcium and phosphate. Throughout maturation, the texture of the cheeses made from acidified milk was more crumbly and the force required to fracture them was less.

2.2.4 Syneresis of cheese curd

Syneresis of curd refers to expulsion of whey because of the contraction of the curd and can be considered as a continuation of the coagulation process. Syneresis in cheesemaking is initiated by cutting the curd, and enhanced by stirring and by increasing the temperature and acidity of the curds and whey (Marshall, 1982).

In terms of cheesemaking, syneresis can be enhanced when the environment of the casein in the curd particles is altered, such as (a) a decrease in solvation or water binding of the casein, (b) shrinkage of the (para)casein micelles due to a reduction in pH or an increase in temperature and (c) rearrangement of the (para)casein micelle network (Walstra *et al.*, 1985).

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 in the milk gel due to the casein-casein interactions is likely to be responsible for syneresis (Lelievre & Creamer, 1978).

Syneresis is initially a first order reaction because the rate depends on the amount of whey in the curd (Fox, 1985); holding curd in whey retards syneresis because of the back pressure of the surrounding whey, whereas removing whey promotes syneresis. When the curd is reduced to 70% of its initial volume, syneresis becomes dependent on factors other than the volume of whey (Fox, 1985). Marshall (1982) considers that hydrophobic interactions within the casein network are probably responsible for the advanced stages of syneresis. This is in accord with the promotion of syneresis by reduced pH and low levels of $CaCl_2$, which reduce micellar charge and increase hydrophobicity, and by increased temperatures, which increase hydrophobic interactions (Fox, 1985).

Walstra *et al.* (1985) suggested that syneresis results from the contraction of the gel network by a gradual process of realignment and bond interchange, *i.e.* a continuation of coagulation mechanisms. 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 (van Dijk, 1982), and is inversely proportional to the viscosity of the continuous phase and the dimensions of the gel.

Pearse & Mackinlay (1989) 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 that form this curd network might be expected to affect the coagulation and subsequently the syneresis.

2.2.4.1 Factors affecting the rate of syneresis

Syneresis of rennet curd is influenced by several factors. They are as follows.

(1) Composition of the milk: A higher fat content in milk is mostly accompanied by a somewhat slower syneresis (Beeby, 1959; Emmons *et al.*, 1980a; Storry *et al.*, 1983; Grandison *et al.*, 1984a; Lawrence & Gilles, 1987). Marshall (1982) reported that increasing the fat concentration from zero in stages up to approximately twice that in the control progressively decreased the rate of syneresis. Grandison *et al.* (1984b) found a positive correlation between syneresis time, the time required to collect 20 ml whey from curd produced from 50 ml milk, and the fat content of the milk.

There also appears to be some correlation between syneresis and genetic variants of β-lactoglobulin and κ-casein (McLean & Schaar, 1989). Syneresis was less with milk containing β-lactoglobulin B compared with β-lactoglobulin A and with milk containing κ-casein A compared with κ-casein B (McLean & Schaar, 1989). This may be due to the difference in calcium ion activity, which correlates with the genetic variants (Walstra, 1993). Pearse & Mackinlay (1989) reported that syneresis was sensitive to the concentration of β-casein and also to low levels of dephosphorylation of β-casein.

(2) Heat treatment of the milk: Pearse *et al.* (1985) reported that, when milk was heated so that serum proteins denatured, the syneresis rate of renneted milk diminished. They also found that the decrease in syneresis was almost linearly correlated with the extent of β-lactoglobulin denaturation.

(3) Homogenization of the milk: The syneresis rate was significantly reduced on homogenisation of milk (Emmons *et al.*, 1980a; Green *et al.*, 1983; Storry *et al.*, 1983). Green *et al.* (1983) observed that, when Cheddar cheese was made from homogenised milk, casein micelle aggregation occurred more slowly, the protein network in the curd was finer, curd fusion was poor and the rate of whey loss was reduced. Walstra (1993) suggested that this effect is related to the incorporation of micellar casein in the surface

coat of the fat globules, which causes the fat globules to become part of the paracasein network, which in turn may hinder network shrinkage.

(4) Addition of calcium to the milk: Patel *et al.* (1972) found that addition of 0.2% (w/w) anhydrous $CaCl_2$ significantly affected whey expulsion. Curd from this milk contained 71.53% moisture as compared with 72.54% in the control curd. Marshall (1982) found that when 2 mM $CaCl_2$ was added to the milk, the rate of syneresis increased at all cutting times (*i.e.* at 2, 3, 4 and 5 times the rennet coagulation time). However, a further increase to 4 mM $CaCl_2$ caused an additional increase in the syneresis rate only at the two shorter cutting times studied. At high calcium concentrations, the syneresis rate may decrease, especially if the gel is held for a long period before cutting (Fox, 1987).

(5) Acidity: The syneresis rate is greater for milk acidified to a lower pH before renneting (Emmons *et al.*, 1959; Berridge & Scurlock, 1970; Pearse *et al.*, 1984). Emmons *et al.* (1959) suggested that the syneresis may be further enhanced if the pH decreases during syneresis because the building blocks of the protein network tend to shrink due to the pH reduction. Patel *et al.* (1972) observed that the moisture content of the curd increased linearly from 69.27 to 74.95% as the pH at coagulation increased from 5.2 to 5.8. Marshall (1982) reported that decreasing the pH of the milk from 6.6 to 6.0 increased the rate of syneresis, though the increase in the range pH 6.6-6.3 was greater than that between pH 6.3 and pH 6.0.

(6) Coagulant concentration: Increased syneresis was observed when more rennet was used (Lelievre & Creamer, 1978). Marshall (1982) reported a slight increase in syneresis rate when the concentration of rennet was increased four fold.

(7) Cutting and stirring: Cutting the rennet curd into pieces creates a free surface through which syneresis can occur. The rate of syneresis increases with a decrease in the size of the curd pieces (Walstra, 1993).

Patel *et al.* (1972) observed an increase in whey expulsion with an increase in the rate of stirring. Stirring the mixture of curd and whey enhances syneresis by preventing sedimentation of the curd particles. It also causes some pressure to be exerted on the curd grains and increases the frequency of their collision with each other and with the container walls promoting syneresis (Fox, 1987). Stirring for a longer time results in a lower moisture content in the curd (Walstra, 1993).

(8) Temperature: Temperature greatly affects the syneresis rate of rennet curd. Lawrence (1959) observed that, whereas the syneresis was greater at higher temperatures, the rate of change of syneresis decreased as the temperature increased. Marshall (1982) reported that, when the temperature was raised from 25 to 35 °C, the rate of syneresis more than doubled, but there was a tendency for the rate to fall at 35°C with an increase in the cutting time. Increasing the temperature promotes syneresis but, within the temperature range normally used for Cheddar cheese cooking, the effect is slight. In cheesemaking, this temperature effect may be negative above 38-40 °C because of the inhibitory effect of higher temperatures on acid production (Fox, 1987).

2.3 CHEDDARING AND SALTING

Cheddaring and salting of the curd during cheese manufacture result in changes in the composition of the curd. They also induce several changes in the structure and texture of the curd. Heating and stretching during the manufacture of Mozzarella cheese lead to the development of its characteristic fibrous structure. The changes taking place in the curd during these stages are briefly described.

2.3.1 Changes during cheddaring of cheese curd

During the manufacture of Cheddar cheese, cheddaring provides a holding period during which the necessary degree of acidity is developed and further whey is released from

the curd. This loss of whey is controlled by the acidity and temperature of the curd and mechanical handling of the curd. The rate of acid development is also influenced by the temperature (Lawrence *et al.*, 1993).

In the cheddaring process, the curd is allowed to flow at a temperature near the cooking temperature. During this process, the curd alters its character considerably, changing from a simple unstructured mass to fibrous striated material (Hall & Creamer, 1972).

During cheddaring, the curd granules fuse under gravity into a solid block (matting). Matting proceeds rapidly under the combined effect of heat and acid. The original rubber-like texture gradually changes into a close-knit texture with the matted curd particles becoming fibrous. The pressure and flow serve to knit, stretch and orientate the network of casein fibres already partly formed in response to the rising acidity (Lawrence *et al.*, 1993). The warmer the curd and the higher its moisture content, the more readily it flows and the finer, longer and denser are the fibres (Czulak, 1959). The process of curd fusion and coalescence of fat globules continues during cheddaring, leading to progressive elimination of interstitial spaces (Brooker, 1979) and the formation of a close-textured cheese curd.

Czulak (1959) reported that pH, pressure and temperature during cheddaring could be manipulated to influence the curd structure and that a direct relationship existed between the structure and water-holding capacity of the curd. This was confirmed by Olson & Price (1970) who showed that extension and rapid flow of curd during cheddaring produced a higher moisture content in the resulting cheese.

2.3.2 Heating and stretching of Mozzarella curd

A characteristic of Mozzarella cheese manufacture is a hot water stretching step that results in the orientation of protein fibres (Masi & Addeo, 1986; Paquet & Kalab, 1988; Kiely *et al.*, 1992). Electron micrographs of Mozzarella curd before and after hot water

stretching clearly demonstrate the transformation that occurs from a non-oriented matrix of protein and fat globules to a highly oriented fibrous structure (Masi & Addeo, 1986). McMahon *et al.* (1993) reported the presence of columns of serum and emulsified fat between the oriented protein fibres.

Mozzarella curd does not stretch in hot water until sufficient calcium phosphate has been solubilised from the curd through acidification (Kosikowski, 1982). Mozzarella cheese made using starters is typically drained at pH 6.1. Reduction in pH and loss of minerals continue after draining until the curd becomes stretchable, usually at a curd pH of 5.3-5.1 (Kosikowski, 1982). However, directly acidified Mozzarella is coagulated, cooked and drained at pH 5.6, resulting in a much greater mineral loss at draining (Breene *et al.*, 1964b; Kosikowski, 1982). The curd is ready for immediate stretching, eliminating the need for further acidification during draining.

The stretching temperature has implications for lactic acid bacteria and coagulant survival and proteolysis during aging. Plasmin may be mainly responsible for proteolytic activity in Mozzarella during aging (Creamer, 1976a; Lawrence *et al.*, 1983, 1987). Plasmin is not inactivated under the time/temperature/pH conditions likely to occur during stretching (Dulley, 1972).

2.3.3 Salting of cheese curd

Salting plays a major role in determining the quality of cheese by controlling (a) the final pH of the cheese, (b) the growth of microorganisms, (c) proteolysis of the caseins, and (d) the overall flavour and texture of the cheese. It also influences the moisture content of the cheese. The level of salt-in-moisture (S/M) controls the rate of proteolysis of the caseins by chymosin, plasmin and bacterial proteases (Lawrence *et al.*, 1993).

The only prerequisite for salt absorption by cheese is the existence of a S/M gradient between the cheese and the salting medium. However, the quantity of salt absorbed

depends on the intrinsic properties of the cheese, the conditions of salting and the duration of salting. As the different procedures of salting all involve salt absorption via an impeded diffusion process, the general factors affecting salt uptake by cheese apply equally to granules or milled curd pieces on mixing with dry salt and moulded cheeses that are brine and/or dry salted (Guinee & Fox, 1993).

2.3.3.1 Salting of Cheddar cheese curd

When dry salt is distributed over the surface of freshly milled curd, a part of it dissolves in the moisture on the surface, creating a very thin layer of saturated brine. The S/M gradient between the brine and the moisture in the cheese results in movement of salt into the cheese curd and water out of the curd. Some water is also "squeezed out" of the curd due to localised surface contraction (salting-out of the protein matrix) as a result of the contact of the curd with the saturated brine. The moisture level in the curd, which influences whey release, affects the rate at which the salt on the surface is dissolved (Breene *et al.*, 1965; Sutherland, 1974; Guinee & Fox, 1993).

2.3.3.2 Factors affecting dry salting of cheese curd

The various factors that influence salt uptake and diffusion on dry salting of cheese curd are as follows.

(1) Rate of salting: An increase in the rate of salting increased the rate of salt absorption by cheese thus giving higher levels of salt and S/M and lower levels of moisture (as a result of increased whey loss) in the cheese after salting for a fixed time (Breene *et al.*, 1965; Gilles, 1976; Guinee & Fox, 1993). However, the increase in salt and S/M levels in the cheese was not proportional to the level of salt added, especially at the higher salting rates, because of higher salt losses at increased salting rates and greater water loss from the cheese.

Sutherland (1974) reported that the volume of whey released from the curd and the percentage of added salt lost increased linearly with the level of salt added. On the other hand, the moisture content decreased and the level of salt, level of S/M and pH of the cheeses increased in a curvilinear fashion as the level of added salt was increased.

Gilles (1976) observed that the increase in S/M level in Cheddar curd was not proportional to the increase in the level of dry salt added to the milled curd. This is attributed to increased salt losses with increased salting rates, which reflects the decreasing effect of the driving force (concentration gradient) in raising the quantity of salt absorbed as the S/M level in the cheese approaches that of the brine (Guinee & Fox, 1993).

(2) Extent of mixing of salt and curd: Increasing the duration of mixing salt into the curd from 20 s to 6 min caused a significant increase in salt and S/M levels, *i.e.* from 1.53 to 1.97% and from 4.41 to 5.71% respectively, as the proportion of salt lost was reduced (Sutherland, 1974). Better mixing leads to salt absorption from more surfaces and there is less "free" salt to be lost in the whey during pressing.

(3) Time duration between salting and pressing: By increasing the pre-pressing holding period, salt losses were substantially reduced and consequently the salt and S/M levels were substantially increased. The increase is attributed to a higher total absorption and hence a reduction in the physical loss of salt (Breene *et al.*, 1965; Sutherland, 1974; Gilles, 1976).

(4) Moisture content of the curd: The rate of salt absorption decreased as the initial moisture level increased, resulting in lower salt and S/M values in cheese for a fixed salting rate (Sutherland, 1974; Gilles, 1976). Such decreases were attributed to greater whey and salt losses from the high moisture curds; an increase in curd moisture content from 39.1 to 43.4% caused a 30% increase in the amount of whey drainage and a decrease in salt retention from 59 to 43% of the amount applied (Sutherland, 1974).

(5) Curd particle size at milling: The rate of salt absorption increases with increasing surface area to volume ratio of the curd (Breene *et al.* 1965; Gilles, 1976). In the milled curd, the time required for salt absorption is less as it occurs from many surfaces simultaneously. Gilles (1976) reported that milling the curd to smaller particles increased salt retention.

(6) Temperature of curd and brine: In brining experiments with milled Cheddar chips, Breene *et al.* (1965) observed that, for curd tempered to any temperature in the range 26.7-43.3 °C, salt uptake increased with increasing brine temperature in the same range. However, curd tempered to 32 °C absorbed salt less readily than curd tempered at lower or higher temperatures before brining. This was attributed to a layer of exuded fat on the surfaces of the curd particles at 32 °C which impeded salt uptake; less fat was exuded at lower temperatures whereas at higher temperatures the exuded fat was liquid and dispersed in the brine.

(7) Curd acidity at salting: Curd salted at low acidity (higher pH) was found to retain more salt than more acidic cheeses (Lawrence & Gilles, 1969; Gilles, 1976; Lawrence & Gilles, 1982). As low acid curd normally contains more moisture than high acid curd, more syneresis and higher salt losses could be expected; however, the rate of salt diffusion and salt uptake, all conditions being equal, would be higher in the higher moisture curd chips (Guinee & Fox, 1993). This contradicts the observations of Sutherland (1974) given in Section (4) above.

2.3.3.3 Effect of salt on moisture content of Cheddar cheese curd

The moisture content of Cheddar cheese curd is influenced by syneresis of the curd during different stages of manufacture such as cutting, cooking, dry stirring and cheddaring. Further syneresis occurs on addition of salt after milling and during pressing.

It is generally accepted that there is an inverse relationship between the levels of moisture and salt in cheese. A considerable volume of whey is released from the Cheddar curd following salting and during pressing (Sutherland, 1974). The amount of whey released is directly related to the amount of salt added to the curd; roughly half of the whey is released during holding following salting and the other half is released on pressing. Although other factors, *e.g.* curd temperature, stirring time after salting, depth of curd in the vats and duration of holding time after salting and before pressing, influence the ratio of whey released during holding after salting to that released on pressing, the overall release of whey was not significantly influenced by these factors (Sutherland, 1974). The moisture content of the cheese was inversely related to the salting rate.

2.3.3.4 Brine salting of cheese curd

The conditions of brining affect not only the salt content of cheese but also the rate at which the cheese is cooled. Because Mozzarella cheese is quite warm as it exits the stretcher (approximately 49 °C internal temperature), brining is used to cool as well as to salt the cheese blocks (Kindstedt, 1993). The temperature of the brine is usually about 4 °C.

When cheese is placed in brine, there is a net movement of NaCl, as Na^+ and Cl^-, from the brine into the cheese as a consequence of the osmotic pressure difference between the cheese moisture and the brine. Consequently, the water in the cheese diffuses out through the cheese matrix so as to restore the osmotic pressure equilibrium (Guinee & Fox, 1993).

The difference between dry salting and brine salting is the availability of water at the surface of the curd. With brine salting, salt absorption begins immediately; release of whey occurs, as in dry salting, but is not a prerequisite for salt absorption (Lawrence *et al.*, 1993).

Geurts *et al.* (1974a) suggested that the penetration of salt into cheese and the concomitant outward migration of water could be described as an impeded diffusion process; NaCl and water molecules move in response to their respective concentration gradients but their diffusion rates are much lower than those in pure solution due to a variety of impeding factors. The diffusion coefficient for NaCl in cheese moisture is approximately 2.31×10^{-10} m^2/s compared with 1.16×10^{-9} m^2/s for NaCl in pure water at 12.5 °C (converted from Geurts *et al.*, 1974a).

The principal factors responsible for impeding NaCl diffusion in cheese, as postulated by Geurts *et al.* (1974a), are as follows.

(1) The effect of the protein matrix on the mass ratios of salt and water migrating in opposite directions. The pores (estimated to be about 2.5 nm wide) of the protein matrix exert a sieving effect on both the inward-diffusing NaCl molecules and the outward-moving water molecules but the effect is more pronounced on the former because of their greater effective diffusion radii, which are approximately twice those of the water molecules. Hence, during brining, the water flux is approximately twice the NaCl flux.

(2) When the NaCl molecules do enter the cheese, the relatively narrow pore width of the protein matrix exerts a frictional effect on the diffusing NaCl and water molecules and reduces their relative diffusion rates from 1 in true solution to approximately 0.5 and 0.75 respectively in cheese moisture.

(3) Frictional effects of protein-bound water. Water binding in cheese (0.1-0.15 g water/g paracasein) (Geurts *et al.*, 1974b) makes approximately 10% of the total cheese moisture unavailable for salt uptake and hence reduces the apparent diffusion coefficient. Furthermore, the protein-bound water reduces the relative pore width of the protein matrix, thus further retarding the movement of NaCl and water molecules.

(4) The high relative viscosity of cheese moisture. The viscosity of cheese moisture is about 1.27 times that of pure water at 12.5 °C due to the presence of dissolved

materials, *e.g.* acids, lactose, salts and nitrogenous compounds. NaCl molecules diffusing through the cheese moisture encounter an increased collision frequency with the dissolved substances, and are also affected by the charge fields of these substances.

(5) Obstructions of fat globules and globular protein particles. On proceeding from one parallel plane to another within the cheese, the diffusing molecules must travel by a circuitous route to bypass obstructing particles.

These various factors help to explain the low diffusion coefficient of NaCl in cheese and its variations with changes in cheese composition and brining conditions.

2.3.3.5 Factors affecting brine salting of cheese curd

The various factors that influence the salt uptake and diffusion on brine salting of cheese curd are as follows.

(1) Concentration gradient: An increase in brine concentration results in higher rates of salt absorption and increased S/M levels in the cheese (Breene *et al.*, 1965; Geurts *et al.*, 1974a; Guinee & Fox, 1986a). However, whereas the rate of NaCl diffusion is scarcely affected by brine concentration in the range 5-20% (Geurts *et al.*, 1974a), the rate of uptake increases at a diminishing rate with increasing brine concentration (Breene *et al.*, 1965; Sutherland, 1974; Guinee & Fox, 1986a).

(2) Cheese geometry: The rate of salt absorption increases with increasing surface area to volume ratio of the cheese (Breene *et al.*, 1965; Gilles, 1976; Guinee & Fox, 1986a). In addition to its influence on the surface area to volume ratio, cheese shape also affects the rate of salt absorption via its effect on: (a) the number of directions of salt penetration from the salting medium into the cheese, and (b) the ratio of planar to curved surface area of the cheese (Geurts *et al.*, 1980; Guinee & Fox, 1986a). In cheeses with approximately equal surface area to volume ratios, the rate of salt absorption by

rectangular-shaped cheeses was higher than that by cylindrical cheese (Guinee & Fox, 1986b).

(3) Salting time: The quantity of salt absorbed increases with salting time (Breene *et al.*, 1965; Godinho & Fox, 1981) but the rate of salt absorption decreases with time due to a decrease in the NaCl concentration gradient between the cheese moisture and the brine (Geurts *et al.*, 1972; Guinee & Fox, 1986a). The quantity of salt taken up by a cheese is proportional to the square root of the brining time (Geurts *et al.*, 1980; Guinee & Fox, 1986a).

(4) Temperature of the curd and brine: Increasing brine temperatures have been found to result in higher mobility of NaCl and higher salt absorption in cheese (Geurts *et al.*, 1974a, 1974b), due partly to an increase in true diffusion and partly to an increase in the effective pore width of the protein matrix as non-solvent water decreases with increasing temperature (Geurts *et al.*, 1974a).

(5) Curd pH: The effect on salt absorption by whole cheeses has not been investigated. The effect of curd pH on salt absorption by Cheddar cheese curd is described in Section 2.3.3.2.

(6) Moisture content of the curd: Geurts *et al.* (1974a, 1974b) showed that the diffusion coefficient and the quantity of salt absorbed by cheese during brine salting generally increased as the moisture content of the curd increased. The higher salt uptake that accompanies increased moisture levels is a consequence of the concomitant increase in the rate (and depth) of penetration into the cheese. This has been attributed (Geurts *et al.*, 1974a) to an increase in the relative pore width of the protein matrix (the volume fraction of the protein phase decreases as the moisture content increases), which reduces the frictional effect on the inward-diffusing NaCl molecules.

2.3.3.6 Effect of brining on moisture content of Mozzarella curd

Moisture loss during brining is dependent on brine temperature, with greater loss occurring as the brine temperature increases. In contrast, salt uptake is affected only slightly by brine temperature (Nilson, 1969).

It is well documented that, within most brine salted cheeses, a decreasing salt gradient is established from surface to centre and is accompanied by a decreasing moisture gradient in the opposite direction (Guinee & Fox, 1993). This is not necessarily true for Mozzarella cheese because of the nature of the thermal conditions that occur during brining (Kindstedt & Kiely, 1990). The large thermal gradients that occur during brining can catalyse an outward migration of water from the warm centre of the cheese toward the colder surface. At the same time, moisture loss from the surface is retarded by the lower temperature of the brine, resulting in less surface dehydration (Nilson, 1969). After brining and during aging, the high salt concentration at the cheese surface continues to promote an outward movement of water, resulting in an increasing gradient of moisture from centre to surface (Kindstedt & Kiely, 1990).

2.4 CHEESE RIPENING

The conversion of milk to cheese curd is only the first stage in the manufacture of most cheese varieties. The cheese curd of many cheese varieties is ripened for periods ranging from a few weeks to 2 or more years. During this period, the carbohydrate, fat and protein components undergo numerous biochemical changes which lead to the development of the appropriate texture, taste and aroma. The ripening involves three primary processes: glycolysis, lipolysis and proteolysis. Proteolysis is the most complex of these phenomena and is the main contributor to the changes in texture during ripening (Fox *et al.*, 1994).

2.4.1 Proteolysis during cheese ripening

Initially, the glycolysis, which alters the pH and the salt balance, is quite important in Cheddar cheese. The proteolysis during ripening is almost entirely responsible for the textural changes in most cheese varieties. The changes arise from the breakdown of the protein network, an increase in pH and greater water binding by the newly formed amino and carboxyl groups (Creamer & Olson, 1982; Fox *et al.*, 1993).

Proteolytic agents in cheeses such as Cheddar generally originate from five sources: the coagulant, the milk, starter bacteria, non-starter bacteria and adjunct starter. The progress of proteolysis in Cheddar cheese can be summarised (Fox *et al.*, 1994) as follows: initial hydrolysis of caseins is caused primarily by residual coagulant (chymosin) and to a lesser extent by plasmin and perhaps cathepsin-D (indigenous milk proteinases), resulting in the formation of large and intermediate-sized peptides which are subsequently degraded by the coagulant and enzymes from the starter and non-starter flora. The production of small peptides and free amino acids results from the action of bacterial proteinases and peptidases.

The role of chymosin and plasmin in the proteolysis in cheese during early ripening is described briefly. Proteolysis due to microorganisms is not reviewed as the experimental cheeses in this study were made without the use of starters.

2.4.1.1 Proteolysis due to action of chymosin

Chymosin is the principal proteinase in traditional calf veIl rennets used for cheesemaking and remains the enzyme of choice. The principal role of chymosin in cheesemaking is to coagulate milk. However, about 6% of the chymosin added to cheese milk is retained in the cheese curd and plays a major role in the initial proteolysis of caseins during ripening (Fox *et al.*, 1994).

The proportion of chymosin retained in the curd is strongly influenced by the pH at whey drainage, increasing as the pH decreases (Holmes *et al.*, 1977; Creamer *et al.*, 1985), and the coagulant activity decreases with an increase in the cooking temperature (Fox *et al.*, 1994).

The primary chymosin cleavage site in the milk protein system is the Phe_{105}-Met_{106} bond in κ-casein and its hydrolysis leads to the coagulation of milk (Dalgleish, 1993). Cleavage of κ-casein Phe_{105}-Met_{106} yields para-κ-casein and glycomacropeptides. Most of the glycomacropeptides are lost in the whey but para-κ-casein remains attached to the casein micelles and is incorporated into the cheese.

Although considerably less susceptible than the Phe_{105}-Met_{106} bond of κ-casein, α_{s1}-, α_{s2}- and β-caseins are readily hydrolysed by chymosin under appropriate conditions. Several workers have reported the action of chymosin on β-casein (Creamer, 1976b; Visser & Slangen, 1977; Mulvihill & Fox, 1978; Carles & Ribadeau-Dumas, 1984) and on α_{s1}-casein (Mulvihill & Fox, 1979; McSweeney *et al.*, 1992, 1993).

β-Casein is hydrolysed by chymosin into three major products of increasing electrophoretic mobilities corresponding to different N-terminal fractions of β-caseins and have been identified as β-I, β-II and β-III (Creamer, 1976b; Visser & Slangen, 1977). Only a very low level of primary hydrolysis of β-casein by chymosin at the most susceptible bond (192-193) was reported by McSweeney *et al.* (1994) and Fox *et al.* (1994).

The hydrolysis of β-casein by chymosin is strongly inhibited by 5%, and completely by 10%, NaCl (Fox & Walley, 1971). The C-terminal region of β-casein is very hydrophobic and undergoes temperature-dependent hydrophobic interactions (Berry & Creamer, 1975). It is likely that such associations occur in cheese and render the chymosin-susceptible bonds, which are located in this region, relatively inaccessible to chymosin (Berry & Creamer, 1975; Fox *et al.*, 1993).

It is generally accepted that chymosin plays a major role in the initial breakdown of α_{s1}-casein, giving rise to the peptide α_{s1}-I (Creamer, 1970; Creamer & Richardson, 1974). The primary site of chymosin action on α_{s1}-casein is Phe_{23}-Phe_{24} (Hill *et al.*, 1974). Cleavage at this site has significance in producing a small peptide, which is further hydrolysed by starter proteinases, and in the softening of Cheddar cheese (Creamer & Olson, 1982). Subsequent hydrolysis of the peptide α_{s1}-I at some unidentified bonds has also been reported (Mulvihill & Fox, 1980; McSweeney *et al.*, 1994; Exterkate & Alting, 1995). The hydrophobic amino terminal segment (residues 14-24) of α_{s1}-casein appears to be important in the structure of rennet curd and cheese (Creamer *et al.*, 1982); softening of the cheese texture during ripening appears to be due to break-up of this network on hydrolysis of α_{s1}- to α_{s1}-I casein by chymosin (Fox, 1987).

In contrast to β-casein, NaCl up to 5% stimulates the hydrolysis of α_{s1}-casein and significant proteolysis occurs in the presence of 20% NaCl (Fox & Walley, 1971). Mulvihill & Fox (1977) found that pH affected the pattern of proteolysis of α_{s1}-casein. Ionic conditions also affected proteolysis (Mulvihill & Fox, 1979).

Para-κ-casein (Green & Foster, 1974) and α_{s2}-casein (Fox *et al.*, 1994) appear to be relatively resistant to chymosin action.

2.4.1.2 Proteolysis due to action of plasmin

Plasmin is the principal indigenous proteinase in milk. It is a blood enzyme that passes into milk. Plasmin is low in normal milk, and most of the enzyme exists as the proenzyme plasminogen (Richardson & Pearce, 1981). Plasminogen activator, also a blood enzyme, increases plasmin activity during storage, mainly in pasteurised milk (Noomen, 1975; Richardson, 1983a). Inhibitors of plasmin and plasminogen activators also pass from blood into milk (Korycka-Dahl *et al.*, 1983). Plasmin and plasminogen activators are associated with casein, but their inhibitors are found in milk serum (Grufferty & Fox, 1988). Being associated with the casein micelles, the plasmin accompanies the micelles into cheese curd (Korycka-Dahl *et al.*, 1983; Richardson,

1983b). Most of the plasmin in milk is retained in cheese and contributes to the ripening of most cheese varieties (Farkye & Fox, 1990). Swiss-type cheeses contain twice as much plasmin activity as Cheddar, which Lawrence *et al.* (1987) suggested is due to the difference in the pH at draining. However, Grufferty & Fox (1988) and Madkor & Fox (1991) found that, in the pH range 4.6-6.6, plasmin remains associated with the casein micelles, which suggests that, within the range relevant to rennet-coagulated cheese, the pH at draining does not affect plasmin retention in cheese (Fox *et al.*, 1993).

Plasmin is a trypsin-like proteinase with a high specificity for peptide bonds containing lysine residues. It is active on all caseins, especially α_{s2}- and β-caseins (Grufferty & Fox, 1988). Plasmin cleaves β-casein, resulting in the formation of γ-caseins and proteose peptones (Gordon *et al.*, 1972; Andrews & Alichanidis, 1983; Eigel *et al.*, 1984; Farkye & Fox, 1992). Richardson & Pearce (1981) found a relationship between plasmin activity in Cheddar cheese and the amount of β-casein degraded.

Plasmin hydrolyses α_{s2}-casein in solution producing about 14 peptides, three of which are potentially bitter (Le Bars & Gripon, 1989). Although plasmin is less active on α_{s1}- than α_{s2}- or β-casein, the formation of λ-casein, a minor casein component, has been attributed to its action on α_{s1}-casein (Aimutis & Eigel, 1982).

Eigel (1977) found no hydrolysis of κ-casein under conditions adequate for the complete hydrolysis of α_{s1}-casein, but Andrews & Alichanidis (1983) reported that hydrolysis of κ-casein by plasmin accounted for 4% of the proteose peptone fraction produced by indigenous plasmin in pasteurised milk stored at 37 °C for 7 days.

Farkye & Fox (1990) investigated the effects of some processing conditions (pH, cooking temperature and method of salting) on plasmin activity in cheese. They found that neither the draining pH nor the method of salting affected the activity. However, plasmin activity increased with increasing cooking temperature.

It has been reported that pasteurisation increases plasmin activity in milk (Noomen, 1975; Richardson, 1983b), possibly by inactivation of plasmin inhibitors or by increasing the rate of activation of plasminogen (Lawrence *et al.*, 1987). Farkye & Fox (1990) proposed that a similar situation occurred in cheese, *i.e.* more plasminogen is converted to plasmin during cooking at high temperatures because the inhibitors of plasminogen activators are inactivated. Also, the inhibitors of the plasmin system are mostly in the serum phase of milk (Reimerdes *et al.*, 1976; Korycka-Dahl *et al.*, 1983) and are probably lost in the whey during cheesemaking.

The combined action of chymosin and plasmin on α_{s1}- and β-caseins could produce quite small peptides. Their specificities are in fact complementary, especially on β-casein which chymosin cleaves primarily toward the C-terminal region and which plasmin cleaves mainly in the N-terminal region (Fox *et al.*, 1994).

2.4.1.3 Factors affecting the rate of proteolysis

The extent of proteolysis in cheese is governed by factors such as the level of residual coagulant, level of starter addition, S/M, temperature of ripening, type of coagulant used, plasmin activity, changes in pH during ripening and duration of the ripening period.

(1) Residual coagulant in cheese curd: Most of the coagulant is lost in the whey at draining. Holmes *et al.* (1977) found the distribution of chymosin between curd and whey to be pH dependent. This was confirmed by Creamer *et al.* (1985) who reported that the lower the pH at draining, the more chymosin was retained in the curd and the greater was the proportion of α_{s1}-casein hydrolysed. The distribution of microbial rennet between the curd and whey was not pH dependent. The overall quantity of residual rennet retained in cheese depended on the amount of whey retained and the retention by pH-dependent absorption by casein in the curd (Lawrence *et al.*, 1987).

(2) S/M: The rate of proteolysis during ripening is markedly affected by the salt to moisture ratio in cheese. Creamer (1971) observed that the hydrolysis of α_{s1}- and β-caseins decreased with an increase in the salt concentration. The degradation of β-casein was much slower in comparison with that of α_{s1}-casein. Thomas & Pearce (1981) related the differences in the texture of plugs, drawn several months after manufacture from regions with different S/M levels in the same cheese block, to proteolysis.

(3) Ripening temperature: Fedrick & Dulley (1984) established that the higher the temperature in the range 8-20 °C, the greater was the extent of casein hydrolysis and change in texture. Lawrence *et al.* (1987) observed that the hydrolysis of β-casein in Cheddar cheese markedly decreased when the temperature of ripening was reduced to 6 °C or less. The hydrolysis of α_{s1}-casein also decreased but to a lesser extent.

(4) pH of the curd: The effect of pH on the rate of proteolysis was studied in a model cheese by Noomen (1978). The overall extent of proteolysis was reported to increase markedly when the pH was greater than 5.8. The relative rate of α_{s1}-casein hydrolysis was greater at low pH than that of β-casein. At pH values greater that 5.6, β-casein was degraded to a greater extent than α_{s1}-casein (Noomen, 1978), presumably as a result of plasmin activity.

(5) Type of coagulant used: de Jong (1976) reported a strong relationship between the degree of proteolysis, particularly of α_{s1}-casein, and the consistency of cheese texture when calf rennet was used. This also held for bovine pepsin, an enzyme that has a specificity similar to that of chymosin but is less proteolytic. Microbial rennets attack α_{s1}-casein at rates similar to chymosin (Edwards & Kosikowski, 1969; Creamer & Olson, 1982) and their use results in cheese with textural characteristics in the early stages of ripening that are almost identical to those of calf rennet cheese.

There are many reports, however, that the texture of cheeses made using coagulants from *Mucor pusillus* and *Mucor miehei*, after long ripening periods, tends to be poorer than or different from that of calf rennet cheese (Birkkjaer & Jøhnk, 1985). This has

been attributed to the greater proteolytic activity of these microbial rennets, which are much less specific than chymosin toward the caseins and, unlike chymosin, readily hydrolyse α_{s2}- and β-caseins. In Cheddar cheese made with rennets from *Endothia parasitica* or *Mucor miehei*, β-casein was considerably more hydrolysed than in calf rennet cheese: in cheese made with *Endothia* rennet, β-casein was even attacked in preference to α_{s1}-casein (Edwards & Kosikowski, 1969). The electrophoretic patterns of casein breakdown by the *Mucor* rennets are different from those obtained with chymosin (Edwards & Kosikowski, 1969; Creamer *et al.*, 1985), showing that cleavage of the casein molecules is related to the coagulant used.

2.4.1.4 Evaluation of proteolysis in cheese

The protein in most cheese varieties is progressively hydrolysed to large peptides, small peptides, amino acids and small organic molecules through the action of proteolytic and other degradative enzymes (O'Keeffe *et al.*, 1976; Fox, 1989). The progression of protein hydrolysis could be followed using various chromatographic techniques, class fractionation with different concentrations of reagents such as trichloroacetic acid and a range of electrophoretic techniques.

Electrophoresis is a powerful tool for following the hydrolysis of individual bonds in the casein molecules during the early stages of cheese maturation (Creamer, 1979). However, only the larger peptides can be adequately visualised in gel electrophoresis which essentially limits the technique to the estimation of the loss of caseins and their major primary products (Creamer, 1991).

Electrophoresis is a method whereby charged molecules in solution, chiefly proteins and nucleic acids, migrate in response to an electrical field. Their rate of migration or mobility through the electrical field depends on the strength of the field, on the net charge, size and shape of the molecules, and on the ionic strength, viscosity and temperature of the medium in which the molecules are moving. As an analytical tool, electrophoresis is simple, rapid and highly sensitive (Andrews, 1983).

Electrophoresis is usually carried out in a slab gel that is formed within a glass sandwich made of two flat glass plates separated by two spacer strips at the edges and clamped together to make a water-tight seal. In most electrophoresis units, the gel is mounted between two buffer chambers containing separate electrodes so that the only electrical connection between the two chambers is through the gel.

Proteins are amphoteric compounds, *i.e.* they contain both acidic and basic residues and their net charge is determined by the pH of the medium they are in. Each protein has its own characteristic charge properties depending on the number and kinds of amino acids carrying amino or carboxyl groups. There is a pH at which there is no net charge on a protein; this pH is called the isoelectric point or pI. In a solution with a pH above its pI, a protein has a net negative charge and migrates toward the anode in an electrical field. Below its pI, the protein is positively charged and migrates toward the cathode. The pH of a solution in an electrophoresis system must be kept constant to maintain the charge, and hence the mobilities, of the proteins. For this reason, the solutions used in electrophoresis must be buffered.

There are two types of buffer systems in electrophoresis, continuous and discontinuous. A continuous system has only a single separating gel and uses the same buffer in the tanks and the gel. In a discontinuous system, a non-restrictive large pore gel, called a stacking gel, is layered on top of a separating gel. Each gel layer is made with a different buffer, and the tank buffers may be different from both the gel buffers. The resolution obtainable in a discontinuous system is much greater than that in a continuous system.

Proteolysis during cheese ripening is readily followed using electrophoresis in gels of polyacrylamide in the presence of high concentrations of urea. Alkaline urea-PAGE and sodium dodecyl sulphate (SDS) PAGE are two of the methods routinely used. SDS-PAGE is useful for quantitating the caseins but differentiation of α_{s1}- and α_{s2}-caseins is often a problem (Creamer, 1991). It is suitable for para-κ-casein but confusing for all

the other proteins. On the other hand, alkaline urea-PAGE is not useful for para-κ-casein but is suitable for everything else.

A mini alkaline urea-PAGE (discontinuous) system (Creamer, 1991) was used in the present study. In the alkaline PAGE method, a solution of the gelling monomer (acrylamide) and the cross-linker (bis-acrylamide or bis) together with the various buffer salts is mixed together with a small amount of initiator or accelerator (such as TEMED, N, N, N', N'-tetramethylethylenediamine) and some catalyst (ammonium persulphate). The gel is formed in a mould and slots are incorporated. The gel is then connected with two buffer chambers, an upper cathodic chamber and a lower anodic chamber. Samples of the cheese that have been dispersed in buffer are placed into the slots, buffer solutions are put into the electrode chambers and the electrical power is connected to the buffer chamber electrodes. The proteins traverse the gel under the influence of the electrical field. The gel is then removed from the apparatus and immersed in a stain solution to fix and stain the proteins in the gel. The excess stain is removed from the gel, which is then examined, subjected to densitometry and photographed (Creamer, 1991).

2.4.2 Cheese rheology

Rheology is formally defined as the study of the flow and deformation of matter. The rheological characterization of cheese is important as a means of determining body and texture for quality and identity. It is also a means of studying the structure of cheese as a function of composition, processing techniques, and storage conditions (Konstance & Holsinger, 1992).

The rheological properties of cheese depend on its composition and microstructure. Casein, fat and water are the three main constituents of cheese and contribute to its structure and texture. Casein forms an open mesh in which fat globules are entrapped and water binds to the protein and fills interstices resulting in a viscoelastic matrix. The ratio of fat to protein in the milk is critical as increases in fat and water contents weaken

the protein structure whereas decreases result in hardening of the cheese (Jack & Paterson, 1992).

2.4.2.1 Rheological changes during cheese ripening

Once the basic structure has been established, ripening generates the final textural character of the cheese. This process is influenced by factors such as maturation conditions (particularly temperature), the nature and amount of coagulant remaining, and the microorganisms and enzymes present in the cheese (Walstra *et al.*, 1987).

The texture of a cheese is determined primarily by its pH and the ratio of intact casein to moisture (Lawrence *et al.*, 1987). A good correlation between the cheese firmness and the quantity of intact casein present was reported by de Jong (1976) for Meshanger cheese and by Creamer & Olson (1982) for Cheddar cheese. The breakdown products of the caseins are mostly water soluble, and Walstra & van Vliet (1982) considered that they did not contribute to the protein matrix of Gouda cheese whereas Creamer & Olson (1982) considered that moisture loss by hydrolysis would harden Cheddar cheese. These differences in view relate to the differences between the two cheese varieties.

Two distinct phases in texture development are observed during cheese ripening. In the first phase, the texture generally changes markedly in the first few weeks of ripening as the cleavage of the Phe_{23}-Phe_{24} bond of α_{s1}-casein by rennet results in the hydrolysis and release of the peptide α_{s1}-I, causing a general weakening of the casein network (de Jong, 1976; Creamer & Olson, 1982; Lawrence *et al.*, 1987). During this period, the rubbery texture of the young cheese curd is rapidly converted to a smoother, more homogeneous product.

In the second phase, the change in texture is relatively slow and is determined mainly by the rate of proteolysis. Proteolysis during this phase is largely controlled by the proportion of residual coagulant and plasmin in the cheese, the moisture content, the salt to moisture ratio and the storage temperature (Lawrence *et al.*, 1987).

The other significant feature of proteolysis is that, as each peptide bond is cleaved, two new ionic groups are generated and each of these will compete for the available water in the system. Thus, some of the water previously available for solvation of the protein chains is incorporated into the new ionic groups. The cheese therefore tends to become relatively harder and more brittle with age and more resistant to slight deformation (Stanley & Emmons, 1977; Creamer & Olson, 1982).

Small changes in the moisture to casein ratio also result in relatively large changes in available moisture, because much of the moisture is bound to the caseins and their degradation products. Even small decreases in water activity (A_w) greatly decrease the rate of proteolytic activity in cheese. Creamer (1971) reported that, at an A_w of 0.983, the rate of β-casein hydrolysis was one-fifth that of α_{s1}-casein. When the A_w was lowered to 0.963, β-casein was only slightly hydrolysed whereas α_{s1}-casein underwent a very slow degradation.

2.4.2.2 Effect of pH on cheese texture

The role of pH in cheese texture is particularly important as the changes in pH are related directly to chemical changes in the protein network of the cheese curd. Although mineral content plays an important role in establishing the characteristic structure (Lawrence *et al.*, 1983, 1984), the texture of Cheddar cheese appears to be more dependent upon pH than any other factor (Lawrence *et al.*, 1987).

Cheddar cheese has a texture that is intermediate between those of the relatively high pH cheeses, which flow readily when a force is applied, and the low pH cheeses, which tend to deform, by shattering, only at their yield point. As the pH decreases towards that of the isoelectric point of paracasein, the protein assumes an increasingly more compact conformation and the cheese becomes shorter in texture and fractures at a smaller deformation (Creamer & Olson, 1982; Walstra & van Vliet, 1982).

Creamer *et al.* (1988) studied the effect of pH on the texture of Cheddar cheese and found a low pH, high calcium cheese to be firmer but more brittle than the control. The high pH, low calcium cheese was more pliant and rubbery, indicating that pH was more important than calcium content, but did not completely preclude calcium effects.

A number of different methods have been employed to study the rheological properties of cheese. In this study, the Bohlin constant stress rheometer, the TAHD texture analyser and the Instron Universal Testing Machine were used to analyse some of textural characteristics of cheese. A method for the rheological evaluation of grated cheese curd was also developed (Section 4.2.5.1).

2.4.2.3 Rheological techniques

In rheology, the relation between the stresses exerted on a material and the corresponding deformations are measured as a function of the time scale of the experiment (van Vliet, 1991). *Stress* is the ratio of the force acting on a surface of a material divided by the area of that surface, and is measured in pascals (Pa). *Strain* is the relative deformation resulting from the application of stress. When the stress is applied tangentially, the strain which results is described as *shear* (Prentice *et al.*, 1993).

The rheological methods of testing may be classified as static methods (where the sample is stressed constantly in the same direction) or dynamic methods (where the sample is stressed in a restricted oscillating manner) (van Vliet, 1991).

2.4.2.3.1 Static measurements

These may be further subdivided based on the type of measurement made.

(1) Measurement of stress when applied strain is constant

These are mostly used for measuring a relaxation time or spectrum. It is assumed that the fixed strain is reached instantaneously although it takes a certain amount of time. For cheese, the relaxation time is dependent on how fast the fixed strain is reached and

also on the strain, for strains greater than 0.05 (Pollak & Peleg, 1980; Luyten, 1988). The relaxation times measured with different apparatus therefore cannot be compared except when the fixed strain and the rate of deformation to obtain it are exactly the same. Because the overall relaxation behaviour of cheese is caused by a whole set of different relaxation phenomena (type and energy content of the protein-protein bonds differ; reconformation processes occur; etc.), interpretation of a relaxation curve is difficult and only comparisons between cheeses can be made (van Vliet, 1991).

(2) Measurement of stress when applied strain rate is constant

These may be used for determination of the viscosity of Newtonian liquids or for determination of the apparent viscosity of non-Newtonian liquids as a function of shear rate or elongation rate. These tests may also be used for measurement of the fracture properties of solid-like materials, as in cheese rheology.

Rheological instruments such as the TAHD texture analyser are used to determine the large strain deformation properties of cheese. Strain is calculated using the equations (Watkinson *et al.*, 1997):

$$\dot{\varepsilon}_0 = v / h_0 \qquad (2.1)$$

$$\epsilon_c = \Delta h_t / h_0 \qquad (2.2)$$

$$\epsilon_h = -\ln (1 - \epsilon_c) \qquad (2.3)$$

and the stress is calculated from

$$\sigma = (1000 F_t / \pi r_0^2) (1 - \epsilon_c) \qquad (2.4)$$

where v = speed of compression (mm/s),

h_0 = initial sample height (mm),

Δh_t = displacement of crosshead at time t (mm),

F_t = force from lubricated compression at time t (N),

r_0 = initial radius of sample (mm),

$\dot{\varepsilon}_0$ = initial strain rate (s^{-1}),

ϵ_c = Cauchy strain,

ϵ_h = Hencky strain, and

σ = stress from lubricated compression test (kPa).

The apparent fracture strain is the strain at the local maximum for stress in the stress versus Hencky strain curve. The apparent modulus of deformability is the slope of the stress versus Hencky strain curve at low strain (typically below 0.03) where the curve is close to a straight line.

(3) Measurement of strain or strain rate when applied stress is constant

These may be used for determination of the viscosity or apparent viscosity by measuring the strain rate. In creep measurements, strain is measured as a function of the time elapsed after the start of the measurement. Measurement of the fracture properties of solid-like materials may also be made (van Vliet, 1991).

2.4.2.3.2 Dynamic measurements

In these dynamic measurements, the applied stress, strain or strain rate is commonly made to vary sinusoidally with time. The time scale of the measurement can be varied by changing the frequency of the oscillation. The strain bonds (linkages, junctions) between the structure elements, which start to break within a time scale, can be determined by slowly increasing the strain. Dynamic measurements are also useful in determining both the elastic and the viscous components in the reaction of a material on an applied stress or strain over a wide range of time scales.

In these dynamic measurements, as the stress is varied sinusoidally at a frequency ω,

$$\sigma_t = \sigma_o \sin(\omega t) \qquad (2.5)$$

where σ_o is the maximum stress. The stress causes a sinusoidally varying shear,

$$\gamma_t = \gamma_o \sin(\omega t - \delta) \qquad (2.6)$$

where γ_o is the maximum deformation and δ is the phase difference between the stress and the shear.

From equations (2.5) and (2.6), the elastic or storage modulus, G′ (a measure of the energy stored in the material), and the viscous or loss modulus, G″ (a measure of the energy dissipated in the material), may be calculated:

$$G' = (\sigma_o / \gamma_o) \cos \delta \qquad (2.7)$$

$$G'' = (\sigma_o / \gamma_o) \sin \delta \qquad (2.8)$$

The ratio between these moduli gives the loss tangent:

$$\tan \delta = (G'' / G') \qquad (2.9)$$

2.5 DEVELOPMENT OF MICROSTRUCTURE IN CHEESE

The development of structure in Cheddar cheese has been comprehensively investigated (Kimber *et al.*, 1974; Brooker, 1979; Kalab *et al.*, 1982).

The addition of lactic acid-producing starter bacteria to milk produces little change to its ultrastructural appearance (Kimber *et al.*, 1974). The casein micelles appear as discrete particles and never make permanent contact because they are mutually repelled by their high negative surface charge. Similarly the fat globules in milk are discrete and

undistorted. Action of the coagulant alters the surface properties of the micelles so that they can form an intermicellar network.

The first step in the formation of the cheese structure is the formation of the casein network. The casein micelles are subjected to specific proteolysis by the coagulant to strip off the hydrophilic portion of the κ-casein (Dalgleish, 1979; Chaplin & Green, 1980), which probably projects from the surface of each particle into the solvent (Walstra, 1979). The denuded micelles then aggregate by a random, diffusion-controlled mechanism (Dalgleish, 1979; Green & Morant, 1981) at a rate independent of their size. Chains of micelles are formed first, but these gradually link together to build up a network (Green *et al.*, 1978).

By the time the milk forms a soft gel, adjacent micelles in large interconnecting aggregates will have undergone partial fusion and formed a three-dimensional network, enclosing the fat globules and starter bacteria.

When the curd is cut into small pieces and cooked, syneresis of the milk gel is accompanied by several changes in the curd structure (Brooker, 1979). The fusion of the micelles becomes complete so that the contours of individual particles can no longer be detected and adjacent portions of the convoluted casein network fuse. The interstitial spaces are correspondingly reduced and the enclosed bacteria and fat globules are brought into closer contact, both with each other and with the casein network (Brooker, 1979). Cooking causes the casein network formed during gelation to form strands, some 1-2 μm across, and some of these are linked by casein bridges (Kimber *et al.*, 1974). Whey drains through channels that have been formed between the protein strands; during whey drainage, the spaces between the strands are reduced in size and the matrix becomes more compact, pressing and distorting the fat globules, which partially retain their individuality although some coalescence occurs (Kimber *et al.*, 1974).

When the curd is cut, fat globules at the new surface are exposed and some of the fat globules and bacteria are lost into the whey. This leaves a thin superficial layer at the

granule surface that is depleted of fat. During cheddaring, the superficial layers of adjacent curd granules fuse leading to the formation of casein-rich junctions (Kalab *et al.*, 1982). Starter bacteria are trapped in the casein network near the fat-casein interface, which has been shown to be the region of highest moisture content in the mature cheese (Kimber *et al.*, 1974).

After the drainage of the whey, the curd becomes compressed under its own weight during the cheddaring process. The casein matrix, formed by progressive fusion of micelles and adjacent aggregates, becomes extensively folded as manufacture progresses and individual micelles lose their identity. The coalescence of many fat globules and the progressive elimination of interstitial spaces are also observed (Kimber *et al.*, 1974). In the cheddaring process, the curd is allowed to flow at a temperature near the cooking temperature (approximately 38 °C). During this process, the curd alters its character considerably, changing from a simple unstructured mass to a fibrous striated material. At the cheddaring temperature, milkfat is normally melted (Norris *et al.*, 1971) although, in the globules, layers of crystalline fat are positioned adjacent to the milkfat globule membrane (Buchheim, 1970). During the cheddaring process, many of these globules rupture and the liquid milkfat is able to form aggregates (Hall & Creamer, 1972). In most fat globules secondary membrane forms the major interface between the lipid phase and the water phase.

The fusion of the casein matrix and the progressive elimination of interstitial spaces continue during the subsequent milling, salting and pressing of the curd. After milling, when the curd particles are dry salted discrete boundaries are set up between the individual particles. This is in contrast to brine-salted cheeses where there is only one boundary, *i.e.* the cheese rind (the exterior of the cheese). The addition of dry salt causes shrinkage of the curd and a rapid rate of release of whey containing calcium and phosphate, particularly in the first few minutes of pressing (Lawrence *et al.*, 1993). It has been suggested that the salted surface of the curd particles acts as a selective permeable membrane, thereby concentrating calcium and phosphate at the surface of the curd particle (McDowall & Dolby, 1936). This deposition results in the phenomenon

of seaminess in Cheddar cheese (Czulak, 1963; Al-Dahhan & Crawford, 1982), a condition in which the junctions of the milled curd particles are visible after pressing. Seaminess in some cases persists in mature cheese (Lawrence *et al.*, 1993).

Several alterations in the ultrastructural appearance of Cheddar cheese take place during maturation. The structural details become less clear during maturation; some fat globules are still apparent in mature cheese but in many cases the boundaries between them have disappeared and many are surrounded by "debris", probably hydrolysed protein (Kimber *et al.*, 1974). Green *et al.* (1981) observed during ripening that the microstructure became more open and less well defined, especially in the 28-week old cheese, presumably reflecting degradation of the protein network by proteolysis. Loss of structural detail during ripening has also been reported by other investigators (Dean *et al.*, 1959; Kalab, 1977; Stanley & Emmons, 1977).

Brooker *et al.* (1975) observed crystalline inclusions in ripening and mature Cheddar cheese which they identified as a variety of calcium salts, particularly phosphates and lactates. Their location in spaces between the fat and casein phases in the cheese suggests that they develop from the pockets of residual whey (Kalab, 1993).

2.5.1 Microscopic examination of cheese

The rheological properties and sensory attributes of cheese are closely related to its structure (Stanley & Emmons, 1977; Emmons *et al.*, 1980b; Green & Manning, 1982; Green *et al.*, 1986). The structure of a cheese is dependent upon the manufacturing procedure, the age and the chemical composition of the cheese (Lawrence *et al.*, 1984; Creamer *et al.*, 1988).

Knowledge of the structure, *i.e.* the spatial arrangement of the major structural elements (casein, fat and water), and their interactions is essential to gain a proper understanding

of the behaviour of cheese. The spatial arrangement of the structural elements can be studied by visual observation techniques, such as light and electron microscopy.

2.5.2 Microscopy techniques

Microscopy and imaging are very appropriate techniques for evaluating food structure because they are the only analytical methods that produce results in the form of images rather than numbers, and are basically an extension of the visual examination of foods. Microscopy techniques vary in the method of image production, resolution and type of signal detected, and give a particular type of structural information that is unique to the technique used (Kalab *et al.*, 1995).

The microstructure of cheese has been examined by light and electron microscopy techniques. The resolving power of the naked eye is only about 0.2 mm. Using a light microscope, the resolution may be taken down to 200 nm. Scanning electron microscopy (SEM) improves the resolution to better than 10 nm, and details as small as 0.5 nm may be distinguished with transmission electron microscopy (TEM) (Kalab & Caric, 1990).

2.5.2.1 Light microscopy

The compound microscope is the basic instrument used in light microscopy, and can be easily adapted with accessories to perform other optical microscopy methods (Dziezak, 1988). The optical system of the compound microscope consists of: the objective and ocular (or eyepiece), two separate lenses aligned in series; a condenser; and a light source (typically an incandescent bulb) (Evans, 1973). The objective is the most important component for resolution. The condenser has a number of functions, the most important being to collect light from the source and concentrate it to illuminate the object.

In conventional light microscopy, illumination is transmitted sequentially through a condenser, the specimen and the objective, producing a real image that is upside down and reversed, and magnified within the microscope tube. The real image is then magnified again by the ocular lens, which produces either a virtual image that appears to be approximately 25 cm from the eye, or a real image on photographic film placed above the microscope tube (Dziezak, 1988). If the specimen is not highly coloured, contrast must be introduced to make it visible. This is commonly achieved by the use of dyes or stains which react specifically with particular components, *e.g.* fat, of the specimen.

2.5.2.2 Confocal microscopy

The confocal laser scanning microscopy (CLSM) is a fluorescence microscopy technique in which a monochromatic laser light is used to scan the sample and focused images of layers from within a relatively thick sample are recorded so that a series of images is obtained (Heertje *et al.*, 1987).

The specimen preparation usually involves placing a few crystals of a fluorescent dye on to the surface and allowing it to dissolve and diffuse into the sample at an appropriate temperature. Cheese consists of hydrated protein in which fat droplets are embedded. The microstructure of both components can be made visible by impregnation of the fluorescers Rhodamine or Fast Green to stain protein and Nile Red or Nile Blue to stain fat. The sample is observed after laser excitation.

The instrument uses a focused laser beam to scan a sub-surface layer of the specimen in such a way that information from this focal plane passes back through the specimen and is projected on to a pinhole (confocal aperture) in front of a detector. Only the light from a defined focal plane in the specimen is able to pass through the confocal aperture, reach the detector and produce an image which is effectively an optical slice of the dyed specimen (Brooker, 1995). By moving the specimen up and down relative to the focused laser light, a number of optical sections can be obtained.

Using CLSM, larger samples of food can be sectioned optically and the images, focused at predetermined planes in the sample, can then be stacked using computer software and reconstituted to reveal a three-dimensional structure. This technique markedly increases the depth of focus (Kalab, 1995).

Among the drawbacks of CLSM is its lower resolution (about 150 nm). High resolution, good quality images of microstructure, such as those obtained using thin sections in electron microscopy, cannot be obtained using CLSM. It is also not quantitative and can observe only the dye present in the sample.

2.5.2.3 Electron microscopy

In electron microscopy techniques, a high energy beam of electrons is used in place of light, with magnetic fields instead of optical lenses and phosphorescent screens (or electronic monitors) and photographic plates to display the images. There are two major modes of electron microscopy - SEM and TEM.

2.5.2.3.1 Scanning electron microscopy

SEM is used to examine the topography to a resolution of about 10 nm, the size of a small casein micelle but not a whey protein. SEM can handle larger specimens than is possible with TEM.

Sample preparation for SEM typically involves fixing in glutaraldehyde, dehydration, mounting on SEM stubs using a silver-based cement and sputter-coating with a layer (approximately 20 nm thick) of gold. The sample is scanned with an electron beam. Secondary electrons emitted from the surface of the specimen are measured via a positively charged collector and the resultant image is displayed on the cathode ray tube. The depth to which the focused electron beam can penetrate can be controlled by varying the accelerating voltage (Dziezak, 1988).

2.5.2.3.2 Transmission electron microscopy

In TEM, the image forms when a beam of accelerated electrons is focused by a series of lenses to pass through the specimen, and then through another series of lenses which give rise to the final magnified image.

The preparation of a specimen for analysis by TEM is relatively complex and involves steps to avoid development of artefacts or cross-contamination. The common method involves sequential fixation, dehydration, embedding, sectioning and staining (Hood & Liboff, 1982). The fixation step stabilises the structure of the specimen. Thin (15-90 nm) sections of samples are embedded in epoxy resin or platinum-carbon replicas of the sample are placed in the path of the electron beam, and the enlarged image is observed on a fluorescent screen or photographed on film. The electrons are transmitted through the sample with varying degrees of absorption. Differences in the electron density of structures stained in the resin sections with heavy metal salts (*e.g.* uranium or lead) or differences in thickness of the metal replica due to differences in the angles at which the metal is deposited on the fractured sample result in the formation of the image (Bozzola & Russell, 1992).

Cheese has been examined routinely using light and electron microscopy techniques. In the present study, CLSM was used to observe the changes in the structure of curd with changes in pH and time. A method to stain and observe the water phase in cheese was also developed (Section 4.2.6.1).

2.6 WATER IN CHEESE

Cheese may be considered as a continuous matrix of a swollen proteinaceous mass, interspersed with fat globules (Walstra & van Vliet, 1982). Water exists in cheese as a continuous phase dispersed throughout the porous casein matrix (Guo & Kindstedt, 1995). At a higher water content, the protein matrix is more swollen and the protein

concentration in the matrix is lower (Luyten, 1988). Cheddar cheese contains 1.4-1.5 g water/g protein (Geurts *et al.*, 1974b).

The amount of water associated with proteins is dependent on several factors. The equilibrium voluminosity of paracasein micelles at room temperature and physiological pH was roughly estimated to correspond to 1.4 g water/g protein; the voluminosity would be lower for a lower pH and for a higher temperature (Walstra, 1993).

Snoeren *et al.* (1984) observed a relation between the hydration of casein micelles and pH, with a "peak" in the voluminosity of the casein micelles at pH 5.45. Creamer (1985) carried out a similar study in which the effect of pH, NaCl, $CaCl_2$ and rennet action on the solvation of the casein micelles was measured. Solvation of the micelle pellets obtained on centrifuging aliquots of milk was calculated as the ratio of the mass of water in the pellet to the mass of dry pellet (*i.e.* g water/g dry pellet). Renneting decreased the proportion of water of solvation between pH 4.8 and pH 6.6. Addition of NaCl (5% w/v) to the mixture after rennet action and prior to pH adjustment and centrifugation increased solvation in this pH range. However, when $CaCl_2$ (0.04% w/v) was added to the milk mixture in addition to NaCl, the micelle solvation decreased at higher pH but not at pHs 4.8 or below. The study indicated that water retention by chymosin-treated micelles decreased slightly with decreasing pH in the range from 6.7 to 5.6 but increased as the pH decreased from 5.6 to 5.25 and that water retention increased markedly when the salt concentration was increased. The equilibrium water content of rennet-treated casein micelles was greater at pH 5.2 than at pH 5.6.

van Hooydonk *et al.* (1986) reported that the voluminosity of casein in normal skim milk was at a maximum around pH 5.3 and that it decreased after renneting.

The equilibrium water content of casein gels is controlled by the opposing forces of repulsion between like ionic charges on the polymer network and the cohesive forces operating (Lelievre & Creamer, 1978). The gradual decrease in solvation as the pH of milk decreases from 6.6 to 5.6 reflects the neutralisation of negative charges on the

protein chains in the polymer network (Creamer, 1985). Rennet treatment lowers the net negative charge on casein micelles (Pearce, 1976) and this is reflected in the lower pellet solvation for rennet-treated casein micelles.

Addition of NaCl to the system increased micelle pellet solvation, possibly by interfering with the cohesive forces holding the micelle together but more probably by displacing calcium or calcium phosphate from the protein matrix with a concomitant increase in the volume of the matrix (Creamer, 1985).

The solvation peak near pH 5.3 is possibly a result of calcium interactions with the phosphate residues on the casein molecules reducing the anticipated monotonic increase in polymer repulsion with increased pH (Creamer, 1985).

2.6.1 Water holding capacity of milk protein structures

The amount of water held by the cheese curd is influenced by the water holding properties of the proteins, and is accomplished by a complexity of reactions between water and milk proteins. Chou & Morr (1979) defined the term "water-holding capacity" as a quantitative indication of the amount of water retained within a protein matrix under certain defined conditions and usually also includes entrapped water.

Hermansson (1986) defined water-holding capacity as the ability of a food structure to prevent water from being released from the three-dimensional structure. The synonyms used for water holding include water hydration, water retention, water absorption, water imbibing and water binding.

Intact casein micelles are capable of binding relatively large amounts of water. Water entrapment in the native micellar structure is partly accomplished by the colloidal calcium phosphate, and also by the hydrophilic nature of the κ-casein and its position in the submicelles. Globular proteins such as β-lactoglobulin display varying degrees

of hydration, depending on denaturation, aggregation and interaction with other proteins (de Wit, 1984; Kinsella, 1984).

As a consequence of heat treatment, the protein is unfolded and may exhibit an increased water-binding capacity. A slight increase in bound water as proteins denature has also been reported. The firmness of the heat-induced network largely determines the water holding, as water is held more effectively in a firm structure than in softer gels (Plock & Kessler, 1992).

The amount of water associated with protein depends on factors such as the amino acid composition, the number of exposed polar groups, surface hydrophobicity, pH value, ionic composition and strength, temperature and concentration (Kinsella *et al.*, 1989).

In Mozzarella cheese, the distribution of water is different from that in most other cheeses as a result of the unusual microstructure caused by stretching (Guo & Kindstedt, 1995). In most cheeses water is finely dispersed throughout the casein matrix; however, in Mozzarella, the casein matrix is oriented into fibrous aggregates that form large open channels or columns that are filled with water and fat droplets (Oberg *et al.*, 1993). Accumulation of water into pockets may contribute to the poor water-holding characteristics of Mozzarella (Guo & Kindstedt, 1995).

Mozzarella cheese is normally stored for a period of 2-3 weeks after manufacture. Fresh Mozzarella often exudes free moisture at the block surfaces and freshly cut surfaces, making it unsuitable for shredding and melting during the first few days after manufacture (Kindstedt, 1995). However, as aging progresses, surface moisture is absorbed back into the block, presumably as a result of increased water-holding capacity of the curd.

2.6.2 Factors influencing the water content of cheese curd

The moisture content of cheese curd is affected by the way the milk is treated before cheesemaking. The moisture content is increased by high temperature pasteurisation of milk, homogenisation of milk or cream and the addition of denatured whey protein. The reasons for the higher moisture content vary, *e.g.* retention of denatured whey protein with a high water-holding capacity or the occlusion effect of insoluble whey protein or homogenised fat. Fat also causes retention of moisture (O'Keeffe, 1984). O'Keeffe (1984) observed that the cheese from skim milk contained 1.05 g moisture/g protein whereas a full cream cheese contained 1.35 g moisture/g protein.

As described earlier, several of the process steps during cheesemaking influence the water content of cheese curd. Together with the effects of heat, agitation, salting and the decrease in pH, the amount of syneresis during cheesemaking determines the water content of the cheese. The processes of syneresis and salting and their effect on cheese moisture have been dealt with in detail in Sections 2.2.4 and 2.3.3 respectively of this review.

2.6.3 Measurement of the water held in cheese curd

The common methods for measuring water-holding properties of foods are based on the application of an external force such as pressure and centrifugation or on capillary suction of a porous material in contact with the sample. When the amount of released water is measured, water is determined either as the water-holding capacity, which is the amount of water bound per gram of protein or dry matter, or as the moisture loss, which is the amount of juice released per gram of sample (Hermansson, 1986).

Several methods are available for measuring the amount of water held in foods (Kneifel *et al.*, 1991; Kneifel & Seiler, 1993). Centrifugation can be used to measure water retention by the cheese curd (Guo & Kindstedt, 1995). In the centrifugation tests, the

sample is centrifuged in a container and either the amount of liquid released or the sample with the remaining water is weighed to determine the quantity of water retained.

In this study, the techniques of mini alkaline urea-PAGE, centrifugation, small and large strain deformation testing (static and dynamic methods) and confocal microscopy were used to determine some of the changes taking place in cheese during the first 2 weeks of ripening.

3.0 OBJECTIVES

During the first few weeks of maturation, the texture of Cheddar-like cheeses changes substantially from a rubbery material, often containing strongly oriented protein fibres from which some moisture can be expelled readily, to a uniform smooth-bodied cheese. In the past, proteolysis, particularly the cleavage of α_{s1}-casein, has been invoked as an important factor in the early changes in cheese texture.

In the present study, the early events that may be responsible for the textural changes were examined in detail with particular emphasis on segregating the effects of proteolysis from the other events that might affect cheese texture.

In order to do this, it was necessary to have a controlled system to study and to have a set of measures to determine the effect of various parameters on the development of cheese texture.

Initially three cheeses that cover the gamut of Cheddar-like cheese characteristics were examined using the general methods of Kindstedt (1993; personal communication) and Guo & Kindstedt (1995) to determine how the quantity and composition of cheese curd and centrifugal serum vary during early cheese maturation.

It was then intended to use appropriate rheological techniques to determine the quantitative changes in cheese texture with time, to use some estimates of proteolysis to determine the relative importance of proteolysis to the textural changes, and to use microscopic examination of cheese curd to determine if the textural changes could be related to visible structural differences.

Finally, it was hoped to apply these techniques to cheeses manufactured using different parameters in order to gain a greater understanding of how these particular parameters could be used to influence the texture of cheese.

4.0 MATERIALS AND METHODS

4.1 MATERIALS

Whole milk (obtained from Tui Milk Products Ltd, Palmerston North, New Zealand), standardised to an appropriate protein to fat ratio, pasteurised at 72°C for 15 s and cooled to 32 °C, was used for cheesemaking.

The starters used were strains of *Lactococcus lactis* subsp. *cremoris*, and were obtained from the Starter Production Unit of the NZDRI.

USP grade lactic acid (Clark Products Ltd, Napier, New Zealand), calf rennet (59 rennet units (RU)/ml; New Zealand Co-operative Rennet Co., Eltham, New Zealand), Rennilase 46L® (Novo Industri, Bagsvaerd, Denmark), glucono-δ-lactone (GDL; Fujisawa Pharmaceutical Co. Ltd, Tokyo, Japan), cheese salt (New Zealand Dairy Salt Standard 1990; Dominion Salt (N.I) Ltd, Mt. Maunganui, New Zealand), β-lactoglobulin (prepared from acid whey by salt fractionation and size exclusion chromatography by Gavin Manderson, NZDRI), Fast Green FCF and Nile Blue (BDH, Poole, England) and tetramethyl- rhodamine-5-(and-6)-maleimide (Molecular Probes Inc., Eugene, Oregon, USA) were used.

The chemicals used for analysis were AR grade.

4.2 METHODS

4.2.1 Cheesemaking

Cheese was made with modifications to the standard Cheddar cheesemaking process. The cheesemaking was further modified as the work progressed in order to minimise the

changes in cheese pH during storage and to alter the pH of the cheese curd after manufacture.

4.2.1.1 Cheesemaking with starter and chymosin

The cheese made with starter and chymosin was used to determine the effect of pH values at draining and salting on the various attributes being studied. The pHs were varied during cheesemaking to cover a wider pH range for the study.

Cheese curd was made with modifications to the standard procedure for making Cheddar cheese. Two 400 l vats were filled with milk and primed with starter at a rate of 4% (w/v). When the milk reached pH 6.3, rennet was added at 16 ml/100 l. The vat contents were mixed and left to set at 32 °C. The coagulum was cut after 20 min using 8 mm cheese knives, and was cooked at 36 °C for vat 1 and at 34 °C for vat 2. The whey was drained out at pH 5.9 for vat 1 and pH 5.6 for vat 2. The curd was cheddared for 30 min and then milled. The curd in vat 1 was divided into two lots and salted at 350 g/100 l milk, for lot 1 when the curd pH was 5.6 and for lot 2 when the curd pH was 5.3. Similarly, the curd in vat 2 was divided into two lots and salted at pH 5.3 and pH 5.0 respectively. After mellowing for 30 min, the four curd lots were transferred into 0.37 m × 0.30 m × 0.18 m hoops lined with cheese cloth and left for 3 h without pressing. The curd blocks were then transferred into plastic bags and stored at 2 °C.

4.2.1.2 Cheesemaking by chemical acidification

Cheese was made by chemical acidification in order to minimise the changes in cheese pH during storage and to study the effect of pH on the various attributes. Starter was not used in the cheese made by this method.

Cheese vats of 100 l capacity were filled with milk at 10 °C and the pH was adjusted to the desired value by the addition of various amounts of dilute (10%) lactic acid. The milk was then warmed to 32 °C and Rennilase 46L® was added at 8 ml/100 l. After 8-

10 min, the coagulum was cut using 8 mm cheese knives and cooked at 36 °C. Stirring was continued for another 60 min. The whey was then drained out and the curd was cheddared for 30 min. After milling, 350 g salt/100 l milk was mixed in and the curd was allowed to mellow for 30 min. The subsequent steps were as in Section 4.2.1.1.

4.2.1.3 Cheesemaking by chemical acidification and addition of GDL

GDL was used to alter the pH of the cheese curd after manufacture to the desired value and to study the effect of this final pH on the various attributes.

Cheese curd was made as described in Section 4.2.1.2. On the next day, the milled, salted and stored curd was grated into pieces about 2 mm × 2 mm × 6 mm in size and mixed with calculated amounts of GDL. The curd was then held at 2 °C for a minimum of 48 h prior to analysis to allow the GDL to fully hydrolyse and diffuse into the curd and to obtain curd samples in the desired pH range. Subsequent storage of the samples was also at 2 °C.

4.2.2 Chemical analyses of cheese curd and centrifugal serum

Samples of cheese curd and centrifugal serum (obtained by the procedure described in Section 4.2.4) were analysed for pH and composition according to standard procedures (New Zealand Ministry of Agriculture and Fisheries, 1979). Fat, total nitrogen, non-protein nitrogen, salt, calcium and sodium contents were determined by the Analytical Chemistry Section of the NZDRI.

(1) pH: A PHM 82 standard pH meter (Radiometer, Copenhagen, Denmark) with an N61 Schott Geräte (Mainz, Germany) electrode was standardised using 0.025 M phosphate buffer of pH 6.88 and 0.05 M potassium hydrogen phthalate buffer of pH 4.00. Grated curd was tightly packed into a 10 ml glass beaker and its pH was determined.

(2) Moisture: The moisture content was determined by drying a known weight of grated curd sample at 100 °C for 16 h, cooling in a desiccator for 1 h and weighing. The content of moisture in non-fat substance (MNFS) was calculated.

(3) Fat: Grated cheese curd (9.0 g) was placed in a Babcock bottle and dissolved with 17.5 ml sulphuric acid, added in three or four portions. The test bottles were placed in a water bath at 65 °C for 5 min and then centrifuged at 165 ***g*** for 5 min. The content of fat-in-dry matter (FDM) was then calculated.

(4) Total nitrogen: The grated cheese sample was analysed using a Kjelfoss (Foss Electric, Hillerød, Denmark) nitrogen analyser. This is a semi-automated protein analyser following the Kjeldahl principle, using hydrogen peroxide to boost digestion. The equipment is calibrated with accurately prepared ammonium sulphate solutions.

(5) Non-protein nitrogen: The sample was solubilised in 0.1 M NaOH. Diluted trichloroacetic acid (TCA; 15%) was then added to give a final concentration of 12% TCA. The precipitated protein was removed by filtration using Whatman No. 42 filter paper and the filtrate was analysed for nitrogen content using the Kjelfoss instrument.

(6) Salt: About 2 g of grated cheese was weighed accurately (± 0.001 g) into a titration sample cup and mixed with 40 ml of dilute (2%) HNO_3 at 65 °C. After 30 min the sample container was attached to the autotitrator measuring electrode and titrated with silver nitrate. The content of salt-in-moisture (S/M) was calculated.

(7) Calcium: About 0.5 g of sample was weighed and mixed with 10 ml of 0.1 M HCl and diluted with the addition of 90 ml of water. Solid NaOH (0.5 g) was added and stirred until it dissolved. The solution was titrated with 0.02 M EDTA solution using Patton and Reeder's indicator.

(8) Sodium: For determination of the sodium content, about 0.05 g of grated cheese was accurately weighed and dispersed in about 50 ml of high purity water and 10 ml of

lithium reference standard was added. The volume was made up to 200 ml and analysed by flame photometery.

(9) Elemental determination by plasma emission spectrometry: The quantities of various mineral elements in the centrifugal serum were measured at the Grasslands Research Centre, New Zealand Pastoral Agriculture Research Institute Ltd, Palmerston North, New Zealand, (Lee *et al.*, 1986) with an ARL 34000 inductively coupled plasma (ICP) atomic emission spectrometer (Science & Technology (NZ) Ltd, Auckland, New Zealand). A standard ARL torch was used in conjunction with a glass cross-flow type nebuliser (GMK) and was fed by a Gilson Minipuls II peristaltic pump. The plasma operating conditions were set to give optimum performance for simultaneous multi-element analysis. Multi-element standards in 2 M HCl for calibrating the ICP spectrometer were prepared from 1000 μg/ml stock solutions of the metal chloride in 2 M HCl made from redistilled constant boiling acid and distilled deionised water.

(10) Values derived from the composition: The MNFS, FDM and S/M contents were calculated as follows:

$$\% \text{ MNFS} = \{M/(100-F)\} \times 100$$

$$\% \text{ FDM} = \{F/(100-M)\} \times 100$$

$$\% \text{ S/M} = (S/M) \times 100$$

where M is the percentage moisture, F is the percentage fat and S is the percentage salt in the cheese curd.

4.2.3 Polyacrylamide gel electrophoresis (PAGE)

The relative amounts of α_{s1}-, α_{s1}-I- and β-caseins were measured using the mini alkaline urea-PAGE technique described by Creamer (1991). The Bio-Rad mini-Protean II

equipment (Bio-Rad Laboratories, Richmond, California, USA) was used with 10 slot gels and a Bio-Rad model 1000/500 power supply.

The cheese curd or serum sample (0.50 g) was mixed with 25 ml of sample buffer (10.8 g Tris-base, 360 g urea, 5.5 g boric acid and 0.92 g EDTA were dissolved in 500 ml of Milli Q water and the solution was made up to 1 litre), held for 1 h, blended using an Ultra-Turrax T25 (Janke & Künkel, Staufen, Germany) at approximately 24 000 rev/min for 20 s, and then centrifuged at 10 000 rev/min at 4 °C for 10 min in a Sorvall RC2 refrigerated centrifuge (Ivan Sorvall Inc., Norwalk, Connecticut, USA). Aqueous subnatant (2 ml) was mixed with 10 µl/ml each of 2-mercaptoethanol and 1% (w/v) bromophenol blue solution and held for 18 h. Each slot in the gel slab was loaded with 5 µl of the mixture. Commercial rennet casein (Anchor Products Ltd, Hautapu, New Zealand) was used as the standard. After staining with Coomassie Blue and destaining, the gels were scanned on a personal densitometer (Molecular Dynamics, Sunnyvale, California, USA) and the integrated densities of the major protein bands were determined.

4.2.4 Centrifugation of cheese curd

Weighed 200 ml centrifugation bottles were filled with about 140 g of well-mixed samples of grated cheese as six replicates and centrifuged at 8500 rev/min (11 086 ***g***) and 25 °C for 75 min using a JA-14 rotor in a Beckman J2-21M centrifuge (Palo Alto, California, USA). The speed, duration and temperature of centrifugation were selected based on the results of the preliminary trials and on the work of Guo & Kindstedt (1995). When the cheese was centrifuged, it separated into three different layers. The curd compacted on to the sides of the bottles and the liquid that separated out had an aqueous lower portion with an upper lipid layer. The weight of curd was measured and the quantity of liquid released (referred to as the centrifugal serum) was estimated by difference. Care was taken to remove the serum from the bottle immediately after centrifugation to prevent its re-absorption into the centrifuged curd. The small quantity of lipid in the serum was allowed to separate out and was sucked out using a pipette.

4.2.5 Rheological analyses of cheese samples

Rheological properties of the cheese curd were determined using a TAHD texture analyser (Stable Micro Systems, Haslemere, UK) and a Bohlin rheometer (Bohlin Instruments Ltd, Cirencester, U.K.). A method was also developed for the rheological evaluation of grated cheese using the Instron Universal Testing Machine (Instron Corporation, Massachusetts, USA). The TAHD texture analyser and the Instron were used to measure large strain events and provide information on fracture properties and on textural properties such as firmness and longness. The Bohlin rheometer was used to measure small strain events and the results were useful in understanding changes in structure.

4.2.5.1 Large strain deformation testing of cheese curd

4.2.5.1.1 Rheological measurements using the TAHD texture analyser

Samples for analysis were obtained from a piece of cheese curd using a core borer (20.0 mm internal diameter) that was mounted on a drill press. The cores were placed on a trough and cut into cylinders of 25.0 mm height by a wire that was firmly attached to a metal frame. The samples were wrapped in polyethylene film to prevent loss of moisture and were allowed to equilibrate to 20 °C over a period of 2 h. After equilibration, they were compressed uniaxially using a TAHD texture analyser with a 50 kg load cell, a resolution of 1 g and an accuracy of 0.025%. The distance measurement had a resolution of 0.001 mm. The TAHD was connected to a personal computer with a rate of transfer of force, displacement and time data of 50 Hz. Temperature was controlled by placing the instrument and sample in a controlled temperature room (20 ± 0.2 °C). Samples were placed between two parallel Teflon plates lubricated with paraffin oil and compressed to 80% Cauchy strain at a crosshead speed of 50 mm/min, and force and displacement were measured as a function of time.

The experimental data were initially analysed using XTRAD software (Stable Micro Systems) and the appropriate data were transferred into software (Master Work

Software, Tawa, New Zealand) written in J, a functional programming language (Iverson Software Inc., Toronto, Canada). Stress and strain were calculated using the software. The relevant equations are given in Section 2.4.2.3.1.

4.2.5.1.2 Rheological measurements using the Instron Universal Testing Machine

A day after the cheese curd was made, a portion of it was grated into pieces about 2 mm × 2 mm × 6 mm and stored at 2 °C. On each day of testing, the required quantity of grated curd was removed and equilibrated at 20 °C for 2 h prior to testing.

A Teflon cylindrical attachment (Fig. 4.2.1) of 120 mm height and 20.0 mm diameter was filled with 18 g of grated cheese at 20 °C. The cheese curd was first compressed and then extruded through an orifice of 4 mm diameter using an Instron Universal Testing Machine at a crosshead speed of 50 mm/min to a final height of 2 mm. The firmness of the curd was defined as the force necessary to extrude the compressed, grated curd through the orifice. The maximum force exerted during the test was recorded as a measure of curd firmness.

4.2.5.2 Small strain deformation testing of cheese curd using a Bohlin constant stress rheometer

Curd stored at 2 °C was cut into 4 mm thick slices using a wire cutter and from these four 30 mm diameter sample discs were obtained using a core borer. The samples were covered with polyethylene film, placed in a nominally water-tight plastic box and equilibrated at 20 °C for 2 h prior to testing.

Rheological measurements of the viscoelastic parameters - storage modulus (G′), loss modulus (G″) and phase angle (δ) - were obtained using a Bohlin CVO constant stress rheometer. The dynamic oscillatory measurements were made using a 25 mm diameter serrated plate system with the gap setting nominally equal to 80% of the sample height at 20 °C. Immediately prior to testing, the disc was cut to 25 mm diameter. Initially, stress sweeps were performed on one of the samples to find the approximate linear

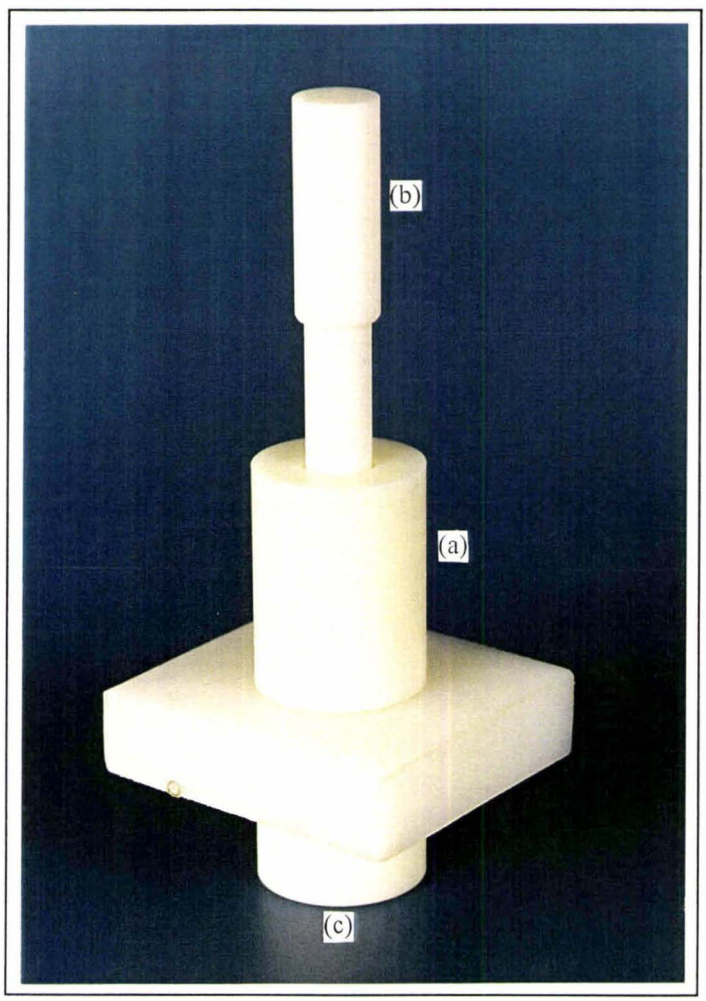

Fig. 4.2.1. *Extrusion attachment used for the rheological evaluation of grated cheese.* A well-mixed sample of grated cheese (18 g) at 20 °C was placed in the cylinder (a) of 120 mm height and 20 mm diameter and the extrusion attachment was fitted to the Instron. The plunger (b) forced the grated cheese out of the 4 mm diameter orifice (c) at a crosshead speed of 50 mm/min to a final height of 2 mm. The change in force on the plunger (b) was measured with time.

viscoelastic region. A strain range of about 1-3 × 10^{-3} was found to be in the higher strain section of the linear region as estimated by a G′ versus strain plot (Fig. 4.2.2). The value of the strain in the linear viscoelastic region determined by the stress sweep was entered as the target strain for subsequent oscillation experiments. The microprocessor in the rheometer is programmed to alter the applied stress until the target strain is reached. Frequency sweeps were performed over the range 0.01-1 Hz and 1 - 3 × 10^{-3} strain. The relevant equations for the determination of storage modulus (G′), loss modulus (G″) and phase angle (δ) are given in Section 2.4.2.3.2.

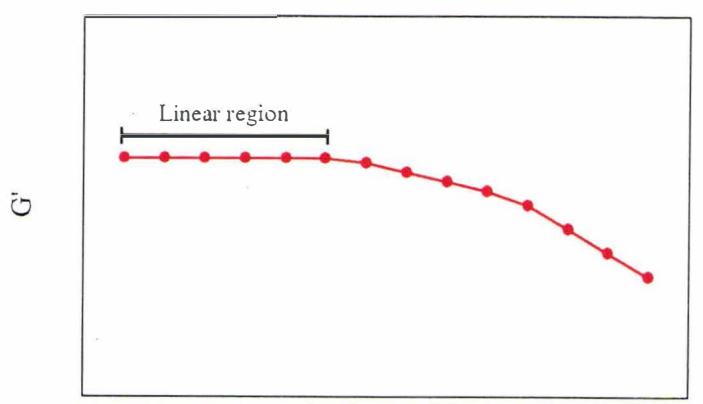

Fig. 4.2.2. *A typical plot of G′ versus strain for the small strain deformation testing of cheese.* Measurements were made at 20 °C with a 25 mm diameter serrated plate system with the gap setting nominally equal to 80% of the sample height using the Bohlin rheometer. A strain range of about 1 - 3 × 10^{-3} was found to be in the higher strain section of the linear region and was entered as the target strain for subsequent oscillation experiments.

4.2.6 Confocal microscopic examination of cheese

For the examination of intact curd, a piece of cheese curd was cut with a razor blade to produce a sample with two parallel surfaces and placed on a microscope slide. Small quantities of the dyes, Fast Green FCF (to stain the protein phase) and Nile Blue (to

stain the lipid phase), were dusted on to the upper surface of the cheese. The cheese sample was then stored overnight in an air-tight plastic box in a refrigerator.

For the examination of grated cheese, strips of glass about 3 mm wide were glued on to a glass slide to form a rectangular well. The well was filled with grated cheese and small quantities of the dyes were dusted on to it. A cover slip was placed on top and secured tightly to the glass slide. Care was taken to ensure adequate contact of the sample with the cover slip but to avoid distorting the pieces of grated curd. Samples were stored in a refrigerator overnight in an air-tight box to allow diffusion of the dyes into the sample.

On the next day, the samples were examined using a Leica TCS 4D confocal laser scanning microscope (Leica, Heidelburg, Germany) with a Leitz DM RBE microscope. A 63X objective was used with oil immersion to examine the samples using the Rhodamine filter block (568 nm) for excitation of Fast Green and the FITC/Nile Red filter block (488 nm) for excitation of Nile Blue.

4.2.6.1 Confocal microscopic examination of the water phase in cheese

A fluorescent dye-protein conjugate was prepared from β-lactoglobulin and tetramethylrhodamine-5-(and-6)-maleimide in 1:1 molar ratio and used in cheese milk so that the water phase in cheese could be observed. For the preparation of conjugate, 100 mg of β-lactoglobulin was dissolved in 10 ml of 0.05 M phosphate buffer (pH 7.0) and to this 2.62 mg of the dye dissolved in 500 µl of dimethyl formamide was added. To the mixture, 6 g of urea was added and stirred. The solution was allowed to stand for 30 min and then dialysed against distilled water. Dialysis was continued for 2 days with three changes of water each day. The solution was chromatographed (FPLC Systems, Amrad Pharmacia Biotech, Uppsala, Sweden) on a Sephadex G-25 column (2.6 cm diameter × 29 cm) at a rate of 1 ml/min in a 20 mM (pH 7.0) phosphate buffer. The dye fraction obtained was freeze dried and stored in an air-tight container in a freezer.

Cheese curd containing the dye-protein conjugate was prepared from a small quantity of milk in the laboratory. About 5 mg of the conjugate was added to 1 litre of milk adjusted to a protein to fat ratio of 1.5:1. The pH of the milk was adjusted to 5.9 with dilute lactic acid and the milk was warmed to 32 °C. Cheese curd was prepared from the milk according to the procedure described in Section 4.2.1.2. The confocal microscopic examination of the cheese curd was carried out as described in Section 4.2.6.

5.0 RESULTS

5.1 AN EXPLORATORY STUDY OF CHEDDAR, MOZZARELLA AND CHESHIRE CHEESES MADE USING STANDARD MANUFACTURING PROTOCOLS

5.1.1 Introduction

The main objective of this research project was to gain an understanding of the processes that affect the changes in cheese structure during the early stages of maturation.

The initial trial was carried out to determine if a serum phase could be isolated from several varieties of cheese and whether the quantity and composition of the serum changed with time. Cheddar, Mozzarella and Cheshire cheeses prepared using the standard manufacturing protocols at the pilot plant of the NZDRI were used. The changes in water distribution with time were determined by a centrifugation technique. Standard analytical methods and urea-PAGE were used to determine changes in composition of the serum phase.

5.1.2 Composition of cheeses

The compositions of the Cheddar, Cheshire and Mozzarella cheeses used are shown in Table 5.1.1 and are typical of the cheeses normally manufactured in New Zealand. Mozzarella cheese had a higher moisture content and a lower fat content than Cheddar or Cheshire cheese. The pH of the Cheshire cheese was much lower than that of the other two cheeses. The calcium content of the cheese and that of the non-fat cheese solids (*i.e.* mostly casein) were higher in Cheddar cheese than in the other two varieties. The content of non-fat cheese solids was calculated from the quantities of fat and

moisture present. The differences in composition were a consequence of the differences in the manufacturing protocols of the three cheese varieties.

Table 5.1.1 *Compositions on day 1 of the standard Cheddar, Mozzarella and Cheshire cheeses*

Component	Cheese variety		
	Cheddar	Mozzarella	Cheshire
Fat (g/kg)	345	210	345
Moisture (g/kg)	359	488	363
Salt (g/kg)	18.8	13.2	20.8
pH	5.31	5.31	4.96
Calcium in cheese (mmol/kg)	191	157	136
Calcium in non-fat cheese solids (mmol/kg)	645	520	466
MNFS (g/kg)	548	618	554
FDM (g/kg)	538	410	542
S/M (g/kg)	52.3	27.0	57.3

Cheshire cheese was also analysed by urea-PAGE. The quantities of β-, α_{s1}- and α_{s1}-I caseins were estimated from the quantities of dye bound to the casein fractions after urea-PAGE. The relative amounts of β-, α_{s1}- and α_{s1} -I-caseins (arbitrary units) in Cheshire cheese on the day of manufacture were 1130, 1436 and 48 respectively (Fig. 5.1.1). The various bands in the gel were identified by comparison with the photograph of the mini alkaline urea-PAGE gel given by Creamer (1991).

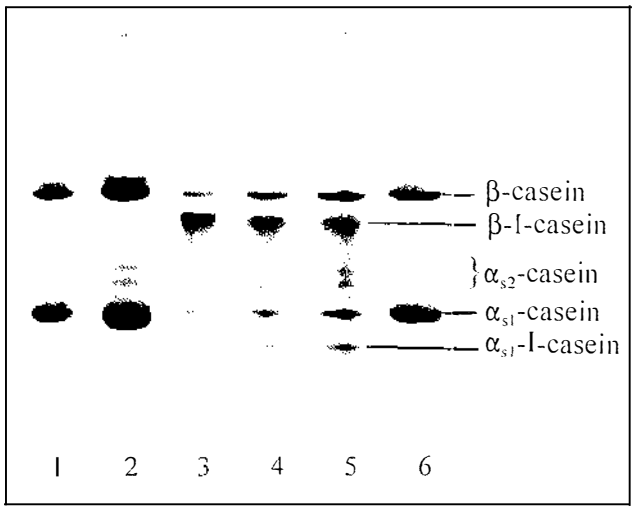

Fig. 5.1.1. *Analyses of Cheshire cheese and its centrifugal serum using mini alkaline urea-PAGE.* Lanes 1 and 6 are commercial rennet casein standard; lane 2 is standard Cheshire cheese before separation of the serum; lanes 3, 4 and 5 are the centrifugal serum from standard Cheshire cheese on days 1, 2 and 4 after manufacture respectively. The major casein fractions are identified.

5.1.3 Centrifugation of cheese

The quantity of centrifugal serum, *i.e.* the amount of serum that could be obtained on centrifugation of the cheese curd, for Cheddar cheese was 7.90 g/kg on day 1 after manufacture. There was no serum on subsequent days. The quantity of centrifugal serum from Mozzarella cheese (Table 5.1.2) was greater than that from Cheshire cheese (Table 5.1.3). The quantities of centrifugal serum from both Mozzarella and Cheshire cheeses decreased gradually with time. There was no release of centrifugal serum from Mozzarella cheese after day 12, and from Cheshire cheese after day 5.

5.1.4 Changes in composition of cheese and centrifugal sera

The pH of both Mozzarella and Cheshire cheeses varied with time (Tables 5.1.2 and 5.1.3). The centrifugal sera obtained were analysed for calcium and nitrogen contents.

On day 4, the calcium content in Cheshire cheese serum was 91.1 mmol/kg (Table 5.1.3) compared with 62.4 mmol/kg in Mozzarella cheese serum (Table 5.1.2). The lower pH of Cheshire cheese resulted in increased solubility of calcium and its movement into the serum phase. The calcium contents of the centrifugal sera from both cheeses increased with time.

Table 5.1.2 *Changes in composition of Mozzarella cheese and its centrifugal serum with time*

Component	Number of days after manufacture			
	4	6	8	12
pH of cheese	5.31	5.34	5.28	5.29
Centrifugal serum (g/kg cheese)	155	120	68.5	4.00‡
Total nitrogen in serum (g/kg)	9.20	11.3	13.7	-
Non protein nitrogen in serum (g/kg)	0.60	0.60	0.60	-
Calcium in serum (mmol/kg)	62.4	65.7	69.6	-
β-Casein in serum (a.u.)†	4344	5175	7040	-
α_{s1}-Casein in serum (a.u.)†	1212	1596	2241	-
α_{s1}-1 Casein in serum (a.u.)†	4798	5131	5811	-

† Arbitrary units of dye bound to the protein bands in PAGE analysis.

‡ Insufficient quantity for chemical analysis.

The quantities of total nitrogen in the sera decreased with time for both cheeses although the nitrogen concentration of the sera appeared to increase with time (Tables 5.1.2 and 5.1.3). The quantities of total nitrogen in the serum from a kilogram of cheese were 1.43 g on day 4 and 0.94 g on day 8 for Mozzarella (calculated from the values of total nitrogen and centrifugal serum in Table 5.1.2) and 0.25 g on day 1 and 0.16 g on day 4 for Cheshire (calculated from the contents of total nitrogen and centrifugal serum in Table 5.1.3). As the amount of water associated with the cheese solids increased, resulting in a reduction in the quantity of centrifugal serum, increased proportions of nitrogen also moved into the solid phase, causing a lowering of the nitrogen content of

the serum. A similar effect was also observed with the non protein nitrogen content of the serum.

Samples of the centrifugal sera from Mozzarella and Cheshire cheeses were analysed by urea-PAGE. The urea-PAGE gel of the Cheshire centrifugal serum is shown in Fig. 5.1.1. The concentrations of the major casein fractions in the centrifugal serum tended to increase with time for both Mozzarella (Table 5.1.2) and Cheshire (Table 5.1.3) cheeses. However, the quantities of the casein fractions in the serum varied with the type of casein. Whereas the quantities of β- and α_{s1}-I-caseins in the serum increased, the quantity of α_{s1}-casein either decreased slightly or remained about constant. In Cheshire serum, for example, the quantities (arbitrary units) of β-casein were 1152 on day 1 and 1672 on day 4; the quantities of α_{s1}-casein were 1509 on day 1 and 1256 on day 4; and those of α_{s1}-I casein were 289 on day 1 and 664 on day 4. The action of chymosin on α_{s1}-casein resulted in an increased quantity of α_{s1}-I casein in the serum.

Table 5.1.3 *Changes in composition of Cheshire cheese and its centrifugal serum with time*

Component	Number of days after manufacture			
	1	2	4	5
pH of cheese	5.12	5.20	5.14	5.06
Centrifugal serum (g/kg cheese)	68.6	64.0	40.0	17.0‡
Total nitrogen (g/kg)	3.70	4.40	4.10	-
Non protein nitrogen (g/kg)	1.30	1.60	2.00	-
Calcium (mmol/kg)	84.9	90.9	91.1	-
β-Casein (a.u.)*	168	285	418	-
α_{s1}-Casein (a.u.)*	220	278	314	-
α_{s1}-I Casein (a.u.)*	42.2	92.8	166	-

* Arbitrary units of dye bound to the protein bands in PAGE analysis.

‡ Insufficient quantity for chemical analysis.

5.1.5 Conclusions

The centrifugation method of Guo & Kindstedt (1995) was found to be effective in separating an aqueous phase from Cheshire and Mozzarella cheeses and the quantity of serum decreased with time for both cheese varieties. The lack of sufficient expressible serum for Cheddar cheese under the conditions used indicated that the manufacturing process used did not result in enough serum that could be centrifuged out from the cheese. In order to study the changes in water distribution in Cheddar cheese, it therefore became necessary to modify the Cheddar cheese manufacturing process such that a cheese with a higher moisture content was obtained.

Changes in the quantity and composition of the centrifugal serum could not be attributed to any one particular factor because of the changes in pH with time (because of continuing starter activity; Tables 5.1.2 and 5.1.3) and the variability in the continuing proteolysis by chymosin. Clearly, greater control of some of these factors was needed to segregate the separate effects. The influence of pH on the quantity of centrifugal serum could be investigated by manufacturing cheeses of different pH values and examining the changes taking place during ripening.

5.2 STUDY OF CHEDDAR-LIKE CHEESES MADE WITH DIFFERENT DRAINING AND SALTING pH VALUES

5.2.1 Introduction

The results of the previous trial (Section 5.1) showed that centrifugal serum could be obtained when Mozzarella and Cheshire cheese were made using the standard manufacturing processes. However, in order to study changes in water distribution with time in Cheddar-like cheeses, the manufacturing process needed to be modified such that an increased amount of moisture was retained in the cheese curd. Consequently, the milk for cheesemaking was standardised to a protein to fat ratio of about 1.5:1 (similar to that for Mozzarella cheese), the cooking temperature was reduced from 38°C to 34 or 36 °C and the curd was cheddared and salted but not pressed. The protein to fat ratio used was much higher than the normal 0.8:1 in Cheddar cheese in order to increase the moisture holding capacity of the cheese, resulting from the greater proportion of protein in the cheese curd.

In addition, the influence of pH on the quantity of centrifugal serum was determined by examining four cheeses made at different draining and salting pH values. Cheese made at a draining pH of 5.9 was salted at pH values of 5.6 and 5.3, and cheese made at a draining pH of 5.6 was salted at pH values of 5.3 and 5.0. The details of the cheesemaking are given in Section 4.2.1.1.

5.2.2 Composition of cheeses

The average moisture contents of the cheeses made with a draining pH of 5.9 and salting pH values of 5.6 and 5.3 were 484 and 459 g/kg respectively. The pH of the cheeses varied with time (Tables 5.2.1 and 5.2.2). The average moisture contents of the cheeses made with a draining pH of 5.6 and salting pH values of 5.3 and 5.0 were 511

and 482 g/kg respectively. The pH values of the cheeses are shown in Table 5.2.3. The cheese samples were not analysed for the other constituents.

Table 5.2.1 *Changes in composition of the cheese made with a draining pH of 5.9 and a salting pH of 5.6 and its centrifugal pellet and serum*

Component	Number of days after manufacture				
	1	3	5	7	9
pH of cheese	5.34	5.24	5.31	5.20	5.26
Centrifugal serum (g/kg cheese)*	66.4 ± 3.21	42.2 ± 4.29	43.3 ± 4.06	31.9 ± 2.46	1.70 ± 0.30‡
Total nitrogen in serum (g/kg)	5.10	8.70	11.5	13.6	-
Calcium in serum (mmol/kg)	86.6	89.7	104	102	-
Sodium in centrifugal pellet (mmol/kg)	344	394	412	431	466

* Values are mean ± standard deviation for six observations.

‡ Insufficient quantity for chemical analysis.

5.2.3 Centrifugation of cheese

The results for the cheeses made with a draining pH of 5.9 and salting pH values of 5.6 and 5.3 are shown in Tables 5.2.1 and 5.2.2 respectively. The quantity of centrifugal serum released from the cheese on day 1 decreased with a decrease in the pH at salting from 5.6 to 5.3. The quantity of centrifugal serum decreased gradually with time and there was no release of serum after day 9 from the cheeses made at both salting pH values.

The quantity of centrifugal serum on day 1 from the cheese made with a draining pH of 5.6 decreased with a decrease in the pH at salting from 5.3 to 5.0 and there was no release of centrifugal serum from day 3 onwards for both these cheese samples (Table 5.2.3). A decrease in the quantity of centrifugal serum was thus observed with a decrease in the pH at both draining and salting.

Table 5.2.2 *Changes in composition of the cheese made with a draining pH of 5.9 and a salting pH of 5.3 and its centrifugal pellet and serum*

Component	Number of days after manufacture				
	1	3	5	7	9
pH of cheese	5.28	5.16	5.19	5.19	5.22
Centrifugal serum (g/kg cheese)*	48.8 ± 3.17	27.2 ± 2.64	29.7 ± 3.18	27.3 ± 1.24	1.37 ± 0.33‡
Total nitrogen in serum (g/kg)	0.73	1.01	1.20	1.38	-
Calcium in serum (mmol/kg)	92.6	112	99.6	105	-
Sodium in centrifugal pellet (mmol/kg)	439	448	481	492	478

* Values are mean ± standard deviation for six observations.

‡ Insufficient quantity for chemical analysis.

5.2.4 Changes in composition of centrifugal pellet and centrifugal serum

The calcium content of the centrifugal serum was determined to see if calcium migrated from the curd to the serum phase. To assess if there was a corresponding movement of sodium, its content in the centrifugal pellet was also determined. There was an increase in the calcium content of the centrifugal serum with a decrease in the cheese pH whereas the sodium content of the centrifugal pellet tended to increase in the samples of cheese

in which the centrifugal serum decreased with time (Tables 5.2.1 and 5.2.2), suggesting that salt might have migrated from the serum to the curd phase whereas calcium migrated in the opposite direction. The calcium content of the centrifugal serum and the sodium content of the centrifugal pellet tended to increase with time for the pH values studied (Tables 5.2.1 and 5.2.2). The concentrations of total nitrogen tended to increase with time in the sera of both cheeses, but the quantity of nitrogen in the serum essentially remained constant. However, the quantity of nitrogen in the serum from a kilogram of cheese with a draining pH of 5.9 was much higher in the cheese made with a salting pH of 5.6 (0.33 g on day 1; calculated from the values of total nitrogen and centrifugal serum in Table 5.2.1) than in the cheese made with a salting pH of 5.3 (0.04 g on day 1; calculated from the values of total nitrogen and centrifugal serum in Table 5.2.3).

Table 5.2.3 *Composition of the cheeses made with a draining pH of 5.6 and salting pH values of 5.3 and 5.0 and their centrifugal sera*

Component	Number of days after manufacture			
	1	3	1	3
	Salting pH 5.3		Salting pH 5.0	
pH of cheese	5.12	5.13	5.08	5.06
Centrifugal serum (g/kg cheese)†	17.3 ± 1.75	-‡	13.5 ± 2.02	-‡
Total nitrogen in serum (g/kg)	6.70	-	7.00	-
Calcium in serum (mmol/kg)	94.2	-	94.7	-
Sodium in centrifugal pellet (mmol/kg)	366	430	502	507

† Values are mean ± standard deviation for six observations.

‡ No centrifugal serum obtained.

Samples of centrifugal pellet and serum were analysed by alkaline urea-PAGE. The values for the dye bound to the major casein fractions for the samples from cheese made with a draining pH of 5.9 and a salting pH of 5.6 are shown in Fig. 5.2.1 (a, c). The ratio of α_{s1}-I- and β-casein to α_{s1}-casein was greater in the centrifugal serum than in the pellet. This indicates the hydrolysis of α_{s1}-casein to the peptide α_{s1}-I and movement of

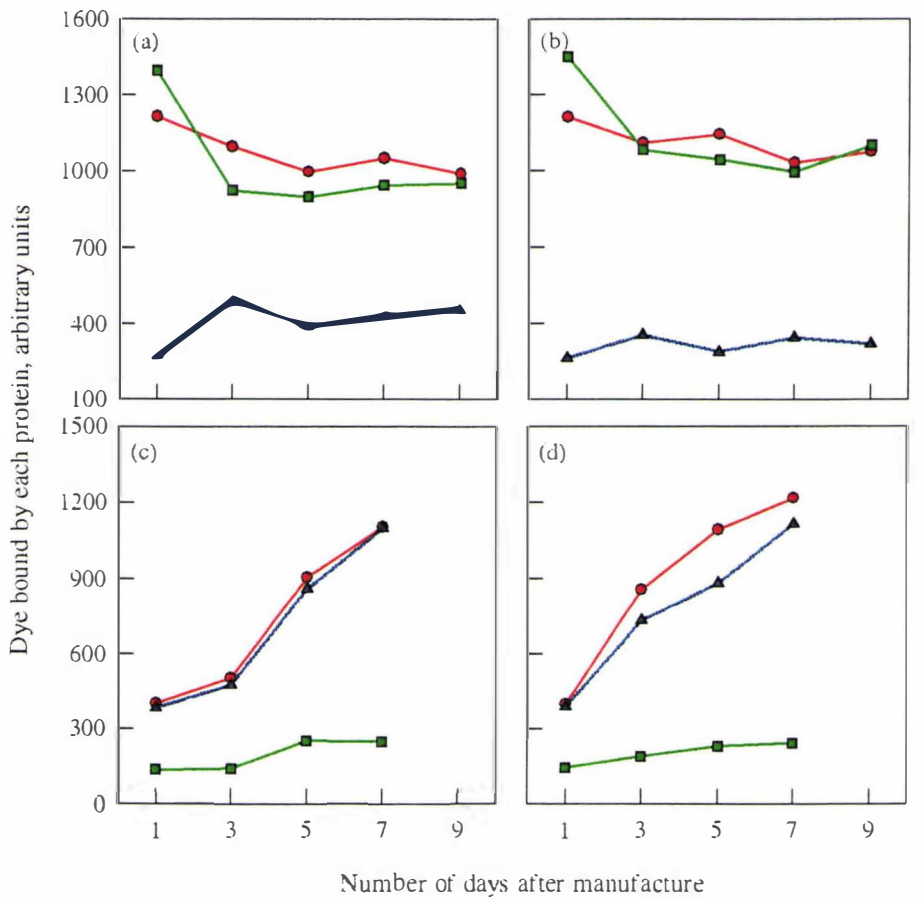

Fig. 5.2.1. *Changing concentrations of* α_{s1}-, α_{s1}-I- *and* β-*caseins with maturation time of centrifugal pellet and centrifugal serum from the cheese curds of different draining and salting pH values.* Cheese curd was centrifuged at 11 086 *g* and 25 °C for 75 min and the pellet and the serum obtained were analysed by mini alkaline urea-PAGE: ●, β-casein; ■, α_{s1}-casein; ▲, α_{s1}-I casein. (a) Centrifugal pellet and (c) centrifugal serum of cheese made with a draining pH of 5.9 and a salting pH of 5.6. (b) Centrifugal pellet and (d) centrifugal serum of cheese made with a draining pH of 5.9 and a salting pH of 5.3.

both the peptide and the dissociated β-casein into the serum. Similar trends were also observed for the samples from cheese made with a draining pH of 5.9 and a salting pH of 5.3 (Fig. 5.2.1 (b, d)). The amounts of β- and α_{s1}-casein in the centrifugal pellet decreased due to proteolysis. The quantities of intact caseins in the serum tended to increase with time.

The inconsistencies in some of the observations made in this trial could have been due to differences in the size of the curd pieces in the bag, and the fact that the curd was not pressed during manufacture. These may have contributed to a variation in composition, *e.g.* in lactose or moisture content.

5.2.5 Conclusions

As a result of a deliberate attempt to increase the moisture content of Cheddar cheese from about 36% (Table 5.1.1), samples of cheese with moisture contents between 46 and 51% were obtained. This allowed the changes in water distribution within the curd matrix to be investigated over a 2-week period of ripening.

The quantity of centrifugal serum was found to be lower for cheeses that had lower pH values at both draining and salting. Although the quantity of serum that was centrifuged out of the curd seemed to be affected by the pH, a definite conclusion could not be drawn because of the continuing changes in the curd pH during the period of study. The various biochemical changes taking place in the cheese during ripening, such as proteolysis (Fig. 5.2.1), continued to contribute to the changes in cheese composition. In order to determine if the changes in water distribution within the curd matrix were dependent on time alone or were correlated (as indicated by the variation in the quantity of centrifugal serum) with cheese pH, it would be necessary to prepare cheese curd samples with different pH values and keep the pH values of the cheese curds constant during the period of study.

5.3 STUDY OF CHEESE CURDS MADE WITH DIFFERENT SET pH VALUES

5.3.1 Introduction

On the basis of results obtained in the previous trials (Section 5.2), the cheese manufacturing process was further modified so that the cheese pH would be constant during the period of study. The objective was to develop a functioning model system that could be used to study the influence of pH on cheese properties. The products obtained by such modified processes would henceforth be referred to as cheese curds.

Cheese curds were made using lactic acid in place of starter, and using Rennilase 46L® instead of calf rennet to ensure a more constant retention of the coagulant enzyme in the cheese curd (Creamer *et al.*, 1985). The quantity of Rennilase 46L® used was less than that of calf rennet in order to have a similar set-to-cut time. However, the set-to-cut time varied with the pH of the cheese milk when Rennilase 46L® was used, the time being shorter at lower pH values. The details of cheese manufacture are given in Section 4.2.1.2.

In the present trial, cheese curds made with set pH values of 6.00, 5.70, 5.40 and 5.10 were analysed for changes in composition and water distribution with time as before (Section 5.2).

5.3.2 Composition of cheese curds

The compositions of cheese curds made at different set pH values are shown in Table 5.3.1 together with the composition of standard Cheddar cheese as given by Lawrence *et al.* (1993). The cheese curds had a higher MNFS content because of the

modifications made to the standard procedure (Section 5.2.1). The cheese curds made also had lower FDM and S/M contents than standard Cheddar cheese.

Table 5.3.1 *Compositions of the standard Cheddar cheese and the cheese curds made with different set pH values*[§]

Component	pH at setting				Standard Cheddar cheese
	6.00	5.70	5.40	5.10	
Moisture (g/kg)	483	466	462	470	340[†]
Fat (g/kg)	205	210	210	210	370[†]
Salt (g/kg)	21.3	19.5	17.8	17.6	17.0[†]
pH	5.95	5.73	5.42	5.10	5.20[†]
MNFS (g/kg)	604	586	579	577	520 - 540[‡]
FDM (g/kg)	409	391	387	393	520 - 560[‡]
S/M (g/kg)	45.8	41.9	38.7	37.5	47 - 57[‡]

[§]Composition on day 1 of experimental cheese curds

[†]Typical composition of Cheddar cheese

[‡] From Lawrence *et al.* (1993).

The changes in pH values of the cheese curds made with set pH values of 6.00 and 5.70 are shown in Tables 5.3.2 and 5.3.3 respectively. The pH values of the cheese curds remained essentially constant with time. Minimal changes in pH values were also observed for cheese curds made with set pH values of 5.40 and 5.10.

5.3.3 Changes in composition of cheese curds, centrifugal pellet and centrifugal serum

The results for the centrifugation of cheese curds made with set pH values of 6.00 and 5.70 are shown in Tables 5.3.2 and 5.3.3 respectively. There was no release of centrifugal serum even on day 1 from the cheese curds made with set pH values of 5.40

and 5.10, hence there is no data to present. The quantity of centrifugal serum did not vary consistently with time but was greater for the pH 6.00 cheese curd (approximately 70 g/kg; Table 5.3.2) than for the pH 5.70 cheese curd (approximately 35 g/kg; Table 5.3.3) and was considered to be dependent on the pH of the cheese curd.

Table 5.3.2 *Changes in composition of the cheese curd made with a set pH of 6.00 and its centrifugal pellet and serum*

Component	Number of days after manufacture						
	1	3	5	7	9	11	13
pH of cheese curd	5.93	5.94	5.96	5.96	5.98	5.96	6.00
Centrifugal serum (g/kg cheese curd)	69.2	70.4	82.3	61.3	43.8	70.0	79.8
Total nitrogen in serum (g/kg)	2.73	4.78	4.78	6.00	5.33	4.93	5.44
Non protein nitrogen in serum (g/kg)	0.49	0.52	0.60	0.50	0.58	0.70	0.56
Calcium in serum (mmol/kg)	31.2	30.6	33.4	33.5	30.6	32.7	32.3
Sodium in centrifugal pellet (mmol/kg)	690	656	668	563	717	715	634

The calcium content of the serum was greater at a set pH of 5.70 whereas the sodium content of the centrifugal pellet was greater at a set pH of 6.00 (Tables 5.3.2 and 5.3.3). Neither the sodium content of the centrifugal pellet nor the calcium content of the serum varied consistently with time at a set pH of 6.00 (Table 5.3.2) or a set pH of 5.70 (Table 5.3.3). Similar results were also observed for the nitrogen contents of the serum. However, the quantity of nitrogen in the centrifugal serum per kilogram of cheese curd increased with time, the quantities being 0.2 g on day 1 and 0.4 g on day 13 for cheese curds made at both set pH values (calculated from the values of total nitrogen and centrifugal serum in Tables 5.3.2 and 5.3.3). On the other hand, the quantity of

nitrogen in the serum was very similar to that in the serum of the Cheddar-like cheese made using chymosin (Section 5.2.4).

Samples of centrifugal pellet and centrifugal serum were analysed by urea-PAGE. The values for the dye bound to the major casein fractions for the samples from curd made with a set pH of 6.00 are shown in Fig. 5.3.1 (a, c). The ratio of α_{s1}-I- and β-caseins to α_{s1}-casein was greater in the centrifugal serum than in the pellet. Similar trends were also observed for the samples from curd made with a set pH of 5.70 (Fig. 5.3.1 (b, d)).

Table 5.3.3 *Changes in composition of the cheese curd made with a set pH of 5.70 and its centrifugal pellet and serum*

Component	Number of days after manufacture						
	1	3	5	7	9	11	13
pH of cheese curd	5.73	5.73	5.72	5.72	5.72	5.71	5.72
Centrifugal serum (g/kg cheese curd)	37.6	32.9	25.0	33.6	39.0	38.5	38.9
Total nitrogen in serum (g/kg)	6.63	10.3	11.6	12.2	12.3	12.3	12.2
Non protein nitrogen in serum (g/kg)	0.77	0.71	0.80	0.78	0.79	0.80	0.82
Calcium in serum (mmol/kg)	41.7	40.2	44.4	45.8	45.2	45.5	46.4
Sodium in centrifugal pellet (mmol/kg)	496	564	499	506	565	563	552

The concentrations of β- and α_{s1}-caseins tended to decrease whereas that of α_{s1}-I-casein increased with time in the centrifugal pellets of both pH 6.00 and pH 5.70 cheese curds. The concentrations of β- and α_{s1}-I-caseins in the sera tended to decrease after day 7 for pH 6.00 cheese curd (Fig. 5.3.1 (c)) and after day 5 for pH 5.70 cheese curd (Fig. 5.3.1(d)). However, the extent of proteolysis was less than in a normal cheese (Fig. 5.2.1).

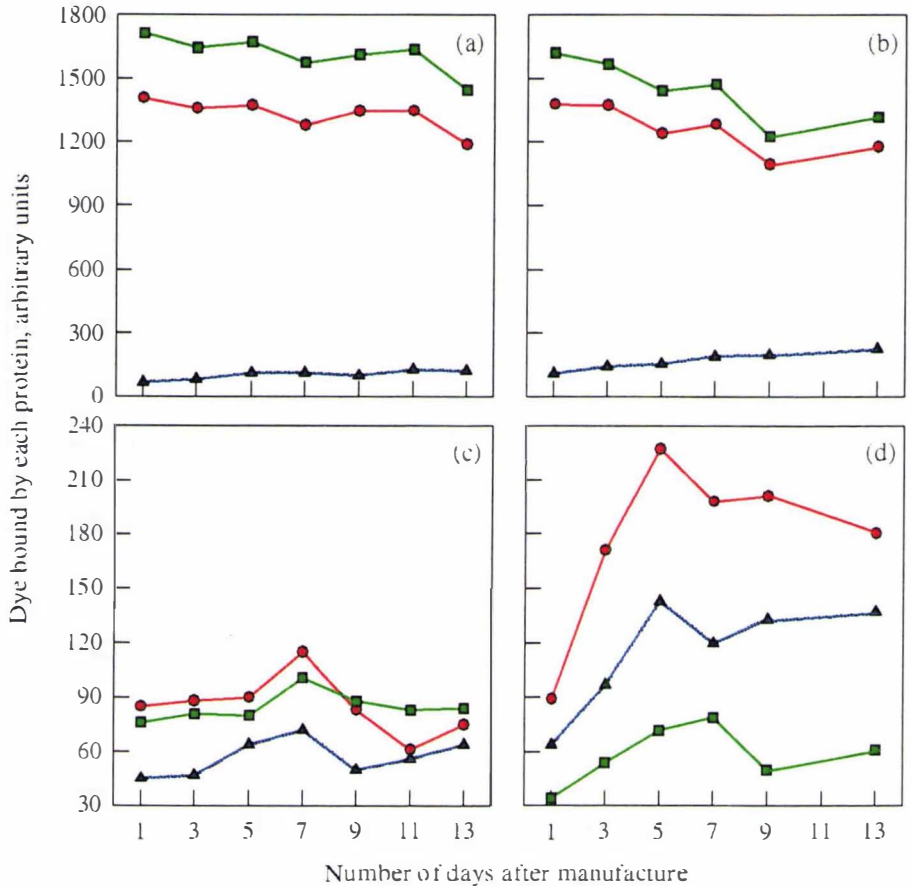

Fig. 5.3.1. *Changing concentrations of* α_{s1}-, α_{s1}-I- *and* β-*caseins with maturation time of centrifugal pellet and centrifugal serum from the cheese curds with different set pH values.* Cheese curd was centrifuged at 11 086 *g* and 25 °C for 75 min and the pellet and the serum obtained were analysed by mini alkaline urea-PAGE: ●, β-casein; ■, α_{s1}-casein; ▲, α_{s1}-I-casein. (a) Centrifugal pellet and (c) centrifugal serum of cheese made with a set pH of 6.00. (b) Centrifugal pellet and (d) centrifugal serum of cheese made with a set pH of 5.70.

5.3.4 Microscopic examination of cheese structure

The samples of curd made with set pH values of 6.00, 5.70, 5.40 and 5.10 were stained with the dyes Fast Green FCF and Nile Blue and examined using a confocal laser scanning microscope by the procedure described in Section 4.2.6. The confocal

micrographs (Fig. 5.3.2) show the continuous protein matrix stained with Fast Green FCF as the fluorescent phase. The dark (non-fluorescent) phase consists mainly of fat and may include void spaces in the structure and water. The microstructure of the pH 6.00 cheese curd appeared to be similar to that of the pH 5.70 cheese curd, but the two were distinctly different from the microstructures of the cheese curds made at pH 5.40 and pH 5.10. The fat appeared to be elongated and oval in shape in the pH 5.40 cheese curd whereas the fat was less evenly distributed for the pH 5.10 cheese curd. The differences in the cheese curd structure were thought to be a result of the differences in the pH at setting.

5.3.5 Conclusions

The use of lactic acid in place of starter cultures provided an effective means of preparing cheese curds with a known pH which did not change with time. Consequently, the influence of pH on the water distribution in the cheese curd and on the compositions of the cheese curd and the centrifugal serum could therefore be studied under controlled conditions. The quantity of centrifugal serum was found to be greatest at higher pH, in the range from 5.1 to 6.0.

A definite reduction in the extent of proteolysis was observed for cheese curds made with different set pH values in comparison with that in a normal cheese because of the modifications made to the cheesemaking process. The influence of proteolysis on the other changes occurring was therefore minimised.

Significant differences in the microstructure of cheese curds made with different set pH values were observed.

It was now established that the set pH influenced the quantity of centrifugal serum. The variation in the quantity of serum with time observed in the earlier trial (Section 5.2) but not in this trial may have been related to the continued decrease in pH between setting

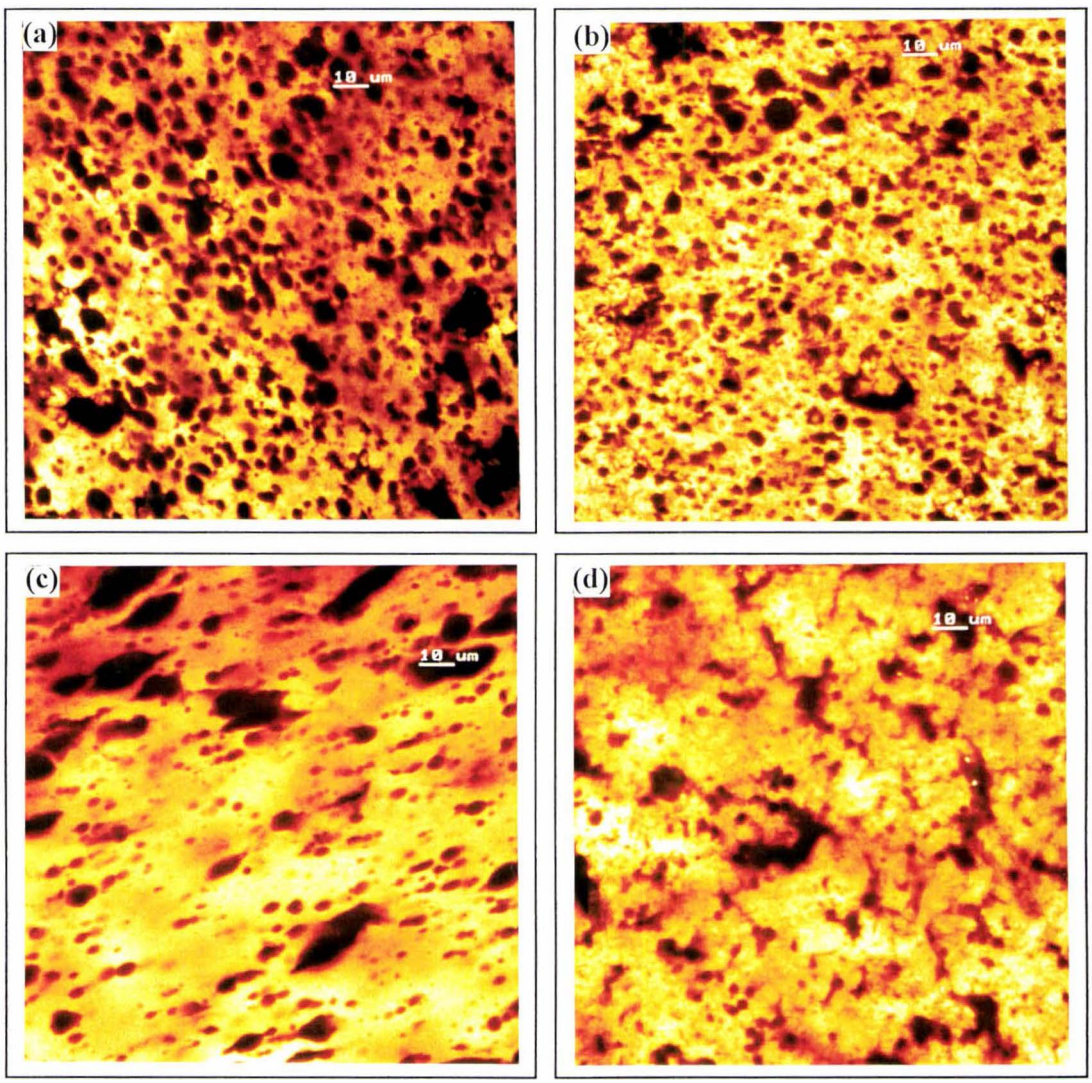

Fig. 5.3.2. *Distribution of protein in cheese as a function of the set pH.* Cheese curds were made from milk adjusted to different pH values at setting using dilute lactic acid: (a) 6.00, (b) 5.70, (c) 5.40 and (d) 5.10. The protein phase of the cheese curd was stained with Fast Green FCF and examined using a Leica TCS 4D confocal laser scanning microscope. Fluorescent areas indicate the protein structure. Scale bar: 10 μm.

and salting. In this trial, there was neither a variation in the quantity of serum with time nor a change in the pH of the cheese curds. The effect of a pH shift, or a drop in pH from that of the pH at setting, on the quantity of centrifugal serum thus needed to be investigated.

5.4 STUDY OF CHEESE CURDS ADJUSTED TO DIFFERENT pH VALUES BY THE ADDITION OF GDL

5.4.1 Introduction

A functioning model system of cheese curds made with different set pH values was developed (Section 5.3) such that there were minimal changes in the pH during storage. A model system in which the pH changed after manufacture also needed to be developed so as to simulate the pH changes in a normal cheese. This was also necessary to determine if a shift in pH after cheesemaking also influenced the changes in water distribution and composition that were observed in the previous trials.

In this trial, the effectiveness of GDL as a pH adjuster was evaluated. GDL was added at levels of 7, 11, 16 and 20 g/kg to cheese curds of set pH 5.90 (according to the procedure described in Section 4.2.1.3) to give samples with pH values of 5.61, 5.43, 5.30 and 5.19 respectively. The changes in water distribution and composition were measured from the third day onwards. The effect of the reduction in pH on the time dependent serum loss was determined.

5.4.2 Composition of cheese curds

The composition of cheese curds made with a set pH value of 5.90 is given in Table 5.4.1. As in the earlier trials (Section 5.3.2), the cheese curds had a higher MNFS content and lower FDM and S/M contents than standard Cheddar cheese (Table 5.3.1). The pH values of the cheese curd samples obtained after mixing with GDL are given in Table 5.4.2. There was no change in the pH of the cheese curds during storage. The moisture contents of the samples of cheese curd (483-487 g/kg) were very similar to that in the pH 5.90 cheese curd. Other components of these samples of cheese curd were not analysed.

Table 5.4.1 *Composition on day 1 of the cheese curd of set pH 5.90 to which GDL was added to alter the pH*

Component	Content
Moisture (g/kg)	483
Fat (g/kg)	215
Total nitrogen (g/kg)	35.0
Non protein nitrogen (g/kg)	1.60
Salt (g/kg)	20.6
pH	5.91
MNFS (g/kg)	615
FDM (g/kg)	416
S/M (g/kg)	42.6

5.4.3 Changes in composition of cheese curds, centrifugal pellet and centrifugal serum

The quantities of expressible serum obtained are shown in Table 5.4.2. The quantity of serum on any day was less for a cheese curd of lower pH value. The quantity of centrifugal serum also decreased with time, the decrease being more pronounced at lower pH values.

The calcium contents of the centrifugal sera are also shown in Table 5.4.2. The calcium content in the serum increased with a decrease in the pH of the cheese curd. Its concentration in the serum also tended to increase with time for all the samples of cheese curd.

Samples of centrifugal pellet and serum were analysed by urea-PAGE. The relative amounts of the casein fractions in the centrifugal pellet are shown in Fig.5.4.1 and those in the centrifugal serum are shown in Fig. 5.4.2. As in the case of cheese curds made

Table 5.4.2 *Composition of cheese curds and centrifugal sera of the curd samples adjusted from pH 5.90 to different pH values with GDL*

Component	Number of days after manufacture					
	3	5	7	9	11	13
(a) pH 5.61 cheese curd						
pH of cheese curd	5.62	5.62	5.61	5.61	5.61	5.61
Centrifugal serum† (g/kg cheese curd)	90.5 ± 1.89	83.9 ± 2.36	70.0 ± 2.18	69.2 ± 1.56	61.0 ± 2.25	54.2 ± 1.64
Calcium in serum‡	60.7	60.9	61.2	62.5	70.2	71.3
(b) pH 5.43 cheese curd						
pH of cheese curd	5.45	5.45	5.43	5.44	5.43	5.43
Centrifugal serum† (g/kg cheese curd)	74.8 ± 1.56	64.7 ± 2.00	50.5 ± 2.14	50.2 ± 2.45	28.5 ± 2.31	17.9 ± 1.76
Calcium in serum‡	70.6	76.8	83.7	99.8	115	124
(c) pH 5.30 cheese curd						
pH of cheese curd	5.30	5.30	5.31	5.31	5.31	5.30
Centrifugal serum† (g/kg cheese curd)	64.1 ± 1.68	55.0 ± 1.67	31.7 ± 2.54	24.5 ± 2.27	9.80 ± 1.48	-¶
Calcium in serum‡	109	112	117	125	133	-
(d) pH 5.19 cheese curd						
pH of cheese curd	5.21	5.20	5.20	5.19	5.19	5.19
Centrifugal serum† (g/kg cheese curd)	52.8 ± 2.16	35.2 ± 1.90	14.1 ± 2.14	2.80 ± 2.36	-¶	-¶
Calcium in serum‡	95.9	130	134	-	-	-

† Values are mean ± standard deviation for six observations.

‡ mmol/kg .

¶ No release of serum on centrifugation of cheese curd.

at different set pH values (Section 5.3.3), the ratios of α_{s1}-I- and β-caseins to α_{s1}-casein were greater in the centrifugal serum than in the pellet for all the samples. The

concentrations of β- and α_{s1}-caseins in the centrifugal pellet decreased with time whereas the α_{s1}-I-casein content increased with time, indicating proteolysis of α_{s1}-casein and loss of β-casein to the serum. However, the extent of proteolysis was minimal over the period of time.

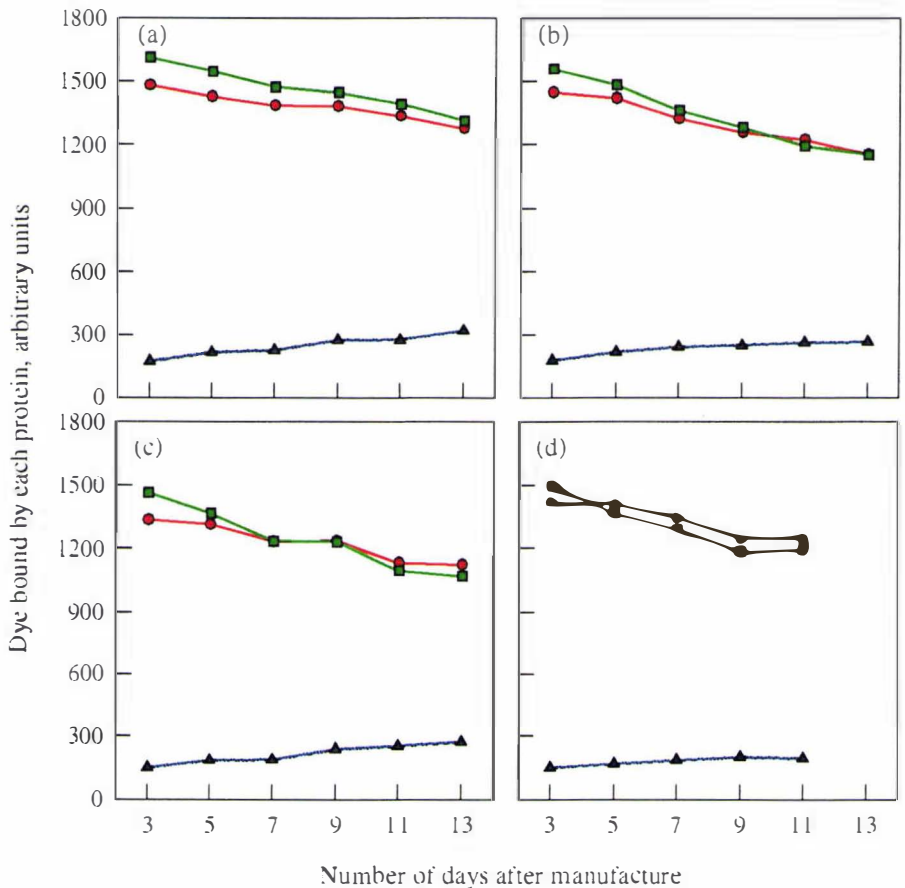

Fig. 5.4.1. *Changing concentrations of α_{s1}-, α_{s1}-I- and β-caseins with maturation time of centrifugal pellets from the cheese curds adjusted to different pH values using GDL.* Cheese curd was centrifuged at 11086 *g* and 25 °C for 75 min and the pellet obtained was analysed by mini alkaline urea-PAGE: ●, β-casein; ■, α_{s1}-casein; ▲, α_{s1}-I-casein. Centrifugal pellets from cheese curds adjusted to different pH values (a) 5.61, (b) 5.43, (c) 5.30 and (d) 5.19 using GDL were analysed.

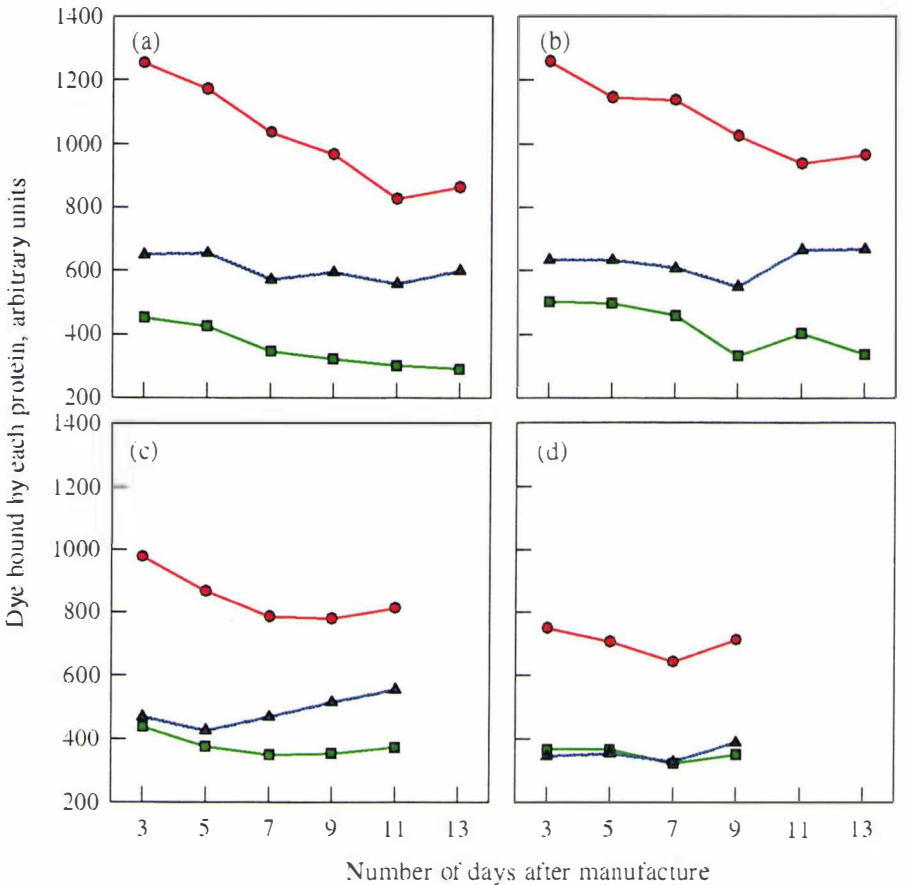

Fig. 5.4.2. *Changing concentrations of* α_{s1}-, α_{s1}-I- *and* β-*caseins with maturation time of centrifugal sera from the cheese curds adjusted to different pH values using GDL.* Cheese curd was centrifuged at 11086 *g* and 25 °C for 75 min and the serum obtained was analysed by mini alkaline urea-PAGE: ●, β-casein; ■, α_{s1}-casein; ▲, α_{s1}-I-casein. Centrifugal sera from cheese curds adjusted to different pH values (a) 5.61, (b) 5.43, (c) 5.30 and (d) 5.19 using GDL were analysed.

5.4.4 Microscopic examination of cheese structure

The four samples of cheese curd were stained with Fast Green FCF and Nile Blue and examined using a confocal laser scanning microscope by the procedure described in Section 4.2.6. The confocal micrographs (Fig. 5.4.3) show the continuous protein matrix stained with Fast Green FCF as the fluorescent phase. The microstructures of the four samples of cheese curd appeared to be very similar.

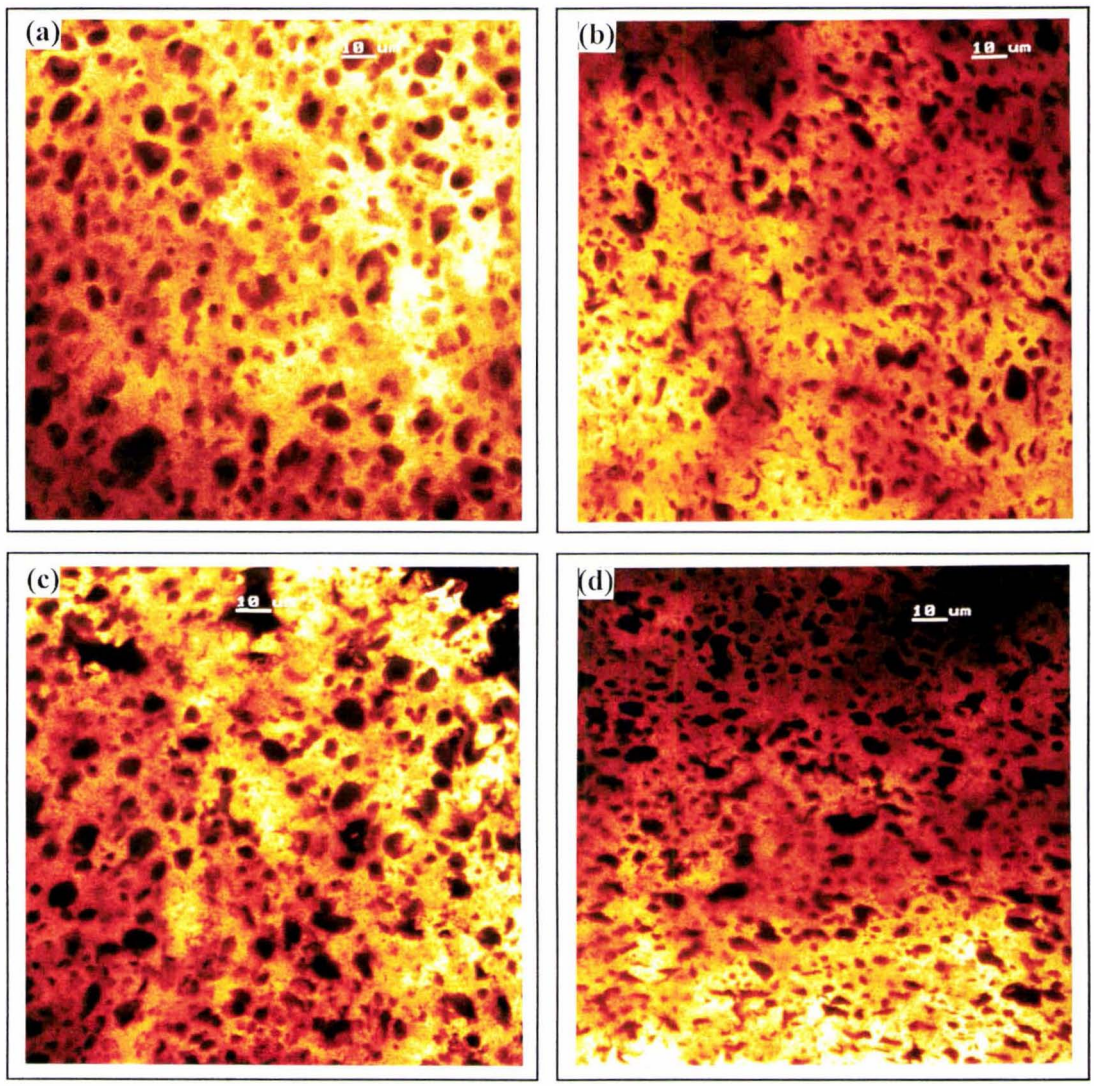

Fig. 5.4.3. *Distribution of protein in cheese as a function of the pH obtained with the addition of GDL.* Cheese curd was made with a set pH of 5.90 and then adjusted to different pH values using GDL: (a) 5.61, (b) 5.43, (c) 5.30 and (d) 5.19. The protein phase of the cheese curd was stained with Fast Green FCF and examined using a Leica TCS 4D confocal laser scanning microscope. Fluorescent areas indicate the protein structure. Scale bar: 10 μm.

Differences in the microstructure of cheese curd samples were expected because of the differences observed in water-holding properties. However, the differences may have been too subtle for detection by the confocal microscopy technique used.

5.4.5 Conclusions

The use of GDL to alter the pH of the cheese curd after it was made at a particular set pH provided a model system by which the influence of the pH shift on the changes in water distribution and composition could be studied. The quantity of centrifugal serum was again affected by the final pH of the cheese curds, the quantity being lower for a cheese curd of lower pH value.

The quantity of serum also decreased with time for all samples, unlike the samples of cheese curd made at different set pH values (Section 5.3) where the quantity of serum essentially remained constant with time. The decrease in the quantity of serum with time was more rapid in the samples with a greater difference between the set and final pH values (pH shift).

As in the case of cheese curds made with different set pH values, a significant reduction in the extent of proteolysis was observed for the pH-altered samples of cheese curd (using GDL) in comparison with a normal cheese.

No appreciable differences in the microstructures of the cheese curds with altered pH values were observed. Differences in microstructure were expected because of the changes in the water holding properties observed. However, these differences may have been too subtle for detection by the confocal microscopy technique used.

5.5 EVALUATION BY VARIOUS TECHNIQUES OF CHEESE CURDS MADE WITH DIFFERENT SET pH VALUES

5.5.1 Introduction

Preparation of cheese curds at set pH values of 6.00, 5.70, 5.40 and 5.10 (Section 5.3) provided a model system that could be used to explore more techniques. As the pH of the cheese curds remained essentially constant during storage, the influence of pH on changes in water distribution and composition could be studied.

In the earlier trial (Section 5.3), centrifugal serum could be obtained from only pH 6.00 and pH 5.70 cheese curds and not from those made at pH 5.40 and 5.10. In this trial, therefore, cheese curds were made in a narrower pH range. Cheese curds were made at set pH values of 6.05, 5.90, 5.75, 5.60 and 5.45. Changes in the quantity and composition of the centrifugal serum were determined. Samples of cheese curd were also analysed for some rheological properties using large and small strain deformation techniques.

5.5.2 Composition of cheese curds

The compositions of the cheese curds made with various set pH values are shown in Table 5.5.1. The FDM, MNFS and S/M contents varied in a manner similar to that for the samples in the earlier trial (Section 5.3.2) in comparison with standard Cheddar cheese.

5.5.3 Changes in composition of cheese curds and centrifugal sera

The changes in pH values of cheese curds made at set pH values of 6.05, 5.90 and 5.75 during storage are shown in Tables 5.5.2, 5.5.3 and 5.5.4 respectively. No appreciable

variation in the pH values was observed during the period of storage. Similarly, no variation was observed in the pH values for cheese curds made at set pH values of 5.60 and 5.45.

Table 5.5.1 *Composition on day 1 of the cheese curds made with different set pH values*

Component	pH at setting				
	6.05	5.90	5.75	5.60	5.45
Moisture (g/kg)	483	462	478	460	462
Fat (g/kg)	195	215	210	210	210
Total nitrogen (g/kg)	38.6	39.7	39.6	43.0	43.0
Non-protein nitrogen (g/kg)	0.90	1.40	0.90	1.11	1.11
pH	6.05	5.88	5.74	5.60	5.46
Salt (g/kg)	15.3	21.9	15.3	16.2	19.5
MNFS (g/kg)	600	589	605	582	585
FDM (g/kg)	377	400	402	389	390
S/M (g/kg)	31.7	47.4	32.0	35.2	42.2

Table 5.5.2 *Changes in composition of the cheese curd made with a set pH of 6.05 and its centrifugal serum*

Component	Number of days after manufacture						
	2	4	6	8	10	12	14
pH of cheese curd	6.05			6.04			6.05
Centrifugal serum[1] (g/kg cheese curd)	118 ± 1.9	108 ± 2.3	106 ± 1.8	106 ± 2.2	105 ± 1.6	103 ± 2.5	99.4 ± 2.1
Calcium in serum (mmol/kg)	21.8	20.4	21.5	18.9	19.6	21.3	21.7
Sodium in serum (mmol/kg)	420	424	402	426	419	416	424

[1] Values are mean ± standard deviation for six observations.

Table 5.5.3 *Changes in composition of the cheese curd made with a set pH of 5.90 and its centrifugal serum*

Component	Number of days after manufacture						
	2	4	6	8	10	12	14
pH of cheese curd	5.88			5.90			5.90
Centrifugal serum[†] (g/kg cheese curd)	82.9 ± 2.2	84.7 ± 2.0	87.6 ± 2.6	90.2 ± 1.7	87.1 ± 2.3	90.4 ± 2.0	69.3 ± 2.4
Calcium in serum (mmol/kg)	29.7	32.0	31.4	32.2	29.0	30.1	31.4
Sodium in serum (mmol/kg)	437	428	427	431	428	420	433

[†] Values are mean ± standard deviation for six observations.

Table 5.5.4 *Changes in composition of the cheese curd made with a set pH of 5.75 and its centrifugal serum*

Component	Number of days after manufacture						
	2	4	6	8	10	12	14
pH of cheese curd	5.75			5.75			5.75
Centrifugal serum[†] (g/kg cheese curd)	39.0 ± 2.6	38.9 ± 2.1	36.0 ± 2.2	37.0 ± 2.7	36.7 ± 2.4	30.2 ± 2.1	30.3 ± 2.6
Calcium in serum (mmol/kg)	42.3	44.1	44.0	43.5	42.9	43.3	43.6
Sodium in serum (mmol/kg)	430	425	437	429	424	435	431

[†] Values are mean ± standard deviation for six observations.

The quantity of centrifugal serum from cheese curds made with set pH values of 6.05, 5.90 and 5.75 are shown in Tables 5.5.2, 5.5.3 and 5.5.4 respectively. The quantity of serum decreased with a decrease in pH of the cheese curd from 118 g/kg for pH 6.05 cheese curd (Table 5.5.2) to 39.0 g/kg for pH 5.75 cheese curd (Table 5.5.4) on day 2. The quantities of serum obtained on day 2 from cheese curds made at set pH values of 5.60 and 5.45 were 12.0 and 7.9 g/kg respectively and there was no release of serum on the following days. The effect of set pH on the quantity of centrifugal serum is also

shown in Fig 5.5.1. As in the previous trial (Section 5.3), no consistent time-dependent variation in the quantity of centrifugal serum was observed.

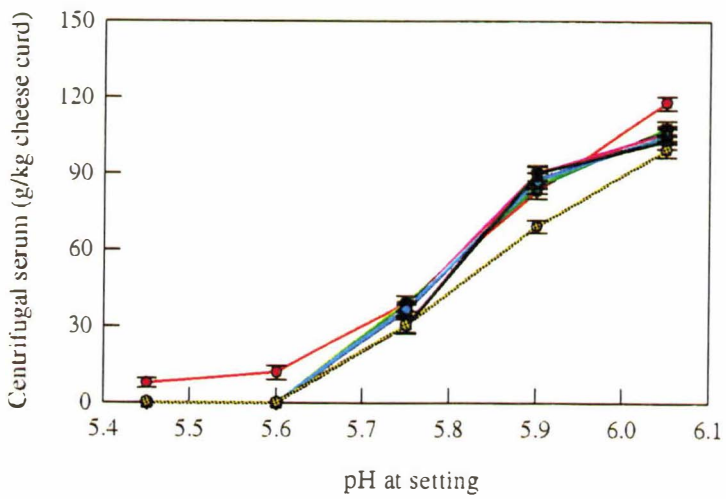

Fig. 5.5.1. *Effect of set pH and time on the quantity of centrifugal serum.* Cheese curds made with different set pH values were centrifuged at 11 086 *g* and 25 °C for 75 min. The quantities of centrifugal serum on different days after manufacture are shown: ⊕, day 2; ■, day 4; ▼, day 6; ▲, day 8; ◇, day 10; ●, day 12; and ○, day 14.

The calcium content of the serum increased with a decrease in the set pH value of the cheese curd (Tables 5.5.2, 5.5.3 and 5.5.4), but there was no consistent variation in the calcium content with time for any of the cheese curds. The sodium contents of the sera were quite similar for the three samples of cheese curd.

5.5.4 Rheological properties of cheese curd

5.5.4.1 Large strain deformation studies

5.5.4.1.1 Evaluation using the TAHD texture analyser

The cheese curd made with a set pH of 5.90 was analysed using the TAHD texture analyser according to the procedure described in Section 4.2.5.1. The results obtained for fracture strain (ϵ_f, or longness, *i.e.* the resistance to crumbling), fracture stress (σ_f,

or firmness) and the modulus of deformability (E_d, or stiffness) are shown in Table 5.5.5.

Table 5.5.5 *Rheological evaluation of the cheese curd samples of set pH 5.90 using the TAHD texture analyser*

Days after manufacture	ϵ_f	σ_f (kPa)	E_d (kPa)
1	1.00 ± 0.06	213 ± 33.5	476 ± 57.1
2	1.14 ± 0.32	199 ± 29.2	513 ± 65.5
3	0.90 ± 0.11	189 ± 40.9	516 ± 50.7
4	1.00 ± 0.09	259 ± 75.8	507 ± 28.9
5	0.98 ± 0.03	206 ± 21.2	562 ± 67.5
6	0.89 ± 0.06	177 ± 21.8	569 ± 36.4
7	0.93 ± 0.09	196 ± 36.1	544 ± 69.9

Values are mean ± standard deviation for four observations.

ϵ_f remained essentially constant during the first 7 days after manufacture. The curd stiffness (E_d) tended to increase with time but a definite conclusion could not be drawn because of the large variation in the results obtained as indicated by the high standard deviations. Some of the inconsistency in the results was due to the test samples being different from one another as a consequence of the constraints of the test procedure. Because the curd was not pressed during cheesemaking, there was no subsequent fusion of curd pieces. Consequently, the curd had to be cut into fairly large pieces (about 30 mm × 30 mm × 30 mm) before salting so that samples that were suitable for analysis using the TAHD could be obtained . Cutting of the curd into large sized pieces may have resulted in uneven distribution of salt and contributed to variations in composition, *e.g.* in moisture and salt contents, between the curd pieces and within the curd pieces. Because of this non-uniformity (which may have been partially responsible for the large standard deviations observed (Table 5.5.5), it was decided not to use this technique in future trials.

5.5.4.1.2 Evaluation using the Instron Universal Testing Machine

Samples of cheese curd of set pH values 6.05, 5.90, 5.75, 5.60 and 5.45 were also analysed for large strain deformation using an Instron Universal Testing Machine. In this technique, a large sample of cheese curd was grated prior to testing and used (Section 4.2.5.1.2); thus the difficulty of inhomogeneity of samples encountered while using the TAHD was overcome. The values for the maximum force exerted on the samples (Fig. 5.5.2), a measure of curd stiffness, were highest for the cheese curd set at pH 5.90. No consistent variation with time in the values was observed for any of the cheese curd samples (Fig. 5.5.2).

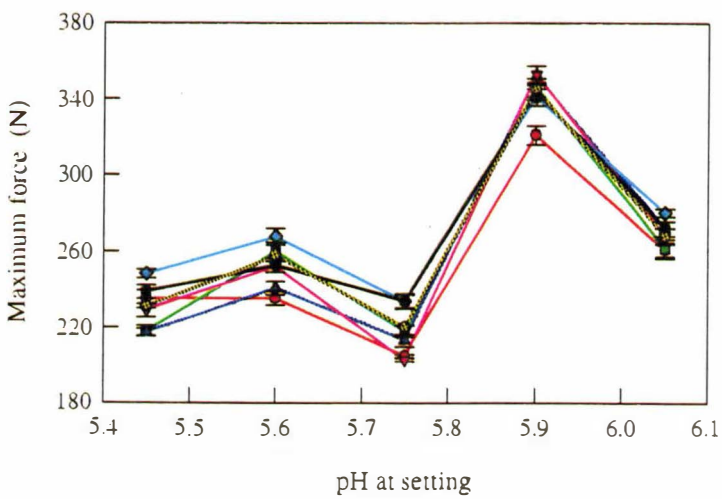

Fig. 5.5.2. *Effect of set pH and time on the rheological properties of cheese, as determined using the Instron.* Cheese curds were made with different set pH values, grated and stored at 2 °C. On each day of testing, the required quantity of grated cheese was equilibrated at 20 °C for 2 h in a water-tight box prior to testing. The maximum force exerted to compress and extrude the cheese curd through the orifice of the extrusion attachment (Fig. 4.2.1) using the Instron was determined on different days after manufacture: ●, day 2; ■, day 4; ▲, day 6; ▼, day 8; ◆, day 10; ⬢, day 12; and ○, day 14.

5.5.4.2 Small strain deformation studies

Samples of cheese curd made with different set pH values were analysed for storage modulus (G′, a measure of stiffness), loss modulus (G″, a measure of viscosity) and phase angle (δ, a measure of how liquid-like the sample is) (Fig. 5.5.3). The values for

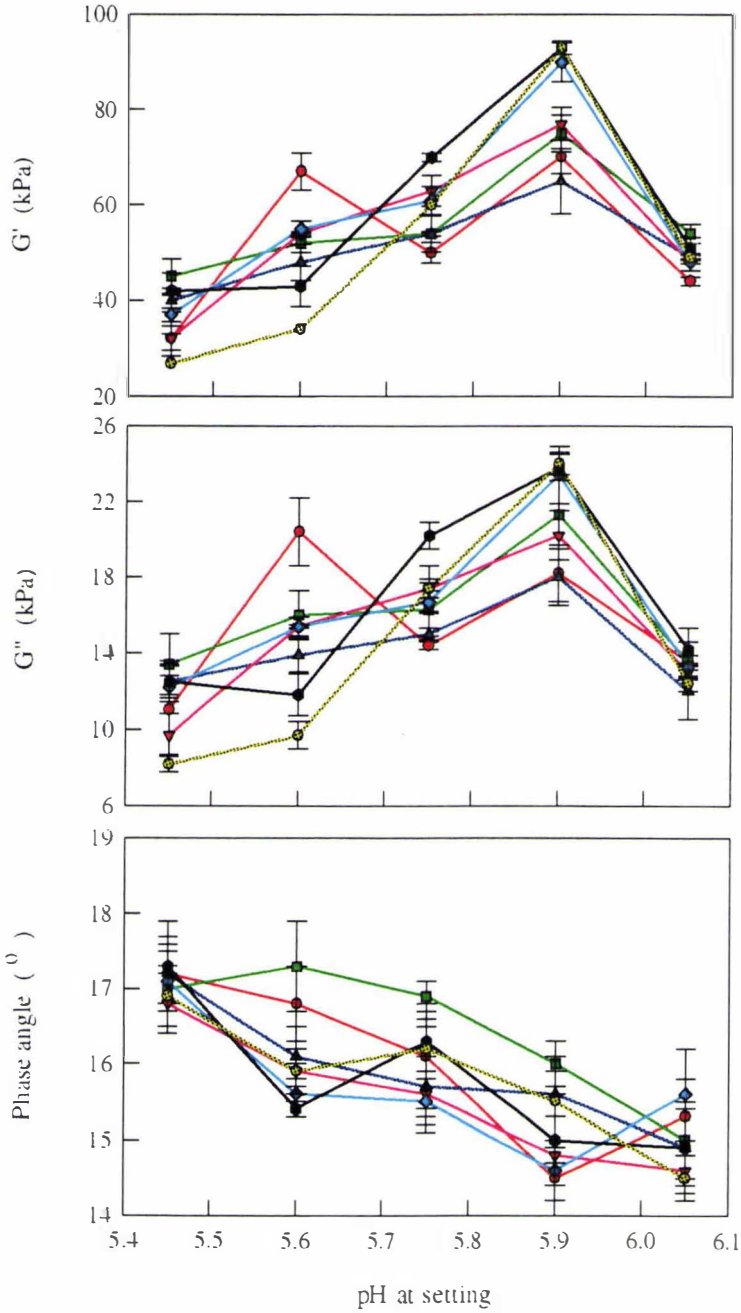

Fig. 5.5.3. *Effect of set pH and time on the rheological properties of cheese, as determined using the Bohlin.* Cheese curds made with different set pH values and stored at 2 °C were cut into 4 mm thick slices. Sample discs of 30 mm diameter obtained from these slices using a core borer were covered with polyethylene film and equilibrated at 20 °C for 2 h in a water tight box prior to testing. Samples were trimmed to 25 mm at the time of testing. Measurements of storage modulus (G′), loss modulus (G″) and phase angle (δ) were obtained using a Bohlin rheometer with a 25 mm diameter serrated plate system and a gap setting equal to 80% of the sample height at 20 °C on different days after manufacture: ●, day 2; ■, day 4; ▲, day 6; ▼, day 8; ◇, day 10; ⬢, day 12; and ○, day 14.

G′ and G″ tended to increase with an increase in cheese curd pH from 5.45 to 5.90 and decreased for samples set at pH 6.05. The curd samples showed an increased solid like behaviour as the set pH increased, as evidenced by lower values for δ at higher pH values (Fig. 5.5.3).

5.5.5 Conclusions

The quantity of centrifugal serum decreased with a decrease in the pH at setting of the cheese curd. However, the quantity of serum did not vary appreciably with time for the cheese curds of higher pH values. As in the earlier trial (Section 5.3), no serum was obtained from cheese curds of lower pH values.

Samples of cheese curd were rheologically evaluated using different techniques. Large variations were observed in the results obtained using the TAHD as a consequence of the constraints of the test procedure. Because of these difficulties, rheological evaluation using the TAHD was not carried out in subsequent trials. Both the maximum force from the large strain method on the Instron and G′ (stiffness) from the small strain method on the Bohlin showed a maximum for cheese curd of set pH 5.90. Cheese curd also exhibited a more solid-like behaviour at pH 5.90, as indicated by the lowest value of phase angle.

Differences in water distribution as well as the rheological properties of the cheese curds were influenced by the pH, although no apparent correlation between the two was evident. It was decided to determine whether a change in the pH of the cheese curd after manufacture had a similar effect on the rheological properties of the cheese curd.

5.6 EVALUATION BY VARIOUS TECHNIQUES OF CHEESE CURDS ADJUSTED TO DIFFERENT pH VALUES USING GDL

5.6.1 Introduction

It was shown in Section 5.4 that addition of GDL at 7, 11, 16 and 20 g/kg to pH 5.90 cheese curd resulted in cheese curds with pH values of 5.61, 5.43, 5.30 and 5.19 respectively and that the quantity of centrifugal serum decreased with time although the pH of the curd did not decrease. In the present trial, GDL was added to pH 5.90 cheese curd at similar levels to obtain cheese curds with pH values of 5.68, 5.60, 5.38 and 5.26. The samples were examined for changes in water distribution and composition and some rheological properties.

5.6.2 Composition of cheese curds

The composition of the cheese curd of set pH 5.90 and of the cheese curds of different final pH values obtained on addition of GDL are shown in Table 5.6.1. Although the levels of GDL added were the same as in the earlier trial (Section 5.4), the final pH values obtained were different, probably due to differences in the initial composition of the cheese curds (cf. Tables 5.4.1 and 5.6.1). The composition of the cheese curd was altered to some extent upon the addition of GDL. However, no consistent variations in the compositions of different samples were observed with changes in pH.

5.6.3 Changes in composition of cheese curds and centrifugal sera

There was no change in the pH values of the samples of cheese curd during storage (Table 5.6.2), confirming the results obtained in the earlier trial (Section 5.4).

The quantities of centrifugal serum from the four samples of cheese curd are shown in Table 5.6.2. The quantity of serum decreased with a decrease in the pH of the cheese curd from 110 g/kg for pH 5.68 cheese curd to 76.7 g/kg for pH 5.26 cheese curd on day 3. Similar variations in the quantity of serum were also observed on other days.

The rate of decrease in the quantity of serum was more rapid for cheese curds of lower pH values (Table 5.6.2). There was no release of serum after day 9 for pH 5.38 cheese curd and after day 7 for pH 5.26 cheese curd. The effect of pH and time on the quantity of centrifugal serum is also shown in Fig. 5.6.1.

Table 5.6.1 *Compositions on day 3 of the cheese curd of set pH 5.90 and of the cheese curds with altered pH values obtained from it by the addition of GDL*

Component	Curd of pH 5.90	Target pH values of the cheese curds			
		5.68	5.60	5.38	5.26
Moisture (g/kg)	488	485	485	487	487
Fat (g/kg)	205	205	205	195	190
Total nitrogen (g/kg)	35.6	35.9	36.0	34.8	35.1
Non protein nitrogen (g/kg)	1.70	1.98	1.71	1.67	1.85
pH	5.91	5.68	5.60	5.38	5.26
Salt (g/kg)	20.3	20.7	18.6	20.9	20.2
MNFS (g/kg)	614	610	610	605	601
FDM (g/kg)	400	398	398	380	370
S/M (g/kg)	41.6	42.7	38.2	42.9	41.5

The calcium content of the serum increased with a decrease in the pH of the cheese curd. The concentration of calcium in the serum also tended to increase with time for the cheese curd samples of pH 5.68 and pH 5.60. No consistent trends were observed for the sodium content of the centrifugal sera.

Table 5.6.2 *Composition of cheese curds and centrifugal sera of the curd samples adjusted to different pH values with GDL*

Component	Number of days after manufacture					
	3	5	7	9	11	13
(a) pH 5.68 cheese curd						
pH of cheese curd	5.68		5.67		5.68	
Centrifugal serum[a] (g/kg cheese curd)	110 ± 1.50	109 ± 2.62	107 ± 2.56	97.4 ± 1.80	79.4 ± 2.34	71.4 ± 2.64
Calcium in serum[a]	56.9	57.3	58.8	59.5	60.2	61.7
Sodium in serum[a]	477	459	458	458	461	463
(b) pH 5.60 cheese curd						
pH of cheese curd	5.61		5.60		5.61	
Centrifugal serum[a] (g/kg cheese curd)	91.4 ± 2.56	80.0 ± 2.18	68.1 ± 2.40	47.4 ± 1.45	16.9 ± 2.43	-[b]
Calcium in serum[a]	71.1	75.2	76.1	77.4	-	-
Sodium in serum[a]	418	427	407	413	-	-
(c) pH 5.38 cheese curd						
pH of cheese curd	5.40		5.38		5.38	
Centrifugal serum[a] (g/kg cheese curd)	82.6 ± 2.28	76.1 ± 2.60	38.0 ± 2.44	9.10 ± 2.76	-[b]	-[b]
Calcium in serum[a]	101	96.6	93.7	-	-	-
Sodium in serum[a]	456	455	449	-	-	-
(d) pH 5.26 cheese curd						
pH of cheese curd	5.25		5.25		5.26	
Centrifugal serum[a] (g/kg cheese curd)	76.7 ± 2.66	63.2 ± 2.90	15.0 ± 2.28	-[b]	-[b]	-[b]
Calcium in serum[a]	106	103	-	-	-	-
Sodium in serum[a]	459	470	-	-	-	-

[a] Values are mean ± standard deviation for six observations. mmol/kg.
[b] No release of serum on centrifugation of cheese curd.

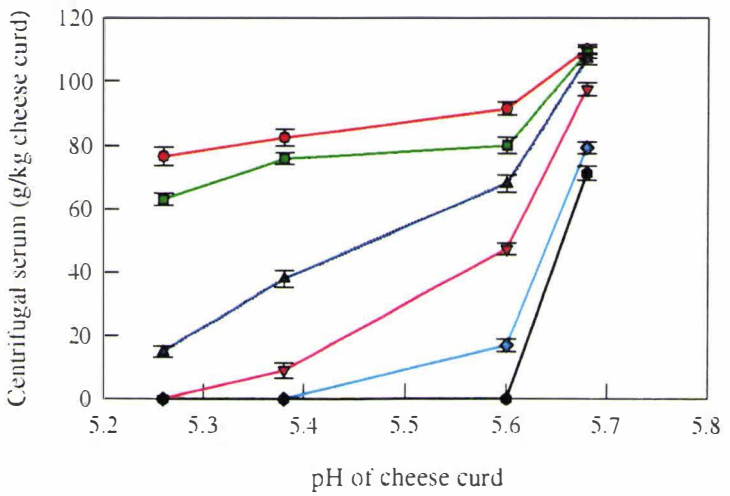

Fig. 5.6.1. *Effect of pH and time on the quantity of centrifugal serum from the cheese curds adjusted to different pH values using GDL.* Cheese curd made with a set pH of 5.90 was adjusted to different pH values using GDL. The samples of cheese curd were centrifuged at 11 086 *g* and 25 °C for 75 min. The quantities of centrifugal serum on different days after manufacture are shown: ●, day 3; ■, day 5; ▲, day 7; ▼, day 9; ◆, day 11; and ⬢, day 13.

5.6.4 Rheological properties of cheese curds

5.6.4.1 Large strain deformation studies

The samples of cheese curd of different final pH values were analysed using the Instron Universal Testing Machine by the procedure described in Section 4.2.5.1. The value of maximum force tended to be higher for cheese curds of higher pH values (Fig. 5.6.2), in which the quantity of centrifugal serum was high and the drop in the quantity of serum with time was minimal (Fig. 5.6.1). For cheese curds of pH values 5.38 and 5.26, the value of maximum force increased on days 11 and 13 and there was no release of centrifugal serum on these days for the two samples. However, no consistent trends in the variation of values was observed with time for any of the cheese curd samples.

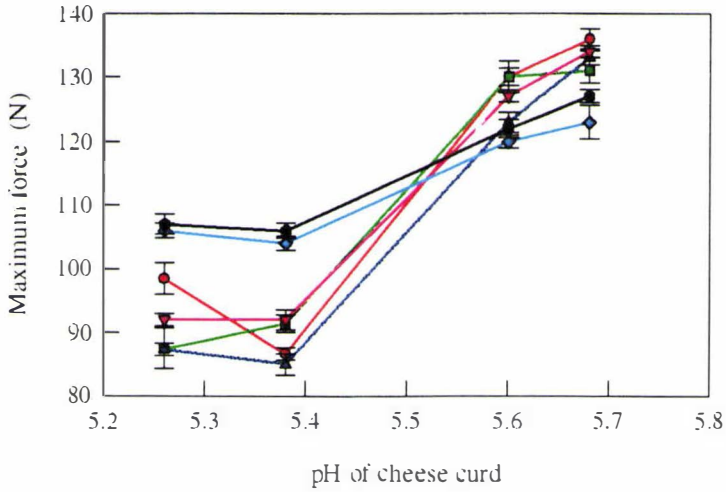

Fig. 5.6.2. *Effect of pH and time on the rheological properties of the cheese curds adjusted to different pH values using GDL, as determined using the Instron.* Cheese curd made with a set pH of 5.90 was grated, adjusted to different pH values using GDL and stored at 2 °C. On each day of testing, the required quantity of grated cheese was equilibrated at 20 °C for 2 h in a water tight box prior to testing. The maximum force exerted to compress and extrude the cheese curd through the orifice of the extrusion attachment (Fig. 4.2.1) using the Instron was determined on different days after manufacture: ●, day 3; ■, day 5; ▲, day 7; ▼, day 9; ◆, day 11; and ●, day 13.

5.6.4.2 Small strain deformation studies

The four samples of cheese curd of different final pH values were also analysed using the Bohlin rheometer for G′, G″ and δ (Fig. 5.6.3). No definite conclusions could be drawn from these results because of the large variations observed. Unlike the sample preparation for the Instron where GDL was added to the grated cheese, GDL had to be added to larger pieces of curd (about 30 mm × 30 mm × 30 mm) so that samples could be obtained for measurements using the Bohlin rheometer. This possibly resulted in test samples being different from one another in their composition. The pH at the centre of the piece of cheese curd was also found to be lower by about 0.1 pH units than on the surface on day 3. These differences in composition may have contributed to the variations in the results obtained.

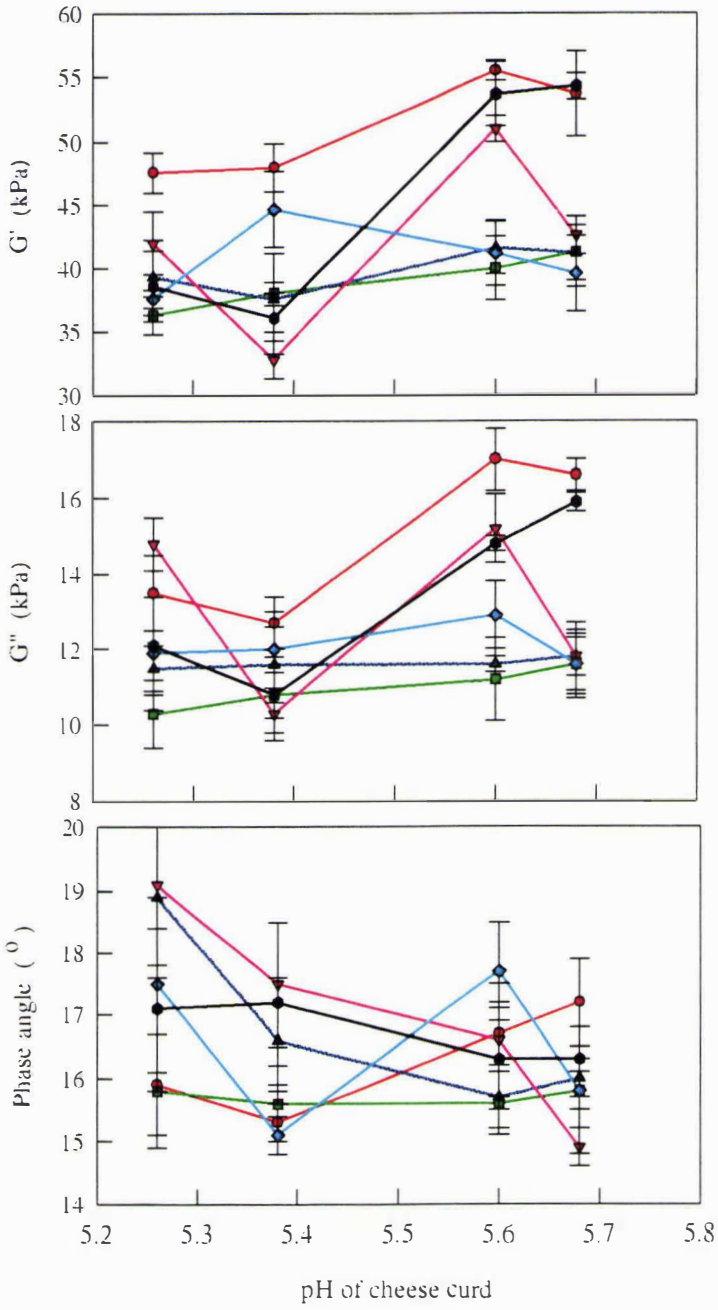

Fig. 5.6.3. *Effect of pH and time on the rheological properties of the cheese curds adjusted to different pH values using GDL, as determined using the Bohlin.* Cheese curds made with a set pH of 5.90, adjusted to different pH values using GDL and stored at 2 °C were cut into 4 mm thick slices. Sample discs of 30 mm diameter obtained from these slices using a core borer were covered with polyethylene film and equilibrated at 20 °C for 2 h in a water tight box prior to testing. Samples were trimmed to 25 mm at the time of testing. Measurements of storage modulus (G'), loss modulus (G") and phase angle (δ) were obtained using a Bohlin rheometer with a 25 mm diameter serrated plate system and a gap setting equal to 80% of the sample height at 20 °C on different days after manufacture: ●, day 3; ▫, day 5; ▲, day 7; ▼, day 9; ◇, day 11; and ●, day 13.

5.6.5 Conclusions

The pH of the cheese curds remained constant during storage which enabled the effect of pH shift on changes in water distribution and rheological properties to be determined. The quantity of centrifugal serum was affected by the pH of the cheese curds as well as by the extent of shift from the original pH. The drop in time-dependent serum loss was greater for curd samples that had undergone a larger pH shift.

Cheese curd samples of higher pH values had a higher maximum force than those of lower pH values in the pH range studied. Small strain deformation testing using the Bohlin rheometer was not a very useful technique for analysing samples in which the pH was altered using GDL.

5.7 SUMMARY OF RESULTS AND THE PLAN FOR THE FINAL TRIAL

Initial studies were performed on Cheddar, Cheshire and Mozzarella cheeses made using the standard manufacturing protocols. A serum phase could be centrifuged out from Cheshire and Mozzarella cheeses but not from Cheddar cheese. The manufacturing process for Cheddar cheese was suitably modified so as to retain more moisture in the cheese curd. This enabled the separation of serum from the cheese and the changes in water distribution with time to be determined.

Studies on Cheddar-like cheeses made at different draining and salting pH values showed pH to be a factor in determining the quantity of serum that could be centrifuged out. The quantity of serum decreased with a decrease in the pH of the cheese. Although the quantity of serum also decreased with time, this could not be attributed solely to the pH as the pH also changed with time.

A model system was subsequently developed in which cheese curds were made at different set pH values. Use of lactic acid in place of starter cultures provided a means of minimising the changes in the pH of cheese curds during storage and of determining the effect of pH on changes in composition and water distribution. The quantity of centrifugal serum was less for a cheese curd of lower pH, but the quantity of serum did not vary appreciably with time. The pH of the cheese curd also seemed to be influencing the changes in its rheological properties. The highest values for the maximum force exerted on the cheese curd samples (Instron) and for the curd stiffness (G′) were both observed for the cheese curd made with a set pH of 5.90 in the pH range studied.

The model system was then modified such that the pH was altered after manufacture. GDL was added at different levels to cheese curd made at a particular set pH value and samples of cheese curd with different final pH values were obtained. The quantity of centrifugal serum was less for cheese curds of lower pH values. The quantity of serum also decreased with time for all samples. The reduction in the quantity of serum was

greatest for cheese curds with the biggest pH shift. The values of maximum force and curd stiffness (G′) were greater for cheese curd of higher pH value in the pH range studied.

The modifications made to the cheesemaking process provided an effective control over the extent of proteolysis during the early stages of ripening. Samples of cheese curd made at different set pH values as well as those adjusted to different pH values with GDL showed a lesser degree of proteolysis than a normal cheese. The evaluation of the extent of proteolysis was discontinued once this was established for both the model curd systems.

Significant differences in the microstructure of cheese curds made with different set pH values was observed, suggesting that the set pH mainly determined the basic structure of cheese. These differences in structure were not evident for the cheese curds adjusted to different pH values using GDL. It is likely that the differences would be detected by other microscopy techniques with a better resolution.

The properties of cheese curds were thus affected both by initial pH and by any subsequent changes brought about in the pH. However, the two effects were not tested on the same lot of cheese curds and needed to be tested separately to gain a better understanding of the phenomena that were occurring. In the trial proposed, cheese curds made at set pH values between 5.3 and 6.6 were to be analysed together with the samples of altered pH values obtained from them. This would enable the relative importance of the two effects on the changes in the properties of the cheese curds to be studied.

5.8 STUDY OF CHEESE CURDS MADE WITH DIFFERENT SET pH VALUES AND ADJUSTED TO DIFFERENT pH VALUES BY THE ADDITION OF GDL

5.8.1 Introduction

Two functioning model systems of cheese curds – one in which cheese curds were made with different set pH values and one in which the pH of cheese curds made at a particular set pH was adjusted to different values with the addition of GDL – were developed in the earlier trials (Sections 5.3 to 5.6). The objective of the present trial was to use the two techniques with the same lots of cheese curds and to study the changes with time using the techniques of centrifugation, rheology and microscopy that were developed earlier (Sections 4.2.4, 4.2.5 and 4.2.6). This was necessary to gain a better understanding of the relative influence of set pH and of a shift in the pH of cheese curds on the changes in water distribution and composition during the early stages of ripening.

The different set pH values at which the cheese curds were made are shown in Table 5.8.1. The quantities of GDL added to these cheese curds to attain the various target pH values and the pH values obtained after mixing with GDL are also shown in the table. The objective was to obtain a whole range of cheese curd samples of similar composition but different final pH, prepared by the two methods, for analysis using the selected techniques. A small strain deformation study of the cheese curds was not carried out because of the difficulties encountered with the pH-adjusted samples in the previous trial (Section 5.6.4.2).

The cheese curd systems (CS) of different set and adjusted pH values studied during this trial were identified with different codes (Table 5.8.1).

Table 5.8.1 *Set and adjusted pH values of the cheese curds made in the final trial*

Code	Set pH at which cheese curds were made	Cheese curds with GDL addition		
		GDL added (g/kg curd)	Target pH	pH obtained
CS1	6.60[*]	9	6.30	6.30
		14	6.10	6.13
		20	5.90	5.85
CS2	6.30[$]	9	6.00	6.00
		16	5.70	5.66
CS3	5.90[¶]	7	5.70	5.71
		12	5.50	5.48
CS4	5.70[§]	7	5.50	5.51
		11	5.30	5.28
CS5	5.50[§]	8	5.30	5.32
CS6	5.30[§]	Nil	-	-

Different superscripts indicate that cheese curds were made on different days.

5.8.2 Composition of cheese curds

The compositions of cheese curds set at different pH values and of the cheese curds of different pH values obtained by mixing them with GDL are given in Tables 5.8.2 to 5.8.7. The slight differences observed in the compositions of the cheese curds may have been due to the modifications made to the standard cheesemaking procedure (Section 5.2.1). However, as in the earlier trials, the MNFS content was higher and the FDM and S/M contents were lower in all samples in comparison with standard Cheddar cheese (Table 5.3.1). The salt and S/M contents tended to be higher in cheese curds of lower pH values (Tables 5.8.4 to 5.8.7). This is contrary to what is reported in the literature, but could have been due to the very different cheese curd systems used in this study.

Table 5.8.2 *Composition on day 3 of the cheese curd set at pH 6.60 and of those with added GDL*

Component	Original curd	Cheese curd after GDL addition		
pH	6.60	6.30	6.13	5.85
Moisture (g/kg)	481	481	483	482
Fat (g/kg)	185	190	185	180
Total nitrogen (g/kg)	37.2	36.7	38.1	39.4
Non protein nitrogen (g/kg)	1.30	1.18	1.20	1.21
Salt (g/kg)	18.0	17.0	17.3	16.5
MNFS (g/kg)	590	594	593	588
FDM (g/kg)	356	366	358	347
S/M (g/kg)	37.4	35.3	35.8	34.2

Table 5.8.3 *Composition on day 3 of the cheese curd set at pH 6.30 and of those with added GDL*

Component	Original curd	Cheese curd after GDL addition	
pH	6.30	6.00	5.66
Moisture (g/kg)	483	485	485
Fat (g/kg)	180	175	180
Total nitrogen (g/kg)	38.1	38.7	37.2
Non protein nitrogen (g/kg)	1.42	1.49	1.34
Salt (g/kg)	17.0	16.1	16.5
MNFS (g/kg)	589	588	591
FDM (g/kg)	348	340	350
S/M (g/kg)	35.2	33.2	34.0

Table 5.8.4 *Composition on day 3 of the cheese curd set at pH 5.90 and of those with added GDL*

Component	Original curd	Cheese curd after GDL addition	
pH	5.90	5.71	5.48
Moisture (g/kg)	484	486	483
Fat (g/kg)	195	200	200
Total nitrogen (g/kg)	38.7	38.8	39.0
Non protein nitrogen (g/kg)	1.12	1.09	1.17
Salt (g/kg)	19.8	18.8	19.1
MNFS (g/kg)	601	608	604
FDM (g/kg)	378	389	387
S/M (g/kg)	40.9	38.7	39.5

Table 5.8.5 *Composition on day 3 of the cheese curd set at pH 5.70 and of those with added GDL*

Component	Original curd	Cheese curd after GDL addition	
pH	5.70	5.51	5.28
Moisture (g/kg)	471	474	473
Fat (g/kg)	215	215	220
Total nitrogen (g/kg)	42.9	41.5	42.0
Non protein nitrogen (g/kg)	0.76	0.70	0.70
Salt (g/kg)	16.9	20.2	20.1
MNFS (g/kg)	600	604	606
FDM (g/kg)	406	409	417
S/M (g/kg)	35.9	42.6	42.5

Table 5.8.6 *Composition on day 3 of the cheese curd set at pH 5.50 and of that with added GDL*

Component	Original curd	Cheese curd after GDL addition
pH	5.50	5.32
Moisture (g/kg)	474	473
Fat (g/kg)	220	225
Total protein (g/kg)	44.6	44.2
Non Protein Nitrogen (g/kg)	0.89	0.82
Salt (g/kg)	20.8	20.9
MNFS (g/kg)	608	612
FDM (g/kg)	418	427
S/M (g/kg)	43.9	44.2

Table 5.8.7 *Composition on day 3 of the cheese curd set at pH 5.30*

Component	Original curd
pH	5.30
Moisture (g/kg)	482
Fat (g/kg)	180
Total nitrogen (g/kg)	43.2
Non protein nitrogen (g/kg)	1.19
Salt (g/kg)	20.3
MNFS (g/kg)	588
FDM (g/kg)	347
S/M (g/kg)	42.1

5.8.3 Changes in composition of cheese curds and centrifugal sera

The changes in quantities of centrifugal serum obtained from the samples of CS1, CS2 and CS3 are shown in Fig. 5.8.1. The quantities of centrifugal serum on day 3 were influenced by the set pH of the cheese curds. The quantity of serum was higher for the cheese curd set at pH 6.30 (127 g/kg; Fig. 5.8.1 (b)) than for the cheese curd set at pH 6.60 (121 g/kg; Fig. 5.8.1 (a)) or pH 5.90 (75.6 g/kg; Fig. 5.8.1 (c)). No serum could be centrifuged out from the cheese curds set at the lower pH values of 5.70, 5.50 and 5.30.

A shift in pH of the cheese curds due to the addition of GDL also influenced the quantity of centrifugal serum. For the cheese curd samples of CS1, the quantity of serum was higher for cheese curds adjusted to lower pH values with GDL than for the cheese curd set at pH 6.60 (Fig. 5.8.1 (a)). The maximum quantity of centrifugal serum obtained was for the cheese curd adjusted to pH 6.13 (137 g/kg). However, the quantities of centrifugal serum were lower for cheese curds adjusted to lower pH values from cheese curd set at pH 6.30 (CS2; Fig. 5.8.1 (b)) and pH 5.90 (CS3; Fig. 5.8.1 (c)). For all the samples of various set and adjusted pH values between 5.48 and 6.60, the maximum quantity of centrifugal serum obtained was for the cheese curd adjusted to pH 6.13. No centrifugal serum was obtained from any of the samples of CS4, CS5 and CS6 cheese curds.

The quantity of centrifugal serum remained about constant with time during the initial period of the study for cheese curds set at pH values of 6.60, 6.30 and 5.90 but decreased for all the samples with GDL-adjusted pH values (Fig. 5.8.1). The decrease in the quantity of serum was relatively small for the samples of cheese curd obtained from cheese curds set at pH 6.60 (Fig. 5.8.1(a)) and pH 6.30 (Fig. 5.8.1 (b)) when compared with those obtained from cheese curd set at pH 5.90 (Fig. 5.8.1 (c)).

The variations in the quantities of centrifugal serum with pH on days 3 and 13 are shown in Fig 5.8.2. A greater drop in the quantity of serum with a decrease in pH on

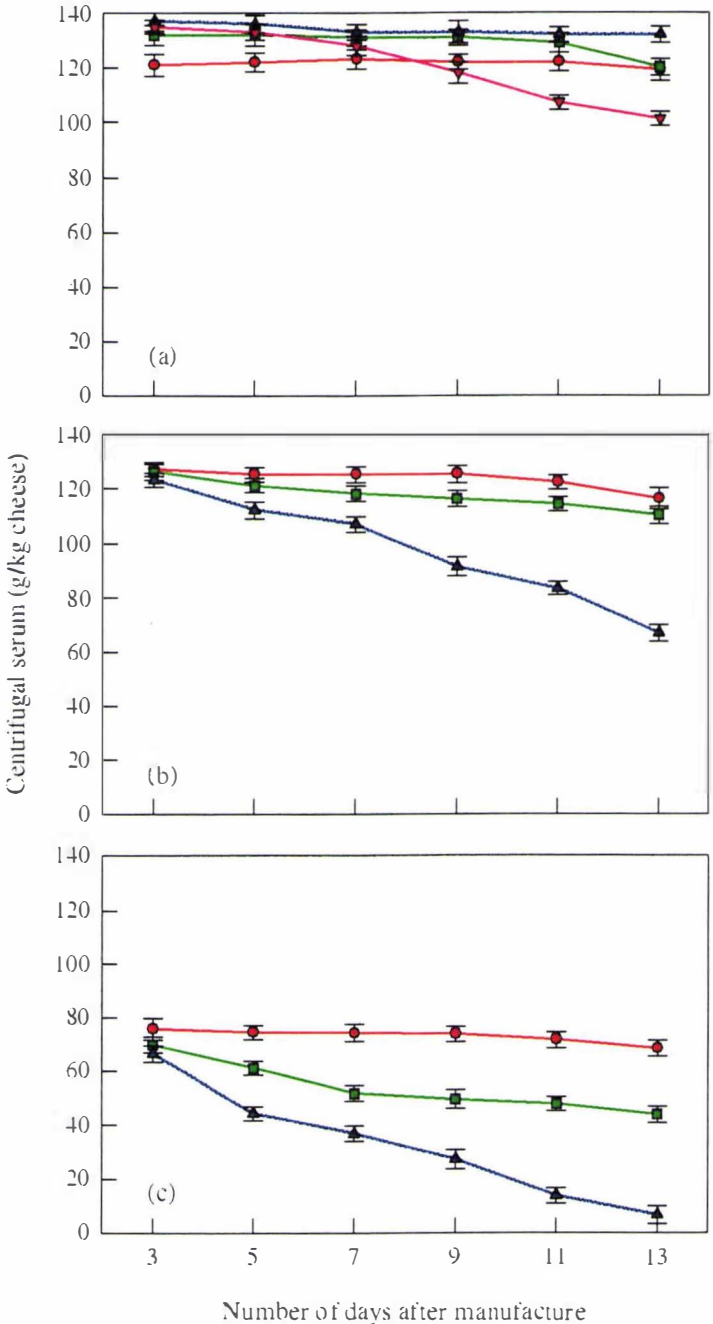

Fig. 5.8.1. *Effect of pH and time on the quantity of centrifugal serum from the cheese curds made with different set pH values and also adjusted to lower pH values using GDL.* Cheese curds were centrifuged at 11 086 *g* and 25 °C for 75 min and the quantities of centrifugal serum on different days after manufacture were determined. Cheese curds were made with different set pH values and then adjusted to lower pH values using GDL as follows: (a) CS1 - cheese curd made with set pH 6.60 (●) and adjusted to pH values 6.30 (■), 6.13 (▲) and 5.85 (▼); (b) CS2 - cheese curd made with set pH 6.30 (●) and adjusted to pH 6.00 (■) and pH 5.66 (▲); (c) CS3 - cheese curd made with set pH 5.90 (●) and adjusted to pH 5.71 (■) and pH 5.48 (▲).

both days was noticed from samples of CS3 (Fig. 5.8.2 (c)) than from the other two sets of cheese curds. This suggested that a shift in pH from pH 5.90 affected the water distribution in the cheese curds to a greater extent than a similar shift from pH 6.60 or pH 6.30.

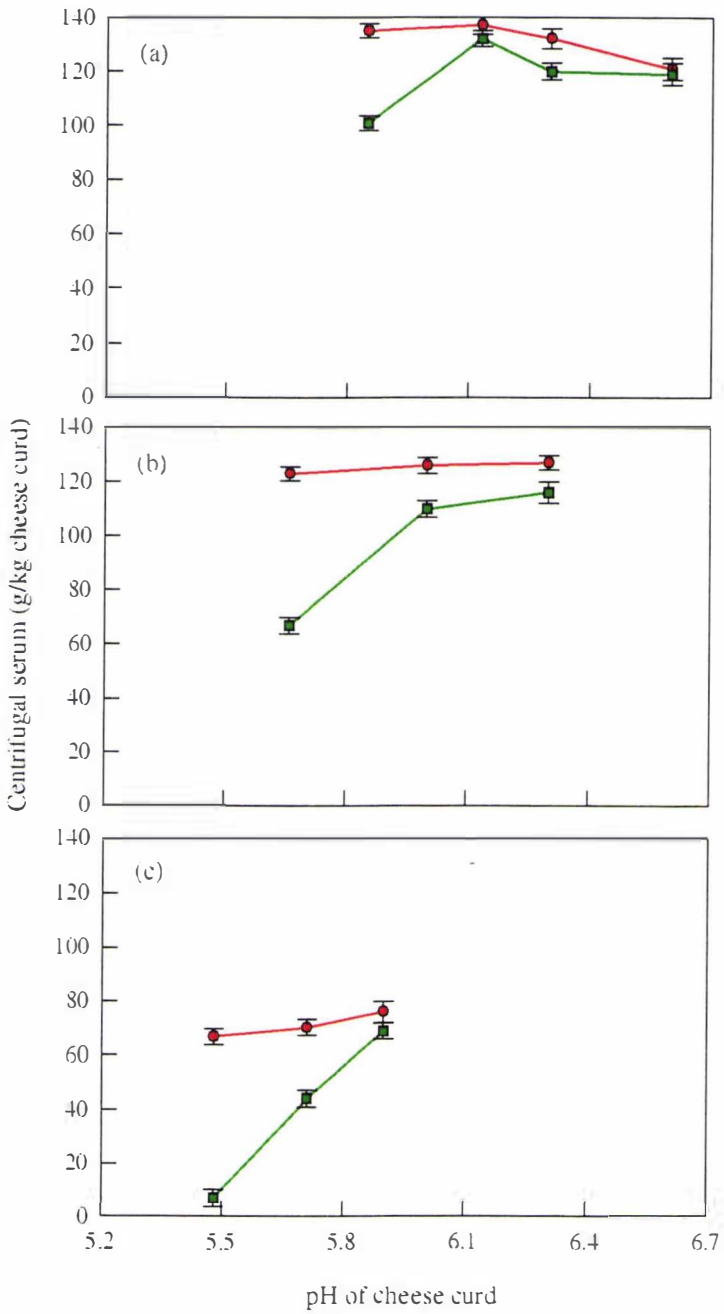

Fig. 5.8.2. *Effect of pH on the quantity of centrifugal serum on days 3 (●) and 13 (■) after manufacture from the cheese curds made with different set pH values and also adjusted to lower pH values using GDL.* Cheese curds were made with different set pH values and then adjusted to various pH values using GDL: (a) CS1, (b) CS2 and (c) CS3. Cheese curds were centrifuged at 11 086 *g* and 25 °C for 75 min and the quantity of centrifugal serum was determined.

The calcium and sodium contents of the centrifugal sera obtained from the cheese curds of CS1, CS2 and CS3 are shown in Fig. 5.8.3. The calcium content of the serum increased with a decrease in the pH of the cheese curds in each of the cheese curd systems. The calcium content of the serum was also higher for the cheese curds with a bigger pH shift. There was an increase in the calcium content of the serum with time for cheese curds of the lowest pH values of CS2 (Fig. 5.8.3 (b)) and CS3 (Fig. 5.8.3 (c)) but the calcium content remained about constant for the other samples of cheese curd.

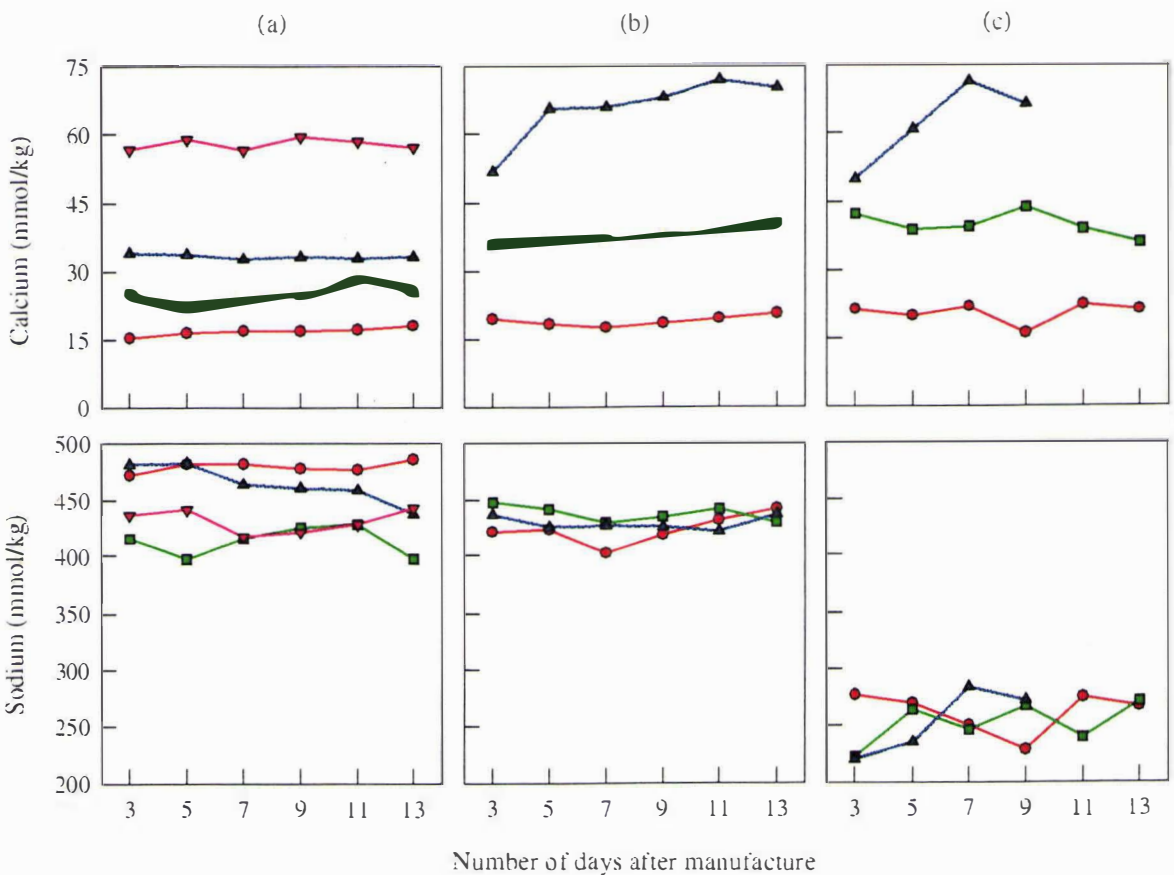

Fig. 5.8.3. *Effect of pH and time on the calcium and sodium contents of the centrifugal serum from cheese curds made with different set pH values and also adjusted to lower pH values using GDL.* Cheese curds were centrifuged at 11 086 ***g*** and 25 °C for 75 min. The calcium and sodium contents of the serum obtained were determined using an ARL 34000 inductively coupled plasma atomic emission spectrometer. Cheese curds were made with different setting pH values and then adjusted to lower pH values using GDL as follows: (a) CS1 - cheese curd made with set pH 6.60 (●) and adjusted to pH 6.30 (■), 6.13 (▲) and 5.85 (▼); (b) CS2 - cheese curd made with set pH 6.30 (⊗) and adjusted to pH 6.00 (■) and pH 5.66 (▲); (c) CS3 - cheese curd made with set pH 5.90 (⊗) and adjusted to pH 5.71 (■) and pH 5.48 (▲).

The sodium content of the serum was lower for cheese curd samples of CS3 (Fig. 5.8.3(c)) than for the other two sets of cheese curds (Fig. 5.8.3 (a), (b)). However, the sodium content of the serum from cheese curds of different pH values did not vary consistently with time. The samples of cheese curd of CS3 had higher salt and S/M contents than the other two sets of cheese curds (Tables 5.8.2, 5.8.3 and 5.8.4) but the quantity of centrifugal serum obtained was lower (Fig. 5.8.1).

5.8.4 Large strain deformation studies of the rheological properties of cheese curds

The samples of cheese curd of different set and adjusted pH values were analysed using the Instron Universal Testing Machine by the procedure described in Section 4.2.5.1. The results obtained for cheese curds of different set pH values are shown in Fig. 5.8.4.

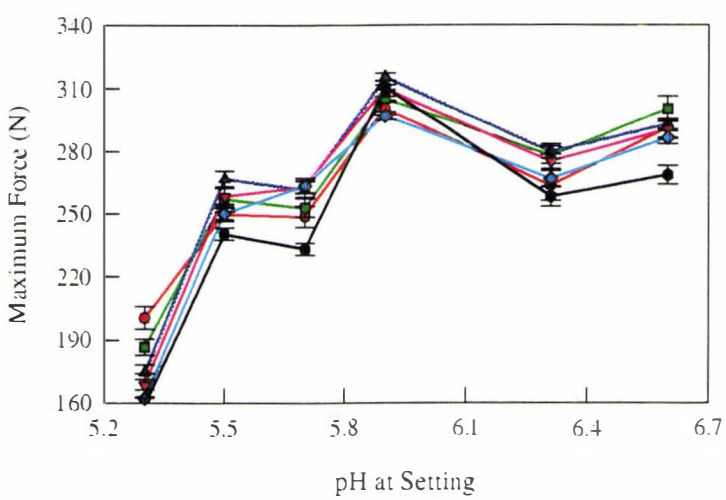

Fig. 5.8.4. *Effect of set pH and time on the rheological properties of cheese, as determined using the Instron.* Cheese curds were made with different set pH values, grated and stored at 2 °C. On each day of testing, the required quantity of grated cheese was equilibrated at 20 °C for 2 h in a water tight box prior to testing. The maximum force exerted to compress and extrude the cheese curd through the orifice of the extrusion attachment (Fig. 4.2.1) using the Instron was determined on different days after manufacture: ●, day 3; ■, day 5; ▲, day 7; ▽, day 9; ◇, day 11; and ●, day 13.

The value of maximum force tended to increase with an increase in the set pH of the cheese curds up to pH 5.90, decreased for cheese curds set at pH 6.30 and tended to increase again for cheese curds set at pH 6.60. However, the maximum force was observed for cheese curds set at pH 5.90. Differences were also observed in the values of the maximum force with time. However, no consistent trends were evident in these values. The results were very similar to those observed in the earlier trial (Section 5.5; Fig. 5.5.2) for the narrower pH range studied.

The results obtained for cheese curds of various set and adjusted pH values of the different cheese curd systems (CS1 to CS6) are shown in Fig. 5.8.5. The results showed essentially similar trends to those observed for cheese curds set at different pH values (Fig. 5.8.4) for cheese curds in similar pH ranges. For example, the maximum force tended to decrease for cheese curds adjusted to pH 6.30 from cheese curd set at pH 6.60 and tended to increase for cheese curds adjusted to pH 6.13 and pH 5.85 (Fig. 5.8.5 (f)).

5.8.5 Microscopic examination of cheese curds

The samples of cheese curd of different set and adjusted pH values were stained with dyes Fast Green FCF and Nile Blue and examined using a confocal laser scanning microscope by the procedure described in Section 4.2.6.

The differences in the microstructure of cheese curd before and after centrifugation were examined (Fig. 5.8.6). The micrographs shown are combined images of the protein and fat phases. The microstructure of the cheese curd adjusted to pH 6.13 with GDL before centrifugation (Fig. 5.8.6 (a)) showed several (dark) regions where there was no fluorescence. There were no fluorescent dyes in these regions which meant that neither fat nor protein was present. Air or water was likely to be present in these areas. The microstructure of the cheese curd after centrifugation (Fig.5.8.6 (b)) showed fewer dark regions, *i.e.* with neither fat nor protein. The protein matrix, in green, appeared much

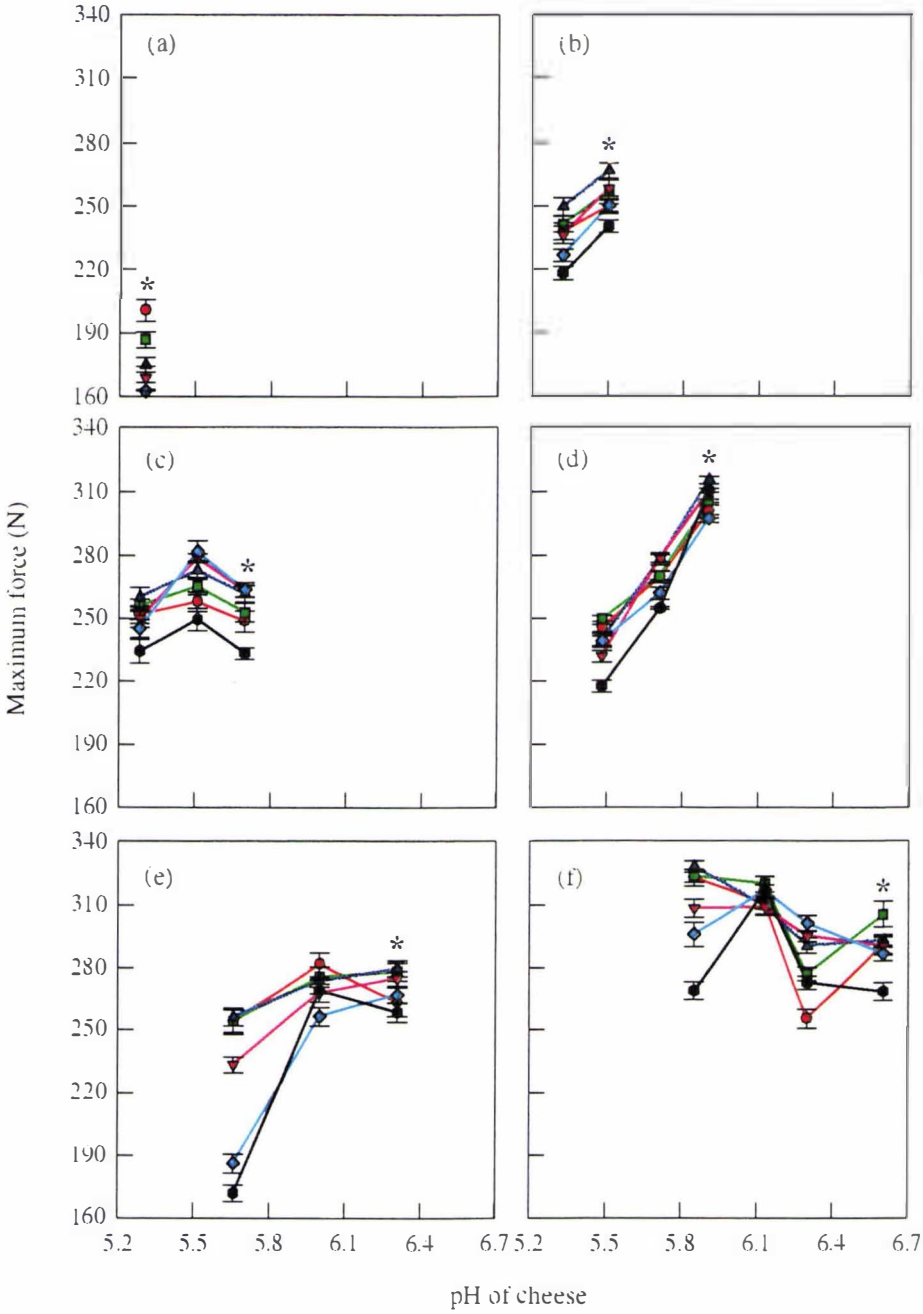

Fig. 5.8.5. *Effect of pH and time on the rheological properties of cheese curds made with different set pH values and also adjusted to lower pH values using GDL, as determined using the Instron.* Cheese curds were made with different set pH values, grated and adjusted to lower pH values using GDL - (a) CS6, (b) CS5, (c) CS4, (d) CS3, (e) CS2 and (f) CS1 - and stored at 2 °C. The initial pH value for each of the cheese curd systems is indicated with an asterisk. On each day of testing, the required quantity of grated cheese was equilibrated at 20 °C for 2 h in a water tight box prior to testing. The maximum force exerted to compress and extrude the cheese curd through the orifice of the extrusion attachment (Fig. 4.2.1) using the Instron was determined on different days after manufacture: ●, day 3; ■, day 5; ▲, day 7; ▼, day 9; ◇, day 11; and ●, day 13.

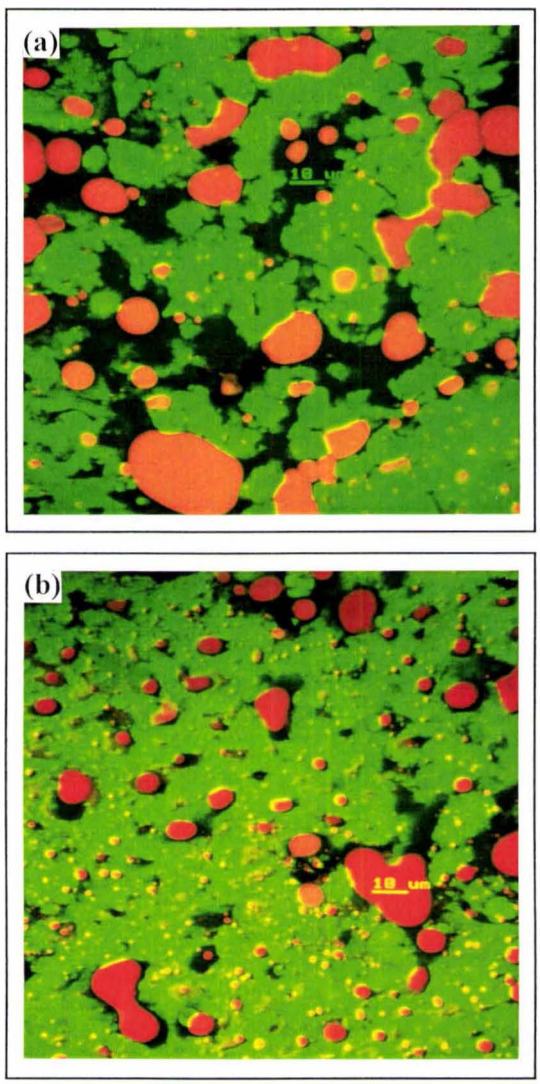

Fig. 5.8.6. *Changes in the distribution of fat and protein in cheese upon centrifugation.* Cheese curd made with a set pH of 6.60 and then adjusted to pH 6.13 using GDL was centrifuged at 11 086 *g* and 25 °C for 75 min. The protein and fat phases of the cheese curd before centrifugation (a) and the centrifugal pellet (b) were stained with Fast Green FCF and Nile Blue respectively and examined using a Leica TCS 4D confocal laser scanning microscope. The micrograph was obtained by merging the images of the protein (appearing green) and fat (appearing red) phases. The dark areas indicate the places where water may be present. Scale bar: 10 μm.

more compact after centrifugation, indicating that the forces of centrifugation drove the matrix closer together, eliminating some of the void spaces, and also drove the centrifugal serum out of the matrix.

The confocal micrographs of cheese curds made at different set pH values (Fig. 5.8.7) showed the continuous protein matrix (stained with Fast Green FCF). As in the earlier trial (Fig. 5.3.2), the structure of the cheese curds seemed to be influenced by the set pH. The fat globules appeared to be globular in cheese curds set at pH 6.30 (Fig. 5.8.7 (a)) whereas they appeared to be larger and irregularly shaped for cheese curd set at pH 5.50 (Fig.5.8.7 (b)). The fat in cheese curd set at pH 5.30 (Fig. 5.8.7 (c)) appeared to be elongated and less evenly dispersed.

The confocal micrographs of cheese curd set at pH 5.70 and those of cheese curds with pH values adjusted using GDL (CS4) are shown in Fig. 5.8.8. The microstructures of the cheese curds adjusted to lower pH values appeared to be similar to those of the cheese curds from which they were obtained. This suggested that the microstructure did not change significantly from that at the set pH if the pH of the cheese curd was altered subsequently.

Differences in the microstructures of a cheese curd from which a serum phase could be centrifuged out (cheese curd set at pH 6.30) and a cheese curd from which no serum was obtained (cheese curd set at pH 5.30) are shown in Fig. 5.8.9. The micrographs are combined images of the protein and fat phases. The micrograph of cheese curd set at pH 6.30 (Fig. 5.8.9 (a)) showed regions where neither fat nor protein was present, indicating a more open structure. The micrograph of cheese curd set at pH 5.30 (Fig. 5.8.9 (b)), from which no centrifugal serum was obtained, did not show the presence of areas in the structure where air or water may have been present.

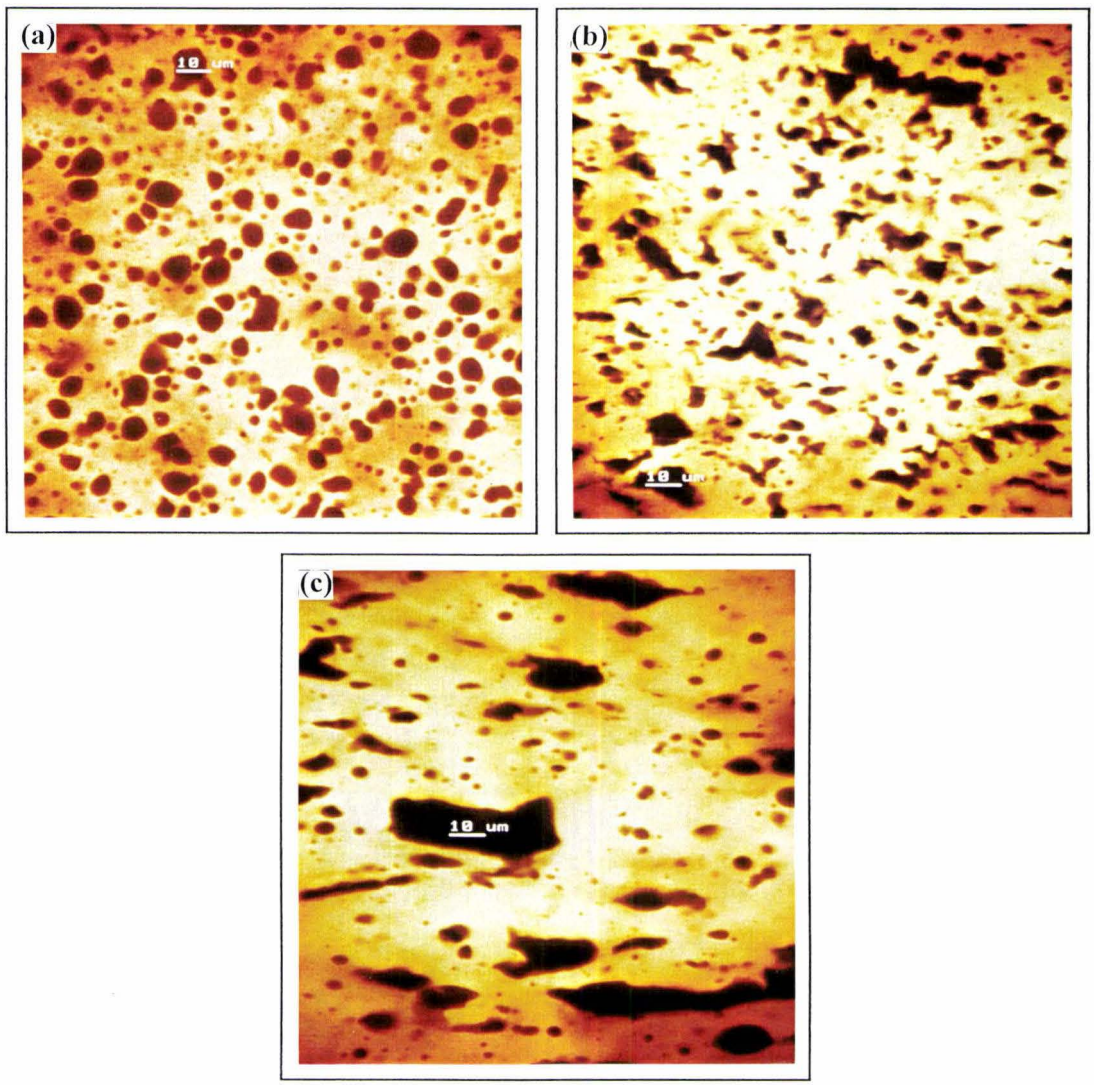

Fig. 5.8.7. *Distribution of protein in cheese as a function of the set pH.* Cheese curds were made from milk adjusted to different pH values at setting using dilute lactic acid: (a) 6.30, (b) 5.50 and (c) 5.30. The protein phase of the cheese curd was stained with Fast Green FCF and examined using a Leica TCS 4D confocal laser scanning microscope. Fluorescent areas indicate the protein structure. Scale bar: 10 μm.

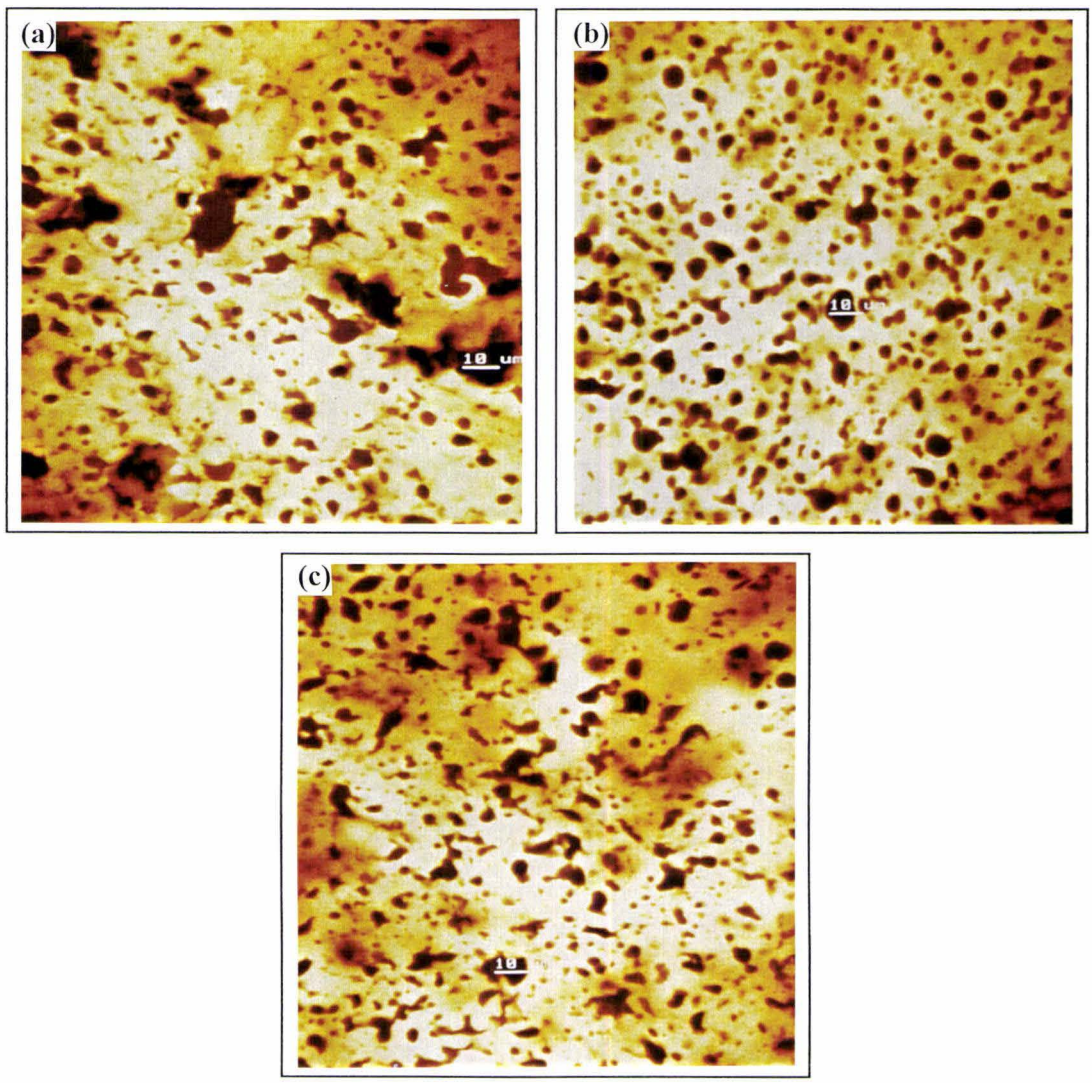

Fig. 5.8.8. *Distribution of protein in cheese as a function of the pH obtained with the addition of GDL.* Cheese curds were made with (a) set pH of 5.70 and then adjusted to (b) pH 5.51 and (c) pH 5.28 using GDL. The protein phase of the cheese curd was stained with Fast Green FCF and examined using a Leica TCS 4D confocal laser scanning microscope. Fluorescent areas indicate the protein structure. Scale bar: 10 μm.

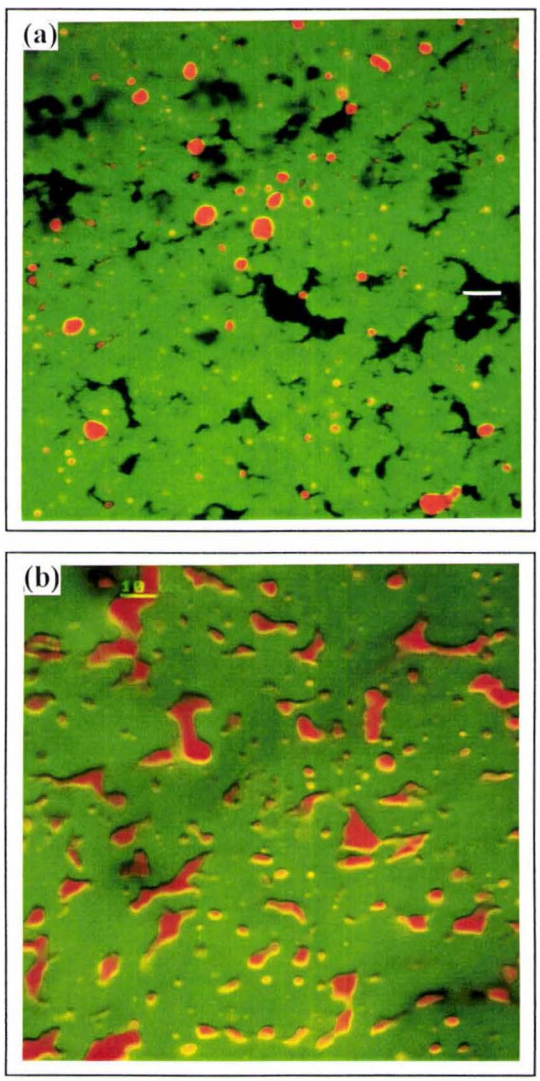

Fig. 5.8.9. *Distribution of fat and protein in cheese as a function of the set pH.* Cheese curds were made from milk adjusted to (a) pH 6.30 and (b) pH 5.30 at setting using dilute lactic acid. The protein and fat phases of the cheese curd were stained with Fast Green FCF and Nile Blue respectively and examined using a Leica TCS 4D confocal laser scanning microscope. The micrograph was obtained by merging the images of the protein (appearing green) and fat (appearing red) phases. The dark areas indicate the places where water may be present. Scale bar: 10 μm.

5.8.6 Conclusions

The quantity of centrifugal serum decreased with a decrease in the set pH of cheese curds between pH 6.30 and pH 5.30. The maximum quantity was obtained from cheese curd set at pH 6.30 whereas no serum could be centrifuged out from cheese curds set at pH 5.70 or lower. The quantity of centrifugal serum remained essentially constant with time during the initial period of the study for cheese curds of all set pH values where no subsequent lowering of pH with GDL was undertaken.

Lowering the pH of the cheese curds with the addition of GDL also affected the quantity of centrifugal serum, the effect being dependent on the initial pH of the cheese curds. The quantities of centrifugal serum obtained were higher for the samples with pH values between 6.30 and 5.85 prepared from cheese curd set at pH 6.60. However, the quantity of centrifugal serum decreased with a decrease in adjusted pH value of samples obtained from cheese curds set at pH 6.30 and 5.90. The quantity of serum also decreased with time for all the samples with adjusted pH values, the decrease being more rapid for samples with lower pH values.

The quantities of centrifugal serum were also different from samples of cheese curd set at a particular pH value and those adjusted to a similar pH value with GDL. For example, the quantity of centrifugal serum was much lower from cheese curd set at pH 5.90 than from cheese curd adjusted to a similar pH value from a set pH of 6.60. Also, although there was no centrifugal serum from cheese curd set at pH 5.50, centrifugal serum was obtained from cheese curd adjusted to a similar pH value from cheese curd set at pH 5.90. All these results supported the findings in the earlier trials.

The set pH of the cheese curds was also found to influence the maximum force and the greatest maximum force was observed for cheese curd set at pH 5.90. The values for maximum force of cheese curds with adjusted pH values using GDL were in the same range as the maximum force of cheese curds of a similar set pH value.

The microstructure of the cheese curds seemed to be determined by the pH at setting as changes in the structure of the cheese curd were apparent when cheese curds were made at different set pH values, but not when the pH was altered from a set pH value. The microstructure of the cheese curds from which a serum phase could be centrifuged out appeared to be less compact and had open spaces in the structure where water may have been present. Such areas were absent from the microstructures of the cheese curd samples from which no centrifugal serum was obtained.

5.9 STAINING AND OBSERVATION OF THE WATER PHASE IN CHEESE

5.9.1 Introduction

The sample preparation for most microscopy techniques involves one or more stages of dehydration; thus the water phase of the sample cannot be observed. In the confocal microscopy technique used earlier in these studies, the sample preparation did not involve dehydration. However, the places where water may have been present in cheese appeared dark in the micrographs as only the protein and fat phases were stained.

A technique to stain and observe the water phase in cheese was then developed. This involved binding of the water-soluble β-lactoglobulin to the dye tetramethylrhodamine-5-(and-6)-maleimide and adding the fluorescent dye-protein conjugate to the milk. The presence of the dye would cause the water phase to fluoresce, which in turn would enable the changes in the water distribution with time to be detected.

A small quantity of cheese curd was made from the milk containing the fluorescent dye-protein conjugate by the procedure described in Section 4.2.6.1.

5.9.2 Microscopic examination of cheese curd

The cheese curd was examined with a confocal laser scanning microscope according to the procedure detailed in Section 4.2.6. The micrographs in Fig. 5.9.1 show the continuous protein matrix (appearing dark brown) and the fat globules (appearing black), together with the brightly fluorescent region indicating the presence of water. Higher quantities of water appeared to be present along the curd boundary. Although the protein phase was not stained, the areas of fluorescence within the protein matrix could have been from the water that was associated with the casein.

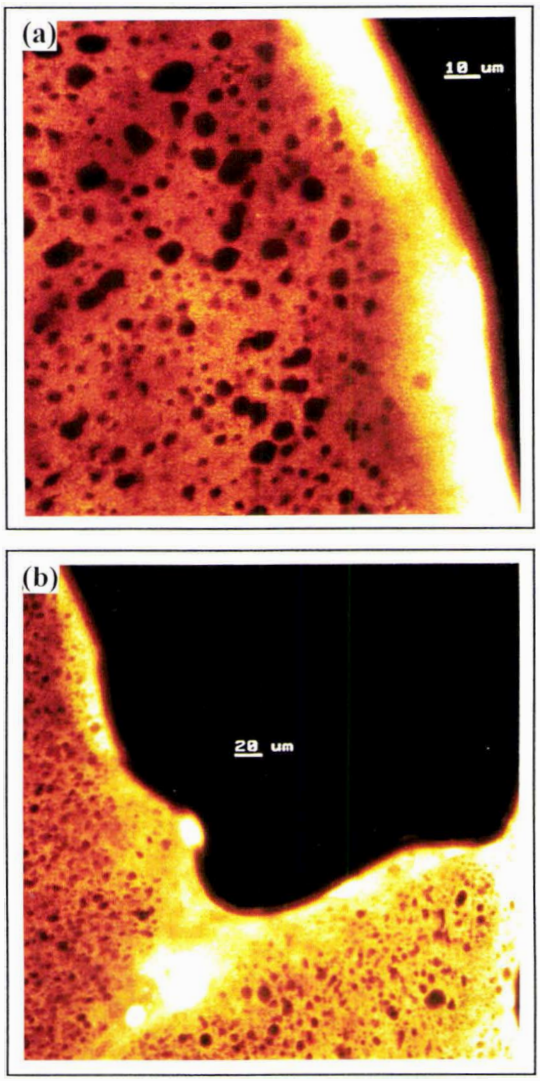

Fig. 5.9.1. *Distribution of water in cheese.* Cheese curd was made from milk to which a fluorescent dye-protein conjugate prepared from tetramethylrhodamine-5-(and-6)-maleimide and β-lactoglobulin in 1:1 molar ratio was added to stain the water phase in cheese. The sample was examined using a Leica TCS 4D confocal laser scanning microscope. The fluorescent areas indicate the presence of water. Scale bar: (a) 10 μm and (b) 20 μm.

Any changes in the size of the brightly fluorescent region with time would have indicated the changes in water distribution occurring in the cheese curd. As more of the water became associated with the casein matrix, as indicated by a reduction in the quantity of the centrifugal serum, there was likely to be a reduction in the size of the fluorescent region. This preliminary finding could be used in conjunction with other staining procedures to examine fat and water or protein and water phases in cheese. Further investigations are necessary to gain a proper understanding of these changes occurring during the early stages of cheese maturation.

5.9.3 Conclusions

The microscopy technique developed was successful in showing the water phase in cheese. A higher quantity of water was found to be present along the curd boundary. A reduction in the quantity of this water was expected with time because of increased water holding by the cheese curd. Further studies need to be carried out in conjunction with other staining techniques to confirm these observations.

6.0 DISCUSSION

6.1 INTRODUCTION

The composition of a cheese is largely determined by the manufacturing conditions. The process of cooking, agitation, pH reduction, salting and syneresis during cheesemaking determines the amount of water present in cheese; the pH at which the whey is drained from the curd determines the proportions of chymosin and plasmin in cheese. The amounts of these components in cheese in turn influence the changes that take place during ripening.

During cheesemaking, nearly 98% of the lactose is removed in the whey (Huffman & Kristoffersen, 1984) and the lactose content of the curd falls rapidly as the fermentation proceeds during manufacture. The cheese curd contains 0.8–1.5% lactose at the end of the cheesemaking process (Fox *et al.*, 1993). Under normal conditions, this residual lactose is metabolised quickly, mainly through the activity of the starter, resulting in a decrease in the pH of cheese. The cheese pH reaches a minimum in about 14 days after manufacture (Lawrence *et al.*, 1987).

Hydrolysis of a small fraction of α_{s1}-casein by the residual rennet has been reported to be contributing to the rapid changes in cheese texture during the first 1-2 weeks of ripening (Lawrence *et al.*, 1987). However, there is a less rapid change in the quantities of intact α_{s1}- and β-caseins in cheese during this period. The presence of nearly 85% intact α_{s1}-casein in 14-day-old cheese and about 95% intact β-casein after 10 weeks of ripening has been reported for Cheddar cheese (Lawrence *et al.*, 1987). Nevertheless, the textural changes during the initial stages of ripening have been ascribed to proteolysis.

Factors other than proteolysis also influence the rheological properties of cheese. During the manufacture of Cheddar cheese, the coagulum is cut into pieces, whey is drained and the curd pieces are pressed together as in cheddaring, milled and salted

before being pressed into hoops. The curd boundaries formed as a result of these operations, especially salting, may result in weaknesses along and around which the structure fails (Green *et al.*, 1985) resulting in different rheological effects.

The rate of acid development during manufacture also has a significant effect on the textural properties of cheese. The changes in pH are related directly to chemical changes in the protein network of the cheese curd. As the pH of cheese curd decreases, there is a concomitant loss of colloidal calcium phosphate from the casein submicelles and, below about pH 5.5, a progressive dissociation of the sub-micelles into smaller casein aggregates (Hall & Creamer, 1972; Roefs *et al.*, 1985).

Changes other than those resulting from proteolysis and pH changes also seem to take place in cheese during the early stages of ripening. Guo & Kindstedt (1995) reported dramatic increases in water-holding capacity of one type of Mozzarella cheese during the first 2 weeks of aging, as evidenced by steep decreases in the quantity of "expressible serum". Decreases in the quantity of expressible serum were accompanied by increases in the protein content, particularly the content of intact β-casein, in the serum.

The earlier studies (de Jong, 1978b; Creamer & Olson, 1982; Walstra & van Vliet, 1982; Lawrence *et al.*, 1987; Fox *et al.*, 1993, 1994) showed proteolysis and the changing pH to be primarily responsible for the major changes observed in cheese during ripening. It was of interest to determine whether the physical changes observed by Guo & Kindstedt (1995) for Mozzarella cheese also occurred in a Cheddar-like (dry salted, unstretched) cheese and to study the influence of these changes on the other attributes of cheese.

In order to do this, proteolysis in cheese during ripening had to be minimised so that the effects of other changes could be determined. This was achieved by eliminating the use of starters in cheesemaking. Although proteolysis due to the residual rennet and plasmin continued to occur, the results of PAGE showed that proteolysis in cheese was

reduced to a great extent in comparison with that in a normal cheese (Figs. 5.2.1 and 5.3.1). Cheesemaking without the use of starters also prevented fermentation and the continuing drop in pH during manufacture. In this study, cheese curds of required pH were obtained by adjusting the pH of the cheese milk to the desired value with dilute lactic acid. The pH remained essentially constant after the initial adjustment.

Changes in composition and water distribution, microstructure and some of the rheological attributes of cheese were studied using the various evaluation techniques. Initial studies on standard Cheddar cheese showed that serum was not readily centrifuged out of the cheese curd. It was necessary to increase the moisture content of the cheese so that changes in water-holding properties could be studied using centrifugation. Cheese was made from milk of a higher protein to fat ratio, at a lower cooking temperature and without pressing (Section 5.2.1) which resulted in the retention of more moisture in the cheese curd.

Preparation of the cheese curd without pressing led to difficulties in obtaining representative samples for rheological measurements. It was necessary to grate the cheese curd into small pieces (about 2 mm × 2 mm × 6 mm) in order to obtain a representative sample. As no standard method was available for the rheological testing of grated cheese curd, a method was developed and used in this study (Section 4.2.5.1.2).

Two model systems of cheesemaking with modifications to the standard Cheddar cheesemaking process were developed. The changes in pH over the period of manufacture for a normal Cheddar cheese and the cheese curds made by the two model systems are summarised in Fig. 6.1.1. As typical examples, cheese made by chemical acidification to a set pH of 6.30 (Fig. 6.1.1 (b)) and cheese made by chemical acidification to a set pH of 5.90 and then adjusted to different pH values using GDL (Fig. 6.1.1 (c–g)) are shown. The two model systems developed during the study differed from the standard Cheddar cheesemaking process to a certain extent, but the

modifications made to the process were necessary to have effective control over the several changes taking place in cheese during the initial period of ripening.

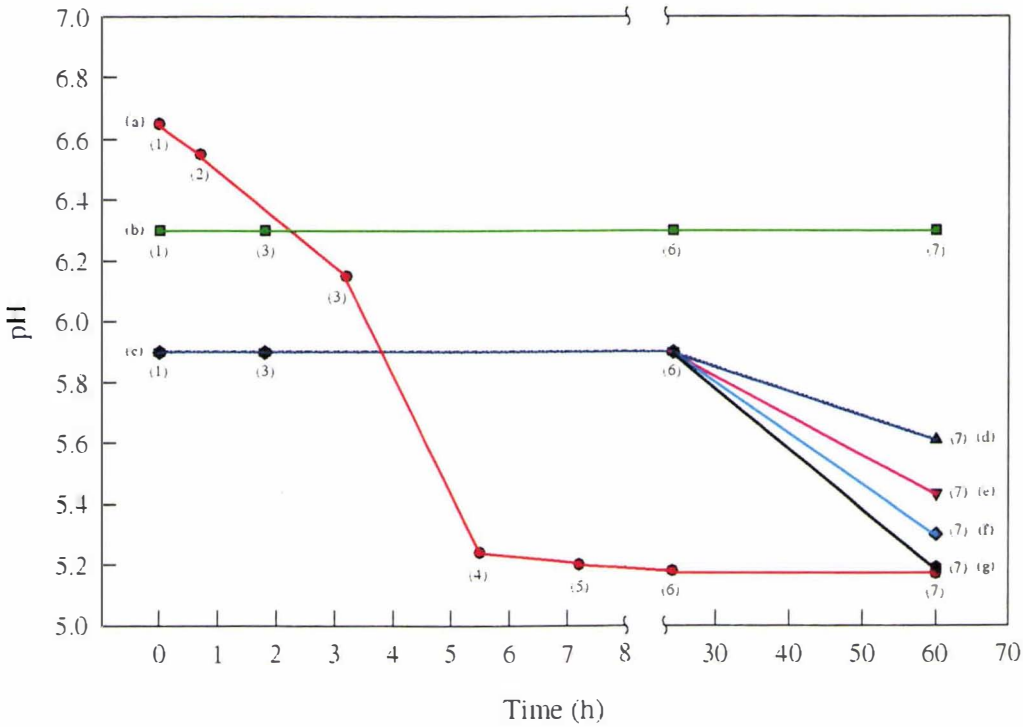

Fig. 6.1.1. *Comparison of the model systems of cheesemaking with the standard Cheddar cheesemaking process.* The pH values at different stages of manufacture – (1) setting; (2) cutting; (3) draining; (4) salting; (5) pressing; (6) day 1; and (7) day 3 – for cheese curds made by (a) the standard Cheddar cheesemaking process (●), (b) chemical acidification to pH 6.30 (■), and (c) chemical acidification to pH 5.90 and addition of GDL at different levels to achieve pH values of (d) 5.61 (▲), (e) 5.43 (▽), (f) 5.30 (◇) and (g) 5.19 (⬢) are shown to differentiate the three processes.

In one of the model systems, cheese curds were prepared at different set pH values such that the pH remained essentially constant during the 2-week period of study. This provided a means of determining the effect of pH on the changes in cheese curd characteristics. The quantity of centrifugal serum decreased with a decrease in the pH of the cheese curd. However, the quantity of centrifugal serum did not vary with time. The microstructure and the rheological properties were influenced by the pH of the cheese curds. The rheological properties also varied with time.

In the other model system developed, the pH of the cheese curd was reduced after manufacture with the addition of various amounts of GDL. The samples of cheese curd of different final pH values were analysed as before. The quantity of centrifugal serum was again less for cheese curds of lower pH values and the quantity also decreased with time for all samples. No appreciable changes in cheese microstructure were observed when the pH was lowered to different values from a particular set pH value. However, rheological properties were influenced by the adjusted pH value of the cheese curds, with the values for maximum force (Instron) of these samples attaining those of cheese curd of a similar set pH value.

The results for the samples of cheese curd where the pH was adjusted using GDL suggested that the cheese curds tended to attain a new equilibrium consistent with what would have been had they been made at this lower pH initially. The results of the study are discussed in the following sections in light of this hypothesis.

6.2 CHANGES IN THE QUANTITY OF CENTRIFUGAL SERUM

Several studies involving various cheeses have shown that, when cheese is compressed, an aqueous phase along with a small quantity of liquid fat can be obtained (Sandberg *et al.*, 193●; Monib, 1962; Morris *et al.*, 1988; Wilkinson *et al.*, 1994). Pressing as a method of separation of the aqueous phase is difficult to control and is not practical for the quantitative study of a large number of cheese samples. Guo & Kindstedt (1995) developed an easier, faster and more repeatable procedure, based on high speed centrifugation, that gave comparable results to the press method. The centrifugation method used in this study was based on the preliminary trials for this work and on the work of Guo & Kindstedt (1995).

The quantity of serum that could be centrifuged out of cheese curds was influenced by the pH of the curds. During the course of this study, cheese curds in the pH range from 5.10 to 6.60 were prepared and analysed for the quantity of centrifugal serum. The

behaviour of the cheese curds for this attribute differed depending on whether the pH remained as at setting or was obtained with the addition of GDL. For samples made with different set pH values in the range from 5.30 to 6.60, the quantity of serum increased with an increase in pH from 5.70 up to 6.30 and no centrifugal serum was obtained from cheese curds below pH 5.70 (Tables 5.3.2 and 5.3.3 and Figs. 5.5.1 and 5.8.1). However, when the pH of cheese curds was reduced in steps from pH 5.90 down to pH 5.19 using GDL, centrifugal serum continued to be obtained (Table 5.4.2 and Figs. 5.6.1 and 5.8.1).

Although a decrease in the quantity of serum with time was expected (Geurts *et al.* 1974a, 1980), very little variation in the quantity of centrifugal serum with time was observed from cheese curds of different set pH values (Tables 5.3.2, 5.3.3, 5.5.2, 5.5.3 and 5.5.4 and Fig. 5.8.1). However, the quantity of serum decreased for cheese curds with added GDL (Tables 5.4.2 and 5.6.2 and Fig. 5.8.1). The decrease in the quantity of centrifugal serum was more pronounced for samples with a bigger drop in pH. There was no release of serum from these samples of lower pH values after the first few days (Tables 5.4.2 and 5.6.2) as was observed from day 1 for samples of similar set pH values (Sections 5.3.3, 5.5.3 and 5.8.3). The water-holding property of cheese curds with added GDL thus appeared to be changing with time towards that observed for cheese curds of similar set pH values. Relatively higher quantities of centrifugal serum were obtained from these samples on the first few days after manufacture. However, the quantities decreased significantly with time and tended to reach a similar range of values to those observed for cheese curds made with set pH values similar to the pH values of these GDL-added samples.

Guo & Kindstedt (1995) also observed such a decrease in the quantity of centrifugal serum with time for low moisture Mozzarella cheese. During aging, it seems possible that the insoluble casein matrix absorbs water and forms a hydrated gel, accompanied by a progressive solubilisation of intact caseins, under the action of NaCl. Such swelling of the protein gel removes water from the vacuoles of the protein matrix and

could result in the observed decreases in the centrifugal serum during storage (Guo & Kindstedt, 1995).

In any system such as cheese curd, the various components will be distributed so that the system tends to attain a minimum energy (Ramkumar *et al.*, 1997). Once this state is attained, the system is considered to be in equilibrium and each region within the system will be indistinguishable from any other region. During the formation of the cheese curd from milk, there are a number of severe disruptions to the system, namely micelle destabilisation by rennet action, curd formation with consequent separation of a whey phase, pH decrease with a consequent redistribution of minerals between the casein-bound state in the micelle (and gel protein matrix) and the serum phase, further mineral changes with the admixture of solid salt with the curd and physical deformation of the cheese curd during processes such as cheddaring and stretching.

The initial milk can be considered to be an equilibrium system that adapts to the addition of salt and acid, or to temperature changes, reasonably quickly. However, once there are two phases, curd and whey, then attainment of a new equilibrium state requires movement of material between the phases as well as within each phase. For example, addition of salt to moist curd may not affect the inner regions of the curd particles for some time because of the diffusion time (Guinee & Fox, 1993). Indications of the final equilibrium positions can be obtained using model systems of finely divided curd material. For example, van Hooydonk *et al.* (1986) reported that the voluminosity of casein in normal skim milk was at a maximum around pH 5.3 and that it decreased after renneting, and Snoeren *et al.* (1984) observed a relationship between the hydration of casein micelles and pH, with a "peak" in the voluminosity of the casein micelles at pH 5.45. In a similar study on rennet-treated milk, Creamer (1985) found the peak to be near pH 5.2. Creamer (1985) also noted that addition of NaCl increased the water retention by the chymosin-treated micelles (curd) whereas $CaCl_2$ decreased it. At higher NaCl concentrations, large quantities of water were retained by the renneted micelles at pH 5.2-5.3. In all these studies, the gel or curd was formed under the final environmental conditions and thus the curd characteristics were more likely to represent

the equilibrium condition that curd might attain when placed in an appropriate environment. The rate of attainment of equilibrium is limited by rates of diffusion of various components within the system and this is much slower in a cheese system than in a renneted micelle model system. The earlier study (Creamer, 1985) indicated that water retention by chymosin-treated casein micelles decreased slightly with decreasing pH in the range from 6.7 to 5.6 but increased as the pH decreased from 5.6 to 5.25 and that water retention increased markedly when the salt concentration was increased.

The influence of pH on the quantity of water retained by the cheese curd was also evident in the present study. Centrifugal serum was obtained from cheese curds made with pH values in the range 5.75-6.60 (Figs. 5.5.1 and 5.8.1). However, for cheese curds made with a set pH value in the range 5.30-5.70 (Section 5.5.3), no centrifugal serum was readily obtained, suggesting an increased water holding by the cheese curds in this pH range.

The quantity of centrifugal serum also decreased with time when the pH of cheese curds was adjusted to values in the range 5.20-5.70 using GDL from a higher set pH (Tables 5.4.2 and 5.6.2). The quantity of centrifugal serum decreased at a faster rate for cheese curds of lower pH values. The water holding properties of the cheese curds thus tended to move towards the properties of cheese curds of lower pH values over the period of time. This is likely to be a result of the changes with time taking place in the equilibrium conditions of the cheese curd systems consistent with those for cheese curds of similar set pH values.

6.3 CHANGES IN RHEOLOGICAL PROPERTIES OF CHEESE CURDS

When milk coagulates, the casein micelles aggregate into chains that eventually all link together into a mesh-like structure that encompasses the fat globules. At the time the curd is cut, there is an open network of paracasein micelles linked together into chains and clusters of individual micelles varying in size. As whey is expelled after the curd

is cut, the mesh-like structure shrinks around the fat globules. The protein network becomes more compact and the micelles fuse together with many of the chains forming into thicker strands, with the curd becoming more firm. The presence of fat in the structure modifies and limits the extent of deformation, adding rigidity to the structure. At the same time, the water acts as a low viscosity lubricant between fat and casein. Provided there is sufficient quantity of it, the water occupies all the space between the fat and the casein strands. It is the combination of all these effects that gives rise to the rheological properties of the final cheese (McMahon *et al.*, 1993).

Lawrence *et al.* (1987) observed that the texture of a cheese is determined primarily by its pH and the ratio of intact casein to moisture. The influence of the pH of cheese curds on rheological properties such as stiffness was also evident in the present study. Large strain deformation studies of cheese curds in the pH range 5.30-6.60 showed that the maximum force (Instron) increased up to a pH of 5.90 (Figs. 5.5.2 and 5.8.4) and then decreased for higher pH values. A maximum in the value of G′ was also observed for cheese curds set at pH 5.90 (Fig. 5.5.3). The samples of cheese curd also showed an increased solid-like behaviour as the set pH increased from 5.45 to 5.90, as evidenced by lower values for δ at higher pH values (Fig. 5.5.3).

Chemical changes in the protein network of the cheese curd result from changes in the pH (Lawrence *et al.*, 1987). As the pH of cheese curd decreases, there is a concomitant loss of colloidal calcium phosphate from the casein submicelles and, below about pH 5.5, a progressive dissociation of the submicelles into smaller casein aggregates (Hall & Creamer, 1972; de Jong, 1978; Roefs *et al.*, 1985). These changes in the protein network contribute to changes in the rheological properties.

Cheddar cheese has a texture that is intermediate between those of the relatively high pH cheeses, which flow readily when a force is applied, and the low pH cheeses, which tend to deform only at their yield point (Lawrence *et al.*, 1993). SEM has established that cheese consists of a continuous protein matrix but that this matrix is clearly different in the various cheese types (Hall & Creamer, 1972). The structural units in the

protein matrix of Gouda cheese are essentially in the same globular form (10-15 nm in diameter) as in the original milk. In contrast, the protein aggregates in Cheshire cheese are much smaller (3-4 nm) and are apparently in the form of strands or chains, *i.e.* the original submicellar protein aggregates appear to have lost almost all their identity (Lawrence *et al.*, 1993). Cheddar cheese is intermediate between Gouda and Cheshire cheeses, *i.e.* much of the protein in Cheddar cheese is in the form of smaller particles than in Gouda cheese. As the pH decreases towards that of the isoelectric point of paracasein, the protein assumes an increasingly more compact conformation and the cheese becomes shorter in texture and fractures at a small deformation (Creamer & Olson, 1982; Walstra & van Vliet, 1982).

Creamer *et al.* (1988) found that high pH or low calcium cheese was more elastic whereas low pH or high calcium cheese was more brittle than the control. A high pH cheese was more pliant and rubbery (as shown by greater compression before fracturing) irrespective of its calcium content whereas a low pH cheese was firmer but crumbled at low deformations.

Lawrence *et al.* (1987) also reported that the texture of cheese changes markedly in the first 1-2 weeks of ripening as the hydrolysis of a small fraction of α_{s1}-casein by rennet to the peptide α_{s1}-I results in a general weakening of the casein network. Such changes in rheological behaviour were also observed for the samples of cheese studied. Both large strain (Figs. 5.5.2, 5.6.2, 5.8.4 and 5.8.5) and small strain (Figs. 5.5.3 and 5.6.3) deformation studies showed changes in the rheological properties of the cheese curds during the 2-week period of study. However, no definite trends in the results with time could be discerned as a consequence of the modifications to the cheesemaking process (Section 5.2.1) and constraints of the test procedure, especially in the case of the small strain deformation studies. For these studies, the cheese curd had to be cut into fairly large sized pieces (about 30 mm × 30 mm × 30 mm) before salting so that samples could be obtained for testing using the Bohlin rheometer. This may have resulted in uneven distribution of salt and contributed to variations in composition between the curd particles.

Proteolysis during ripening also contributes to textural changes in cheese. The rate of proteolysis is controlled largely by the proportion of residual rennet and plasmin in cheese, the salt to moisture ratio and the storage temperature (Lawrence *et al.*, 1987). A good correlation between cheese firmness and the quantity of intact α_{s1}-casein was reported by de Jong (1976). Hydrolysis of α_{s1}-casein to the peptide α_{s1}-I during the first 7-14 days results in converting the rubbery texture of young cheese into a smoother, more homogeneous product (Lawrence *et al.*, 1987). Further hydrolysis during the subsequent days causes a more gradual change in cheese texture.

The experimental cheese curds prepared during this study underwent minimal proteolysis because of the modifications made in the cheesemaking process (Section 4.2.1). Analysis of centrifugal pellet and centrifugal serum from cheese curds of different set pH values by alkaline urea-PAGE (Fig. 5.3.1) showed much less hydrolysis of the α_{s1}- and β-caseins when compared with that in a normal cheese (Fig. 5.2.1). Similar results for proteolysis were also observed for cheese curds adjusted to lower pH values using GDL (Figs. 5.4.1 and 5.4.2). Although there was no appreciable change with time for the extent of proteolysis in the experimental cheese curds, changes in the rheological characteristics of these samples were observed during the 2-week period of study. These differences in rheological properties must, therefore, be related to structural changes.

Large strain deformation testing showed that rheological properties of cheese curds adjusted to lower pH values using GDL tended towards the rheological properties of the cheese curds made with set pH values in a similar range (Figs. 5.8.4 and 5.8.5). The presence of higher calcium contents in these samples, because of the higher set pH values, may have prevented them from attaining the textural properties of the cheese curds set at these lower pH values.

6.4 CHANGES IN CHEESE MICROSTRUCTURE

Microstructure is a major determinant of cheese texture and consistency (Stanley & Emmons, 1977; Emmons *et al.*, 1980a; Green & Manning, 1982; Green *et al.*, 1986). Different cheese varieties represent variations (of either ingredients or make conditions or both) of a common manufacturing scheme and, by varying processing conditions, composition and structure within a particular cheese type can be manipulated (Kiely *et al.*, 1992).

Because of a pH- and temperature-dependent dynamic equilibrium between micellar and serum casein and ions (calcium and phosphate in particular), the extent of cheese milk acidification is pivotal in determining mineral (*e.g.* calcium and phosphate) retention in rennet curd as well as retention of lactose and proteases (coagulant and plasmin) (Lawrence *et al.*, 1984). The relative proportions of these constituents together with moisture and fat determine the basic cheese structure.

In the present study using confocal laser scanning microscopy, the microstructure was found to be affected by the pH at setting (Figs. 5.3.2 and 5.8.7). Because of the direct acidification method of cheesemaking used, the pH at draining was the same as the pH at setting. Significant differences in structure were observed with variations in the pH of the cheese curds. The fat globules appeared globular in cheese curds of higher pH values whereas they appeared elongated and less evenly dispersed in cheese curds made with lower pH values (Figs. 5.3.2 and 5.8.7). Differences in the quantities of mineral retained (Fig. 5.8.3) and the possible differences in the distribution of caseins resulting from the differences in pH may have resulted in the changes in microstructure observed.

Kindstedt (1993) studied low moisture part skim Mozzarella cheeses made at different draining pH values of 6.40, 6.15 and 5.90. Draining at the lowest whey pH resulted in significantly lower ratios of calcium to total protein in cheese curd at all stages of manufacture and in the final cheese. It was also found that the curd drained at whey pH 6.40 displayed a three-dimensional network of discrete, partially fused paracasein

particles, whereas at a drain pH of 5.90 the curd matrix appeared as an almost completely fused amorphous mass, presumably due in part to greater dissolution of colloidal calcium phosphate from the paracasein matrix at the lower pH. Essentially similar differences in the structure with pH were also observed in this study even for the much wider pH range studied (Figs. 5.3.2 and 5.8.7).

No appreciable differences were observed in the structure of cheese curds adjusted to different pH values from a particular set pH (Figs. 5.4.3 and 5.8.8). Changes in microstructure also are expected to be occurring because of the differences observed in the water distribution and the rheological characteristics over the period of time. It is possible that the changes in structure were too subtle and may not have been detected by the confocal microscopy technique used. The use of other microscopy techniques with a greater resolution are likely to show the differences in structure.

A confocal microscopy technique was developed to observe the water phase in cheese (Section 4.2.6.1). Use of the water soluble dye-protein conjugate in cheese milk provided a means of staining the water in cheese for observation using a microscope. The technique was successful in showing the water in the casein matrix as a distinctly separate phase (Fig. 5.9.1) that could be evaluated for changes with pH and with time. Further studies in conjunction with other staining techniques are necessary to gain a greater understanding of the changes in water and fat or water and protein phases during the early stages of cheese ripening.

6.5 GENERAL DISCUSSION

Literature reports on the changes taking place in cheese during the early stages of ripening (first 2 weeks after manufacture) are rather limited. Several major changes take place in cheese during this period as a result of redistribution of water and minerals, decrease in pH due to the activity of microorganisms, and hydrolysis of α_{s1}-casein by

the residual rennet. Simultaneous occurrence of all these changes also makes determination of the importance of any one aspect extremely difficult.

The development of model systems of cheesemaking in this study provided an effective means of exercising control of some of these changes so that other changes could be measured. Use of direct acidification was essential to prevent changes in cheese pH during storage and to control the changes resulting from the variations in cheese pH. The changes in water distribution and composition, microstructure and some of the rheological attributes with pH and with time were studied.

That significant redistribution of water takes place in cheese curd during early ripening was evident from the variations in the quantity of serum that could be centrifuged out (*e.g.* Fig. 5.8.1). The pH of some of the experimental cheese curds was reduced with the addition of GDL to simulate the changes in pH in a normal cheese. The quantity of centrifugal serum decreased with time for the cheese curds in which there was a decrease in pH after manufacture (Tables 5.4.2 and 5.6.2 and Fig. 5.8.1). A similar redistribution of water probably takes place in the early stages of ripening of a normal cheese. The decrease in the quantity of centrifugal serum may have been a result of the swelling of the insoluble casein matrix at the microstructural level accompanied by a progressive solubilisation of intact caseins under the action of NaCl (Guo & Kindstedt, 1995).

A decrease in the set pH resulted in a lower calcium content of the cheese curds which in turn contributed to variations in their structure and rheological properties. The microstructure of the cheese curds varied appreciably with the pH at setting (Fig. 5.3.2). The changes that are likely to be occurring in the microstructure of cheese curds adjusted to different pH values using GDL (Fig. 5.4.3) could not be detected by the confocal microscopy technique used. Other microscopy techniques, such as TEM, are likely to detect the subtle changes that may be occurring.

The set pH of the cheese curds influenced the rheological properties studied (Figs. 5.5.2 and 5.5.3) with the cheese curd made with a set pH of 5.90 showing the maximum curd stiffness. The rheological properties were also affected when the pH was altered after manufacture with the addition of GDL. The samples of cheese curds adjusted to lower pH values tended to have the rheological characteristics of cheese curds set at these lower pH values (Figs. 5.8.4 and 5.8.5). This suggested that cheese curds tended to attain a new equilibrium consistent with that of cheese curds of a lower pH value. The attainment of a new equilibrium was possibly limited by the presence of a higher calcium content than that present in cheese curds of a lower set pH value.

Proteolysis occurring in the cheese curd and the changing pH were said to be responsible for many of the changes observed during the period of ripening (Lawrence *et al.*, 1987). The present study showed variations in the quantity of centrifugal serum, microstructure and some of the rheological properties in cheese curds even when proteolysis was at a minimum and when the pH essentially remained constant. This suggests that factors other than proteolysis, such as redistribution of water during ripening, also contribute to the changes in the properties of the cheese curd.

6.6 CONCLUSIONS

The changes in curd properties early in the ripening process in a normal commercial Cheddar cheese appear to be a consequence of the changing pH and continuing proteolysis superimposed on the effect of the re-absorption of centrifugal serum into the casein matrix.

Earlier studies had shown proteolysis to be mainly responsible for the changes in rheological properties of cheese observed during the early stages of ripening and that the changes in pH would also result in chemical changes in the protein network of the cheese curd. Guo & Kindstedt (1995) suggested that these chemical changes and the proteolysis were responsible for variations in the quantity of centrifugal serum.

However, the present study showed variations in the water-holding capacity of the cheese curd even when the proteolysis tended to be minimal. This suggests that the physical changes in cheese curd, such as redistribution of water, are also important during the initial stages of ripening and may contribute to the differences in the rheological properties, independent of proteolysis, as observed during this study.

6.7 SCOPE FOR FUTURE WORK

The present study successfully showed the importance of some of the physical changes that occur in the cheese curd during the initial period of ripening in determining the properties of cheese curd. More work to support these findings would help in a better understanding of the processes and provide means by which cheeses of more desirable qualities could be manufactured.

Some of the areas where further work could be done include the following.

(1) Studies to determine the causes for the minimum variation observed in the quantity of centrifugal serum with time from cheese curds made with different set pH values.

(2) Studies on the changes with time in the water phase of the cheese curd as determined by the confocal microscopy technique that was developed. This is likely to provide visual evidence for the changes in the quantity of centrifugal serum observed.

(3) Use of other microscopy techniques (SEM or TEM) to observe the subtle changes in structure that were not detected using confocal microscopy.

(4) Rheological testing of samples of smaller dimensions using Bohlin rheometer in order to avoid large variations in the results.

(5) Similar studies on other varieties of cheese with distinctly different manufacturing processes.

(6) Studies on the influence of an increase in the pH after manufacture of a cheese curd made with a lower set pH using suitable chemical agents (as opposed to the lowering of pH using GDL that was carried out in this study).

7.0 REFERENCES

AIMUTIS, W. R. & EIGEL, W. N. 1982 Identification of λ-casein as plasmin-derived fragments of bovine α_{s1}-casein. *Journal of Dairy Science* **65** 175-181

AL-DAHHAN, A. H. & CRAWFORD, R. J. M. 1982 Some aspects of seam formation in Cheddar cheese. In *Proceedings of the XXI International Dairy Congress, Moscow.* Vol. 1. Book 1. Mir Publishers, Moscow, Russia. p. 389

ANDREWS, A. T. 1983 *Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications.* Clarendon Press, Oxford, England.

ANDREWS, A. T. & ALICHANIDIS, E. 1983 Proteolysis of caseins and the proteose-peptone fraction of bovine milk. *Journal of Dairy Research* **50** 275-290

BEEBY, R. 1959 A method for following the syneresis of the rennet coagulum in milk. *Australian Journal of Dairy Technology* **14** 77-79

BERRIDGE, N. J. & SCURLOCK, P. G. 1970 The influence of various treatments on the drainage of continuously made curd. *Journal of Dairy Research* **37** 417-429

BERRY, G. P. & CREAMER, L. K. 1975 The association of bovine β-casein. The importance of the C-terminal region. *Biochemistry* **14** 3542-5345

BIRKKJAER, H. & JØHNK, P. 1985 Technological suitability of calf rennet substitutes. *Bulletin of the International Dairy Federation* No. 194 pp. 8-10

BOZZOLA, J. J. & RUSSELL, L. D. 1992 *Electron Microscopy - Principles and Techniques for Biologists.* Jones & Bartlett Publishers, Boston, Massachusetts, USA.

BREENE, W. M., PRICE, W. V. & ERNSTROM, C. A. 1964a Changes in composition of Cheddar curd during manufacture as a guide to cheese making by direct acidification. *Journal of Dairy Science* **47** 840-848

BREENE, W. M., PRICE, W. V. & ERNSTROM, C. A. 1964b Manufacture of pizza cheese without starter. *Journal of Dairy Science* **47** 1173-1180

BREENE, W. M., OLSON, N. F. & PRICE, W. V. 1965 Salt absorption by Cheddar cheese curd. *Journal of Dairy Science* **48** 621-624

BROOKER, B. E. 1979 Milk and milk products. In *Food Microscopy*. (Ed. J. G. Vaughan) Academic Press, London, England. pp. 291-311

BROOKER, B. E. 1995 Imaging food systems by confocal laser scanning microscopy. In *New Physico-chemical Techniques for the Characterization of Complex Food Systems.* (Ed. E. Dickinson) Blackie Academic & Professional, London, England. pp. 53-68

BROOKER, B. E., HOBBS, D. G. & TURVEY, A. 1975 Observations on the microscopic crystalline inclusions in Cheddar cheese. *Journal of Dairy Research* **42** 341-348

BUCHHEIM, W. 1970 [The process of fat crystallization in the fat globules of milk. Electron microscopic studies by means of the freeze etching method]. *Milchwissenschaft* **25** 65-70

CARLES, C. & RIBADEAU-DUMAS, B. 1984 Kinetics of action of chymosin (rennin) on some peptide bonds of bovine β-casein. *Biochemistry* **23** 6839-6843

CARLSON, A., HILL, G. C. & OLSON, N. F. 1986 The coagulation of milk with immobilized enzymes: a critical review. *Enzyme and Microbial Technology* **8** 642-650

CHAPLIN, B. & GREEN, M. L. 1980 Determination of the proportion of κ-casein hydrolysed by rennet on coagulation of skim-milk. *Journal of Dairy Research* **47** 351-358

CHOU, D. H. & MORR, C. V. 1979 Protein-water interactions and functional properties. *Journal of the American Oil Chemists Society* **56** 53A-62A

CREAMER, L. K. 1970 Protein breakdown in Gouda cheese. *New Zealand Journal of Dairy Science and Technology* **5** 152-154

CREAMER, L. K. 1971 Beta-casein hydrolysis in Cheddar cheese ripening. *New Zealand Journal of Dairy Science and Technology* **6** 91

CREAMER, L. K. 1976a Casein proteolysis in Mozzarella type cheese. *New Zealand Journal of Dairy Science and Technology* **11** 130-131

CREAMER, L. K. 1976b A further study of the action of rennin on β-casein. *New Zealand Journal of Dairy Science and Technology* **11** 30-39

CREAMER, L. K. 1979 Degradation of casein components during cheese maturation. In *Proceedings of the Fifth International Congress of Food Science and Technology.* (Eds. H. Chiba, M. Fujimaki, K. Iwai, H. Mitsuda & Y. Morita) Elsevier Publishing Company, New York, USA. pp. 340-346

CREAMER, L. K. 1985 Water absorption by renneted casein micelles. *Milchwissenschaft* **40** 589-591

CREAMER, L. K. 1991 Electrophoresis of cheese. *Bulletin of the International Dairy Federation* No. 261 pp. 14-28

CREAMER, L. K. & OLSON, N. F. 1982 Rheological evaluation of maturing Cheddar cheese. *Journal of Food Science* **47** 631-636, 646

CREAMER, L. K. & RICHARDSON, B. C. 1974 Identification of the primary degradation product of α_{s1}-casein in Cheddar cheese. *New Zealand Journal of Dairy Science and Technology* **9** 9-13

CREAMER, L. K., ZOERB, H. F., OLSON, N. F. & RICHARDSON, T. 1982 Surface hydrophobicity of α_{s1}-I, α_{s1}-casein A and B and its implications in cheese structure. *Journal of Dairy Science* **65** 902-906

CREAMER, L. K., LAWRENCE, R. C. & GILLES, J. 1985 Effect of acidification of cheese milk on the resultant Cheddar cheese. *New Zealand Journal of Dairy Science and Technology* **20** 185-203

CREAMER, L. K., GILLES, J. & LAWRENCE, R. C. 1988 Effect of pH on the texture of Cheddar and Colby cheese. *New Zealand Journal of Dairy Science and Technology* **23** 23-35

CZULAK, J. 1959 Some notes on mechanical cheddaring of cheese curd. In *Proceedings of the XV International Dairy Congress, London.* Vol. 2. Richard Clay & Company Ltd, Bungay, Suffolk, England. pp. 829-831

CZULAK, J. 1963 Control of seaminess and of whey drainage in cheese made with Bell-Siro Cheesemaker 3. *Australian Journal of Dairy Technology* **18** 192-194

DALGLEISH, D. G. 1979 Proteolysis and aggregation of casein micelles treated with immobilized or soluble chymosin. *Journal of Dairy Research* **46** 653-661

DALGLEISH, D. G. 1993 The enzymatic coagulation of milk. In *Cheese: Chemistry, Physics and Microbiology. Vol. 1. General Aspects.* (Ed. P. F. Fox) Chapman & Hall, London, England. pp. 69-100

DEAN, M. R., BERRIDGE, N. J. & MABBITT, L. A. 1959 Microscopical observations on Cheddar cheese and curd. *Journal of Dairy Research* **26** 77-82

de JONG, L. 1976 Protein breakdown in soft cheese and its relation to consistency. 1. Proteolysis and consistency of 'Noordhollandse Meshanger' cheese. *Netherlands Milk and Dairy Journal* **30** 242-253

de JONG, L. 1978a The influence of the moisture content on the consistency and protein breakdown of cheese. *Netherlands Milk and Dairy Journal* **32** 1-14

de JONG, L. 1978b Protein breakdown in soft cheese and its relation to consistency. 3. The micellar structure of Meshanger cheese. *Netherlands Milk and Dairy Journal* **32** 15-25

de WIT, J. N. 1984 Functional properties of whey proteins in food systems. *Netherlands Milk and Dairy Journal* **38** 71-89

di MATTEO, M., CHIOVITTI, G. & ADDEO, F. 1982 [Variation in the composition of Mozzarella cheese during storage]. *Scienza e Tecnica Lattiero-Casearia* **33** 197-213

DULLEY, J. R. 1972 Bovine milk protease. *Journal of Dairy Research* **39** 1-9

DZIEZAK, J. D. 1988 Microscopy and image analysis for R&D. *Food Technology* **42**(7) 109-124

EDWARDS Jr., J. L. & KOSIKOWSKI, F. V. 1969 Electrophoretic proteolytic patterns in Cheddar cheese by rennet and fungal rennets: their significance to international classification of cheese varieties. *Journal of Dairy Science* **52** 1675-1678

EIGEL, W. N. 1977 Effect of bovine plasmin on α_{s1}-B and κ-A caseins. *Journal of Dairy Science* **60** 1399-1403

EIGEL, W. N., BUTLER, J. E., ERNSTROM, C. A., FARRELL Jr., H. M., HARWALKAR, V. R., JENNESS, R. & WHITNEY, R. McL. 1984 Nomenclature of proteins of cow's milk: fifth revision. *Journal of Dairy Science* **67** 1599-1631

EMMONS, D. B., PRICE, W. V. & SWANSON, A. M. 1959 Tests to measure syneresis and firmness of Cottage cheese coagulum, and their application in the curd making process. *Journal of Dairy Science* **42** 866-869

EMMONS, D. B., LISTER, E. E., BECKETT, D. C. & JENKINS, K. J. 1980a Quality of protein in milk replacers for young calves. V. Effect of method of dispersing fat on curd formation and whey syneresis. *Journal of Dairy Science* **63** 417-425

EMMONS, D. B., KALAB, M., LARMOND, E. & LOWRIE, R. J. 1980b Milk gel structure. X. Texture and microstructure in Cheddar cheese made from whole milk and from homogenized low-fat milk. *Journal of Texture Studies* **11** 15-34

EVANS, R. J. 1973 Microscopy (chemistry). In *Encyclopaedia of Microscopy and Micro Techniques.* (Ed. P. Gray) Van Nostrand Reinhold Co., New York, USA. p. 342

EXTERKATE, F. A. & ALTING, A. C. 1995 The role of starter peptidases in the initial proteolytic events leading to amino acids in Gouda cheese. *International Dairy Journal* **5** 15-28

FARKYE, N. & FOX, P. F. 1990 Observations on plasmin activity in cheese. *Journal of Dairy Research* **57** 413-418

FARKYE, N. Y. & FOX, P. F. 1992 Contribution of plasmin to Cheddar cheese ripening: effect of added plasmin. *Journal of Dairy Research* **59** 209-216

FARKYE, N. Y., KIELY, L. J., ALLSHOUSE, R. D. & KINDSTEDT, P. S. 1991 Proteolysis in Mozzarella cheese during refrigerated storage. *Journal of Dairy Science* **74** 1433-1438

FEDRICK, I. A. & DULLEY, J. R. 1984 The effect of elevated storage temperatures on the rheology of Cheddar cheese. *New Zealand Journal of Dairy Science and Technology* **19** 141-150

FERNANDEZ, A. & KOSIKOWSKI, F. V. 1986 Physical properties of direct acidified Mozzarella cheese from ultrafiltered whole milk retentates. *Journal of Dairy Science* **69** 643-648

FOX, P. F. 1970 Influence of aggregation on the susceptibility of casein to proteolysis. *Journal of Dairy Research* **37** 173-180

FOX, P. F. 1985 Proteolysis and protein-protein interactions in cheese manufacture. In *Developments in Food Proteins.* Vol. 3. (Ed. B. J. F. Hudson) Elsevier Applied Science Publishers, London, England. pp. 69-112

FOX, P. F. 1987 Cheese manufacture: chemical, biochemical and physical aspects. *Dairy Industries International* **52**(7) 11-13

FOX, P. F. 1989 Proteolysis during cheese manufacture and ripening. *Journal of Dairy Science* **72** 1379-1400

FOX, P. F. & WALLEY, B. F. 1971 Influence of sodium chloride on the proteolysis of casein by rennet and by pepsin. *Journal of Dairy Research* **38** 165-170

FOX, P. F., LAW, J., McSWEENEY, P. L. H. & WALLACE, J. 1993 Biochemistry of cheese ripening. In *Cheese: Chemistry, Physics and Microbiology. Vol. 1. General Aspects.* (Ed. P. F. Fox) Chapman & Hall, London, England. pp. 389-438

FOX, P. F., SINGH, T. K. & McSWEENEY, P. L. H. 1994 Proteolysis in cheese during ripening. In *Biochemistry of Milk Products.* (Eds. A. T. Andrews & J. Varley) The Royal Society of Chemistry, Cambridge, England. pp. 1-31

GEURTS, T. J., WALSTRA, P. & MULDER, H. 1972 Brine composition and the prevention of the defect 'soft rind' in cheese. *Netherlands Milk and Dairy Journal* **26** 168-179

GEURTS, T. J., WALSTRA, P. & MULDER, H. 1974a Transport of salt and water during salting of cheese. 1. Analysis of the processes involved. *Netherlands Milk and Dairy Journal* **28** 102-129

GEURTS, T. J., WALSTRA, P. & MULDER, H. 1974b Water binding to milk protein, with particular reference to cheese. *Netherlands Milk and Dairy Journal* **28** 46-72

GEURTS, T. J., WALSTRA, P. & MULDER, H. 1980 Transport of salt and water during salting of cheese. 2. Quantities of salt taken up and of moisture lost. *Netherlands Milk and Dairy Journal* **34** 229-254

GILLES, J. 1976 Control of salt in moisture levels in Cheddar cheese. *New Zealand Journal of Dairy Science and Technology* **11** 219-221

GODINHO, M. & FOX, P. F. 1981 Ripening of Blue cheese: salt diffusion rates and mould growth. *Milchwissenschaft* **36** 329-333

GORDON, W. G., GROVES, M. L., GREENBERG, R., JONES, S. B., KALAN, E. B., PETERSON, R. F. & TOWNEND, R. E. 1972 Probable identification of γ-, TS-, R- and S-caseins as fragments of β-casein. *Journal of Dairy Science* **55** 261-263

GRANDISON, A. S., FORD, G. D., OWEN, A. J. & MILLARD, D. 1984a Chemical composition and coagulating properties of renneted Friesian milk during the transition from winter rations to spring grazing. *Journal of Dairy Research* **51** 69-78

GRANDISON, A. S., FORD, G. D., MILLARD, D. & OWEN, A. J. 1984b Chemical composition and coagulating properties of renneted milks from cows during early lactation. *Journal of Dairy Research* **51** 407-416

GREEN, M. L. & FOSTER, P. M. D. 1974 Comparison of the rates of proteolysis during ripening of Cheddar cheeses made with calf rennet and swine pepsin as coagulants. *Journal of Dairy Research* **41** 269-282

GREEN, M. L. & MANNING, D. J. 1982 Development of texture and flavour in cheese and other fermented products. *Journal of Dairy Research* **49** 737-748

GREEN, M. L. & MORANT, S. V. 1981 Mechanism of aggregation of casein micelles in rennet-treated milk. *Journal of Dairy Research* **48** 57-63

GREEN, M. L., HOBBS, D. G., MORANT, S. V. & HILL, V. A. 1978 Intermicellar relationships in rennet-treated separated milk. II. Process of gel assembly. *Journal of Dairy Research* **45** 413-422

GREEN, M. L., TURVEY, A. & HOBBS, D. G. 1981 Development of structure and texture in Cheddar cheese. *Journal of Dairy Research* **48** 343-355

GREEN, M. L., MARSHALL, R. J. & GLOVER, F. A. 1983 Influence of homogenization of concentrated milks on the structure and properties of rennet curds. *Journal of Dairy Research* **50** 341-348

GREEN, M. L., MARSHALL, R. J. & BROOKER, B. E. 1985 Instrumental and sensory texture assessment and fracture mechanisms of Cheddar and Cheshire cheeses. *Journal of Texture Studies* **16** 351-364

GREEN, M. L., LANGLEY, K. R., MARSHALL, R. J., BROOKER, B. E., WILLIS, A. & VINCENT, J. F. V. 1986 Mechanical properties of cheese, cheese analogues and protein gels in relation to composition and microstructure. *Food Microstructure* **5** 169-180

GRUFFERTY, M. B. & FOX, P. F. 1988 Milk alkaline proteinase. *Journal of Dairy Research* **55** 609-630

GUINEE, T. P. & FOX, P. F. 1986a Transport of sodium chloride and water in Romano cheese slices during brining. *Food Chemistry* **19** 49-64

GUINEE, T. P. & FOX, P. F. 1986b Influence of cheese geometry on the movement of sodium chloride and water during brining. *Irish Journal of Food Science and Technology* **10** 73-96

GUINEE, T. P. & FOX, P. F. 1993 Salt in cheese: physical, chemical and biological aspects. In *Cheese: Chemistry, Physics and Microbiology. Vol. 1. General Aspects* (Ed. P. F. Fox) Chapman & Hall, London, England. pp. 257-302

GUO, M. R. & KINDSTEDT, P. S. 1994 Age-related changes in the water phase of Mozzarella cheese. 2. Evaluation of moisture expelled by centrifugation. *Journal of Dairy Science* **77** (Suppl. 1) 41

GUO, M. R. & KINDSTEDT, P. S. 1995 Age-related changes in the water phase of Mozzarella cheese. *Journal of Dairy Science* **78** 2099-2107

HALL, D. M. & CREAMER, L. K. 1972 A study of the sub-microscopic structure of Cheddar, Cheshire and Gouda cheese by electron microscopy. *New Zealand Journal of Dairy Science and Technology* **7** 95-102

HEERTJE, I., van der VLIST, P., BLONK, J. C. G., HENDRICKX, H. A. C. M. & BRAKENHOFF, G. J. 1987 Confocal scanning laser microscopy in food research: some observations. *Food Microstructure* **6** 115-120

HERMANSSON, A.-M. 1986 Water- and fatholding. In *Functional Properties of Food Macromolecules.* (Eds. J. R. Mitchell & D. A. Ledward) Elsevier Applied Science, London, England. pp. 273-314

HILL, A. R., BULLOCK, D. H. & IRVINE, D. M. 1982 Manufacturing parameters of Queso Blanco made from milk and recombined milk. *Canadian Institute of Food Science and Technology Journal* **15** 47-53

HILL, R. D., LAHAV, E. & GIVOL, D. 1974 A rennin sensitive bond in α_{s1}B-casein. *Journal of Dairy Research* **41** 147-153

HOLMES, D. G., DUERSCH, J. W. & ERNSTROM, C. A. 1977 Distribution of milk clotting enzymes between curd and whey and their survival during Cheddar cheese making. *Journal of Dairy Science* **60** 862-869

HOOD, L. F. & LIBOFF, M. 1982 Starch ultrastructure. In *New Frontiers in Food Microstructure.* (Ed. D. B. Bechtel) AACC, St. Paul, Minnesota, USA. pp. 341-370

HUFFMAN, L. M. & KRISTOFFERSEN, T. 1984 Role of lactose in Cheddar cheese manufacturing and ripening. *New Zealand Journal of Dairy Science and Technology* **19** 151-162

JACK, F. R. & PATERSON, A. 1992 Texture of hard cheeses. *Trends in Food Science and Technology* **3** 160-164

KALAB, M. 1977 Milk gel structure. VI. Cheese texture and microstructure. *Milchwissenschaft* **32** 449-458

KALAB, M. 1993 Practical aspects of electron microscopy in dairy research. *Food Structure* **12** 95-114

KALAB, M. 1995 Practical aspects of electron microscopy in cheese research. In *Chemistry of Structure-Function Relationships in Cheese.* (Eds. E. L. Malin & M. H. Tunick) Plenum Press, New York, USA. pp. 247-276

KALAB, M. & CARIC, M. 1990 Food microstructure - evaluation of interactions of milk components in food systems. In *Proceedings of the XXIII International Dairy Congress, Montreal.* Vol. 2. International Dairy Federation, Brussels, Belgium. pp. 1457-1480

KALAB, M., LOWRIE, R. J & NICHOLS, D. 1982 Detection of curd granule and milled curd junctions in Cheddar cheese. *Journal of Dairy Science* **65** 1117-1121

KALAB, M. ALLAN-WOJTAS, P. & MILLER, S. S. 1995 Microscopy and other imaging techniques in food structure analysis. *Trends in Food Science and Technology* **6** 177-186

KIELY, L. J., KINDSTEDT, P. S., HENDRICKS, G. M., LEVIS, J. E., YUN, J. J. & BARBANO, D. M. 1992 Effect of draw pH on the development of curd structure during the manufacture of Mozzarella cheese. *Food Structure* **11** 217-224

KIMBER, A. M., BROOKER, B. E., HOBBS, D. G. & PRENTICE, J. H. 1974 Electron microscope studies of the development of structure in Cheddar cheese. *Journal of Dairy Research* **41** 389-396

KINDSTEDT, P. S. 1993 Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of Mozzarella cheese. *Critical Reviews in Food Science and Nutrition* **33** 167-187

KINDSTEDT, P. S. 1995 Factors affecting the functional characteristics of unmelted and melted Mozzarella cheese. In *Chemistry of Structure-Function Relationships in Cheese.* (Eds. E. L. Malin & M. H. Tunick) Plenum Press, New York, USA. pp. 27-41

KINDSTEDT, P. S. & KIELY, L. J. 1990 Physical and chemical methods for controlling the body and texture of Mozzarella cheese. In *Proceedings of the 9th Biennial Cheese Industry Conference, Utah State University, Logan, USA.*

KINDSTEDT, P. S., KIELY, L. J., YUN, J. J. & BARBANO, D. M. 1991 Relationship between Mozzarella manufacturing parameters, cheese composition, and functional properties: Impact of coagulant. In *Proceedings of the 28th Annual Marschall Italian Cheese Seminar, Madison, Wisconsin, USA.* pp. 89-97

KINSELLA, J. E. 1984 Milk proteins: physicochemical and functional properties. *CRC Critical Reviews in Food Science and Nutrition* **21** 197-262

KINSELLA, J. E., WHITEHEAD, D. M., BRADY, J. & BRINGE, N. A. 1989 Milk proteins: possible relationships of structure and function. In *Developments in Dairy Chemistry - 4. Functional Milk Proteins.* (Ed. P. F. Fox) Elsevier Applied Science, London, England. pp. 55-95

KNEIFEL, W. & SEILER, A. 1993 Water-holding properties of milk protein products - a review. *Food Structure* **12** 297-308

KNEIFEL, W., PAQUIN, P., ABERT, T. & RICHARD, J.-P. 1991 Water-holding capacity of proteins with special regard to milk proteins and methodological aspects - a review. *Journal of Dairy Science* **74** 2027-2041

KONSTANCE, R. P & HOLSINGER, V. H. 1992 Development of rheological test methods for cheese. *Food Technology* **46**(10) 105-109

KORYCKA-DAHL, M., RIBADEAU-DUMAS, B., CHENE, N. & MARTAL, J. 1983 Plasmin activity in milk. *Journal of Dairy Science* **66** 704-711

KOSIKOWSKI, F. V. 1982 *Cheese and Fermented Milk Foods* Edwards Bros. Inc., Ann Arbor, Michigan, USA

LAWRENCE, A. J. 1959 Syneresis of rennet curd. Part 1. Effect of time and temperature. *Australian Journal of Dairy Technology* **14** 166-169

LAWRENCE, R. C. & GILLES, J. 1969 The formation of bitterness in cheese: a critical evaluation. *New Zealand Journal of Dairy Technology* **4** 189-196

LAWRENCE, R. C. & GILLES, J. 1982 Factors that determine the pH of young Cheddar cheese. *New Zealand Journal of Dairy Science and Technology* **17** 1-14

LAWRENCE, R. C. & GILLES, J. 1987 Cheese composition and quality. In *Milk: The Vital Force. Proceedings of the XXII International Dairy Congress.* D. Reidel Publishing Company, Dordrecht, Netherlands. pp. 111-121

LAWRENCE, R. C., GILLES, J. & CREAMER, L. K. 1983 The relationship between cheese texture and flavour. *New Zealand Journal of Dairy Science and Technology* **18** 175-190

LAWRENCE, R. C., HEAP, H. A. & GILLES, J. 1984 A controlled approach to cheese technology. *Journal of Dairy Science* **67** 1632-1645

LAWRENCE, R. C., CREAMER, L. K. & GILLES, J. 1987 Texture development during cheese ripening. *Journal of Dairy Science* **70** 1748-1760

LAWRENCE, R. C., GILLES, J. & CREAMER, L. K. 1993 Cheddar cheese and related dry-salted cheese varieties. In *Cheese: Chemistry, Physics and Microbiology. Vol. 2. Major Cheese Groups.* (Ed. P. F. Fox) Chapman & Hall, London, England. pp. 1-38

LE BARS, D. & GRIPON, J-C. 1989 Specificity of plasmin towards bovine α_{s2}-casein. *Journal of Dairy Research* **56** 817-821

LEE, J., SEDCOLE, J. R. & PRITCHARD, M. W. 1986 Matrix interactions in an inductively coupled argon plasma optimised for simultaneous multi-element analysis by atomic emission spectrometry. *Spectrochimica Acta* **41B** 217-225

LELIEVRE, J. 1977 Rigidity modulus as a factor influencing the syneresis of renneted milk gels. *Journal of Dairy Research* **44** 611-614

LELIEVRE, J. & CREAMER, L. K. 1978 An NMR study of the formation and syneresis of renneted milk gels. *Milchwissenschaft* **33** 73-76

LUYTEN, H. 1988 The rheological and fracture properties of Gouda cheese. Ph.D. Thesis, Wageningen Agricultural University, Wageningen, Netherlands

MABBITT, L. A., CHAPMAN, H. R. & BERRIDGE, N. J. 1955 Experiments in cheesemaking without starter. *Journal of Dairy Research* **22** 365-373

MADKOR, S. A. & FOX, P. F. 1991 Plasmin activity in buffalo milk. *Food Chemistry* **39** 139-156

MARSHALL, R. J. 1982 An improved method for the measurement of the syneresis of curd formed by rennet action on milk. *Journal of Dairy Research* **49** 329-336

MASI, P. & ADDEO, F. 1986 An examination of some mechanical properties of a group of Italian cheeses and their relation to structure and conditions of manufacture. *Journal of Food Engineering* **5** 217-229

McDOWALL, F. H. & DOLBY, R. M. 1936 Studies on the chemistry of Cheddar cheese making. *Journal of Dairy Research* **7** 156-175

McLEAN, D. M. & SCHAAR, J. 1989 Effects of β-lactoglobulin and κ-casein genetic variants and concentrations on syneresis of gels from renneted heated milk. *Journal of Dairy Research* **56** 297-301

McMAHON, D. J., OBERG, C. J. & McMANUS, W. 1993 Functionality of Mozzarella cheese. *Australian Journal of Dairy Technology* **48** 99-104

McSWEENEY, P. L. H., OLSON, N. F. & FOX, P. F. 1992 Proteolytic specificity of chymosin on bovine α_{s1}-casein. *Journal of Dairy Science* **75**(Suppl. 1) 121

McSWEENEY, P. L. H., OLSON, N. F., FOX, P. F., HEALY, A. & HØJRUP, P. 1993 Proteolytic specificity of chymosin on bovine α_{s1}-casein. *Journal of Dairy Research* **60** 401-412

McSWEENEY, P. L. H., POCHET, S., FOX, P. F.& HEALY, A. 1994 Partial identification of peptides from the water-soluble fraction of Cheddar cheese. *Journal of Dairy Research* **61** 587-590

MODLER, H. W. 1988 Development of a continuous process for the production of Ricotta cheese. *Journal of Dairy Science* **71** 2003-2009

MONIB, A. M. M. F. 1962 The calcium-paracaseinate-phosphate-complex under conditions similar to those in cheese. *Mededelingen Landbouwhogeschool Wageningen* **62**(10) 1-76

MORRIS, H. A., HOLT, C., BROOKER, B. E., BANKS, J. M. & MANSON, W. 1988 Inorganic constituents of cheese: analysis of juice from a one-month-old Cheddar cheese and the use of light and electron microscopy to characterize the crystalline phases. *Journal of Dairy Research* **55** 255-268

MULVIHILL, D. M. & FOX, P. F. 1977 Proteolysis of α_{s1}-casein by chymosin: influence of pH and urea. *Journal of Dairy Research* **44** 533-540

MULVIHILL, D. M. & FOX, P. F. 1978 Proteolysis of bovine β-casein by chymosin: influence of pH, urea and sodium chloride. *Irish Journal of Food Science and Technology* **2** 135-139

MULVIHILL, D. M. & FOX, P. F. 1979 Proteolytic specificity of chymosin on bovine α_{s1}-casein. *Journal of Dairy Research* **46** 641-651

MULVIHILL, D. M. & FOX, P. F. 1980 Proteolysis of α_{s1}-casein by chymosin in dilute NaCl solutions and in Cheddar cheese. *Irish Journal of Food Science and Technology* **4** 13-23

NEW ZEALAND MINISTRY OF AGRICULTURE AND FISHERIES 1979 *Chemistry: Standard Laboratory Methods.* Wellington, New Zealand: MAF Dairy Division

NILSON, K. M. 1969 Solving problems of Mozzarella cheese. *American Dairy Review* **31** 28, 30, 72

NOOMEN, A. 1975 Proteolytic activity of milk protease in raw and pasteurized cow's milk. *Netherlands Milk and Dairy Journal* **29** 153-161

NOOMEN, A. 1978 Activity of proteolytic enzymes in simulated soft cheeses (Meshanger type). 2. Activity of calf rennet. *Netherlands Milk and Dairy Journal* **32** 49-68

NORRIS, R., GRAY, I. K., McDOWELL, A. K. R. & DOLBY, R. M. 1971 The chemical composition and physical properties of fractions of milk fat obtained by a commercial fractionation process. *Journal of Dairy Research* **38** 179-191

OBERG, C. J., McMANUS, W. R. & McMAHON, D. J. 1993 Microstructure of Mozzarella cheese during manufacture. *Food Structure* **12** 251-258

O'KEEFFE, A. M. 1984 Seasonal and lactational influences on moisture content of Cheddar cheese. *Irish Journal of Food Science and Technology* **8** 27-37

O'KEEFFE, R. B., FOX, P. F. & DALY, C. 1975 Proteolysis in Cheddar cheese: influence of the rate of acid production during manufacture. *Journal of Dairy Research* **42** 111-122

O'KEEFFE, R. B., FOX, P. F. & DALY, C. 1976 Contribution of rennet and starter proteases to proteolysis in Cheddar cheese. *Journal of Dairy Research* **43** 97-107

OLSON, N. F. & PRICE, W. V. 1970 Effect of curd deformation during cheddaring on characteristics of Cheddar cheese. *Journal of Dairy Science* **53** 1676-1680

PAQUET, A. & KALAB, M. 1988 Amino acid composition and structure of cheese baked as a pizza ingredient in conventional and microwave ovens. *Food Microstructure* **7** 93-103

PATEL, M. C., LUND, D. B. & OLSON, N. F. 1972 Factors affecting syneresis of renneted milk gels. *Journal of Dairy Science* **55** 913-918

PEARCE, K. N. 1976 Moving boundary electrophoresis of native and rennet-treated casein micelles. *Journal of Dairy Research* **43** 27-36

PEARSE, M. J. & MACKINLAY, A. G. 1989 Biochemical aspects of syneresis: a review. *Journal of Dairy Science* **72** 1401-1407

PEARSE, M. J., MACKINLAY, A. G., HALL, R. J. & LINKLATER, P. M. 1984 A micro assay for the syneresis of cheese curd. *Journal of Dairy Research* **51** 131-139

PEARSE, M. J., LINKLATER, P. M., HALL, R. J. & MACKINLAY, A. G. 1985 Effect of heat induced interaction between β-lactoglobulin and κ-casein on syneresis. *Journal of Dairy Research* **52** 159-165

PLOCK, J. & KESSLER, H. G. 1992 [Whey protein preparations - Gel formation and water binding capacities at neutral pH]. *Lebensmittelindustrie und Milchwirtschaft* **113** 895-899

POLLAK, N. & PELEG, M. 1980 Early indications of failure in large compressive deformation of solid foods. *Journal of Food Science* **45** 825-830, 835

PRENTICE, J. H., LANGLEY, K. R. & MARSHALL, R. J. 1993 Cheese rheology. In *Cheese: Chemistry, Physics and Microbiology. Vol. 1. General Aspects.* (Ed. P. F. Fox) Chapman & Hall, London, England. pp. 303-340

RAMKUMAR, C., CREAMER, L. K., JOHNSTON, K. A. & BENNETT, R. J. 1997 Effect of pH and time on the quantity of readily available water within fresh cheese curd. *Journal of Dairy Research* **64** 123-134

REIMERDES, E. H., KLOSTERMEYER, H. & SAYK, E. 1976 [Milk proteinases. 7. Fractionation of components of the proteinase inhibitor system in milk]. *Milchwissenschaft* **31** 329-334

RICHARDSON, B. C. 1983a Variation of the concentration of plasmin and plasminogen in bovine milk with lactation. *New Zealand Journal of Dairy Science and Technology* **18** 247-252

RICHARDSON, B. C. 1983b The proteinases of bovine milk and the effect of pasteurization on their activity. *New Zealand Journal of Dairy Science and Technology* **18** 233-245

RICHARDSON, B. C. & PEARCE, K. N. 1981 The determination of plasmin in dairy products. *New Zealand Journal of Dairy Science and Technology* **16** 209-220

ROEFS, S. P. F. M., WALSTRA, P., DALGLEISH, D. G. & HORNE, D. S. 1985 Preliminary note on the change in casein micelles caused by acidification. *Netherlands Milk and Dairy Journal* **39** 119-122

SANDBERG, E., HAGLUND, E. & BARTHEL, C. 1930 [Analysis of cheese juice as a method of determining the degree of maturation]. *Lait* **10** 1-21

SCOTT, R. 1986 *Cheesemaking Practice.* Applied Science Publishers Ltd, London, England

SERPELLONI, M., LEFEVRE, P. & DUSAUTOIS, C. 1990 Glucono-delta-lactone in milk ripening. *Dairy Industries International* **55**(2) 35, 37, 39

SHARMA, H. S., BASSETTE, R., MEHTA, R. S. & DAYTON, A. D. 1980 Yield and curd characteristics of Cottage cheese made by the culture and direct-acid-set methods. *Journal of Food Protection* **43** 441-446

SNOEREN, T. H. M., KLOK, H. J., van HOOYDONK, A. C. M. & DAMMAN, A. J. 1984 The voluminosity of casein micelles. *Milchwissenschaft* **39** 461-463

STANLEY, D. W. & EMMONS, D. B. 1977 Cheddar cheese made with bovine pepsin. II. Texture-microstructure-composition relationships. *Canadian Institute of Food Science and Technology Journal* **10** 78-84

STORRY, J. E., GRANDISON, A. S., MILLARD, D., OWEN, A. J. & FORD, G. D. 1983 Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. *Journal of Dairy Research* **50** 215-229

SUTHERLAND, B. J. 1974 Control of salt absorption and whey drainage in Cheddar cheese manufacture. *Australian Journal of Dairy Technology* **29** 86-93

THOMAS, T. D. & PEARCE, K. N. 1981 Influence of salt on lactose fermentation and proteolysis in Cheddar cheese. *New Zealand Journal of Dairy Science and Technology* **16** 253-259

van DIJK, H. J. M. 1982 Syneresis of curd. Ph.D. Thesis. Wageningen Agricultural University, Wageningen, Netherlands

van HOOYDONK, A. C. M., HAGEDOORN, H. G. & BOERRIGTER, I. J. 1986 pH-induced physico-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physico-chemical properties. *Netherlands Milk and Dairy Journal* **40** 281-296

van VLIET, T. 1991 Inventory of test methods. *Bulletin of the International Dairy Federation* No. 268 pp. 16-25

VISSER, S. & SLANGEN, K. J. 1977 On the specificity of chymosin (rennin) in its action on bovine β-casein. *Netherlands Milk and Dairy Journal* **31** 16-30

WALSTRA, P. 1979 The voluminosity of bovine casein micelles and some of its implications. *Journal of Dairy Research* **46** 317-323

WALSTRA, P. 1993 The syneresis of curd. In *Cheese: Chemistry, Physics and Microbiology. Vol. 1. General Aspects.* (Ed. P. F. Fox) Chapman & Hall, London, England. pp. 141-191

WALSTRA, P. & JENNESS, R. 1984 *Dairy Chemistry and Physics.* John Wiley & Sons, New York, USA

WALSTRA, P. & van VLIET, T. 1982 Rheology of cheese. *Bulletin of the International Dairy Federation* No. 153 pp. 22-27

WALSTRA, P. & van VLIET, T. 1986 The physical chemistry of curd making. *Netherlands Milk and Dairy Journal* **40** 241-259

WALSTRA, P., van DIJK, H. J. M. & GEURTS, T. J. 1985 The syneresis of curd. 1. General considerations and literature review. *Netherlands Milk and Dairy Journal* **39** 209-246

WALSTRA, P., LUYTEN, H. & van VLIET, T. 1987 Consistency of cheese. In *Milk: The Vital Force. Proceedings of the XXII International Dairy Congress.* D. Reidel Publishing Company, Dordrecht, Netherlands. pp. 159-168

WILKINSON, M. G., GUINEE, T. P., O'CALLAGHAN, D. M. & FOX, P. F. 1994 Autolysis and proteolysis in different strains of starter bacteria during Cheddar cheese ripening. *Journal of Dairy Research* **61** 249-262

WATKINSON, P., BOSTON, G., CAMPANELLA, O., COKER, C., JOHNSTON, K. A., LUCKMAN, M. & WHITE, N. 1997 Rheological properties and maturation of New Zealand Cheddar cheese. *Lait* **77** 109-120

8.0 PUBLICATION

Journal of Dairy Research (1997) **64** 123–134 *Printed in Great Britain*

Effect of pH and time on the quantity of readily available water within fresh cheese curd

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(*Received 16 October 1995 and accepted for publication 10 June 1996*)

SUMMARY. Some of the textural changes that occur early in cheese maturation may be related to the redistribution of water within the cheese matrix. To examine this, a model cheese curd system was devised and explored. Initially, cheese curd was prepared using starter and chymosin and the curd pH was controlled by varying the draining and salting pH values. The quantity of serum that could be centrifuged from the resultant curd was less for lower pH curd and decreased in volume with time. The curd pH decreased with time. In the protocol finally adopted, milk was acidified with lactic acid and coagulated with Rennilase 46L. After cheddaring, salting and light pressing, the samples of this curd were finely diced and mixed with glucono-δ-lactone to give curd samples with comparable moisture contents, similar casein proteolysis rates but different pH values. The quantity of serum that could be centrifuged from these samples was greater for pH 5·6 curd than for pH 5·2 curd and decreased faster for the lower pH curd. Neither the curd moisture nor the pH changed significantly during curd storage and the casein proteolysis was low. These results for the model curd system are consistent with known water absorption characteristics of casein curd under 'equilibrium' conditions and the effects of pH and mineral salts on this absorption. It was concluded that, during the early stages of cheese ripening, there may be a redistribution of moisture within the cheese, related to the basic properties of the protein matrix and the transient effects of curd salting, rather than as a direct consequence of glycolytic and proteolytic changes.

In the conversion of milk to cheese, one of the first major steps is separation of the fat and casein fractions from the milk serum. In general, a lactic-acid-producing culture is mixed with milk and a milk-coagulating proteinase is added. The casein micelles gradually form into chains that trap the bacteria and the milk fat globules. With time, these chains of micelles thicken by the addition of further micelles and the coalescence of the chains. When the curd is cut, the effects of mild agitation, lowering of the pH and increased temperature allow the curd to synerese, or expel the serum (whey) (Akkerman, 1992; Green & Grandison, 1993). The continuing effects of the intermolecular forces that brought about the coagulation no doubt contribute to curd shrinkage. These forces are relatively weak and the serum seeps out slowly. In Cheddar cheesemaking, the bulk of the whey is run off and the curd is piled up and allowed to slump. This cheddaring creates shearing within the curd with further

expulsion of whey. Cutting of this curd and its vigorous stirring followed by application of dry salt to the curd pieces extracts more moisture, particularly from the surface regions of the curd particles. The serum that was originally evenly dispersed throughout the system becomes concentrated into small vacuoles throughout the curd during syneresis and is then squeezed into larger vacuoles and into elongated pockets during cheddaring and dry stirring (Kimber *et al.* 1974). During Mozzarella cheesemaking, there is a heating and curd stretching step that also results in coalescence of vacuoles that contain serum, bacteria and fat globules (Oberg *et al.* 1993; Tunick *et al.* 1993). String cheese, which appears to have a similar manufacturing process to that of Mozzarella but with a more extreme stretching procedure, accumulates the serum in elongated channels (Taneya *et al.* 1992) that align with the stretch direction.

Recently Guo & Kindstedt (1994, 1995) examined one type of Mozzarella cheese using the mild separation procedure of centrifuging the cheese at 25 °C. They found that the amount of 'expressible serum' reduced as the cheese aged over a period of ~ 2 weeks. In an effort to determine the generality and fundamental basis of this behaviour, we developed a model system in which the pH, moisture content and physical pressing of the curd and the proteolytic action of the milk coagulant could be controlled. The main objective of these experiments was to devise a reliable system with which to study the changes in the water distribution in cheese made using the cheddaring process during the first 2 weeks of maturation.

MATERIALS AND METHODS

Materials

Whole milk (obtained from Tui Milk Products Ltd. Palmerston North), standardized to a protein:fat ratio of 1·5, pasteurized at 72 °C for 15 s and cooled to 32 °C, was used for the cheesemaking trials. The protein:fat ratio in milk was kept high so as to retain more moisture in the cheese curd. The starters used were strains of *Lactococcus lactis* subsp. *cremoris*. A dilute (100 ml/l) solution of USP grade lactic acid (Clark Products Ltd, Napier) was used to adjust the pH of the milk. Calf rennet (59 rennet units/ml: New Zealand Co-operative Rennet Co., Eltham) or Rennilase 46L (Novo Industri, DK-2880 Bagsværd, Denmark) was used for coagulating the milk. Glucono-δ-lactone (GDL: Fujisawa Pharmaceutical Co. Ltd. Tokyo, Japan) was used to adjust the pH of the cheese curds. The chemicals used for analysis were of Analar grade.

Curd making

Trial 1. Curd made with starter and chymosin. Cheese curd was made with modifications to the standard procedure for making Cheddar cheese. Two 400 l vats were filled with milk and primed with starter at 40 g/kg. When the milk reached pH 6·3, rennet was added at 16 ml/100 l. The vat contents were mixed and left to set at 32 °C. The coagulum was cut after 20 min, and was cooked at 36 °C for vat 1 and at 34 °C for vat 2. The whey was drained out at pH 5·9 for vat 1 and pH 5·6 for vat 2. The curd was cheddared for 30 min. The curd in vat 1 was divided into two lots. Salting was at 350 g/100 l milk, lot 1 when the curd pH was 5·6 and lot 2 at pH 5·3. Similarly, the curd in vat 2 was divided into two lots and salted at pH 5·3 and pH 5·0. After mellowing for 30 min, the curd was transferred into 0·37 × 0·30 × 0·18 m hoops lined with cheesecloth and left for 3 h without pressing. The curds were then transferred into plastic bags and stored at 2 °C.

Trial 2. Cheese curd made by direct acidification. Four 100 l vats were filled with milk at 10 °C and the pH was adjusted to 6·0, 5·7, 5·4 and 5·1 by the addition of various amounts of dilute (100 ml/l) lactic acid. The milk was then warmed to 32 °C and Rennilase 46L was added at 8 ml/100 l. After 8–10 min, the coagulum was cut and cooked at 36 °C. Stirring was continued for a further 60 min. The whey was then drained out and the curd was cheddared for 30 min. After milling, 350 g salt/100 l milk was mixed in and the curd was allowed to mellow for 30 min. The subsequent steps were as for Trial 1.

Trial 3. Cheese curd made by direct acidification and addition of glucono-δ-lactone. Cheese curd was made as described for Trial 2 except that the pH of the milk was adjusted to 5·9 with the addition of dilute (100 ml/l) lactic acid before the addition of Rennilase 46L.

On the next day, the milled, salted and stored curd was grated into about $2 \times 2 \times 6$ mm pieces and divided into four lots. Each lot was mixed with a calculated amount of GDL and left for 48 h at 2 °C to equilibrate and to obtain curd samples in the pH range from 5·2 to 5·6. The amounts of GDL added were 7, 11, 16 and 20 g/kg curd, which resulted in curds with pH values of 5·61, 5·43, 5·30 and 5·19 respectively. These curds were then stored at 2 °C.

Centrifugation of cheese curd

Centrifugation bottles were filled with ~ 140 g well-mixed samples of grated curd. Centrifugation was carried out at 11 086 ***g*** and 25 °C for 75 min using a JA – 14 rotor in a Beckman J2 – 21 M centrifuge (Palo Alto, CA 94304, USA). The speed, duration and temperature of centrifugation were selected based on the results of preliminary trials and on the work of Guo & Kindstedt (1995). The weight of liquid released after centrifugation, referred to as the centrifugal serum in this paper, was measured as a difference in weight. Care was taken to remove the centrifugal serum from the bottle immediately after centrifugation to prevent its reabsorption into the centrifuged curd. The lipid in the serum was allowed to separate out and was then removed by pipette. Each curd sample was centrifuged as six replicates.

Analysis of cheese curd, centrifuged curd and centrifugal serum

pH. The grated curd was tightly packed into a 10 ml glass beaker and its pH was determined using a PHM 82 standard pH meter (Radiometer, DK-2400 Copenhagen, Denmark) with an N61 Schott Geräte (D-6500 Mainz, Germany) electrode.

Moisture. The moisture content was determined by drying a known weight of the grated curd sample at 100 °C for 16 h, cooling in a desiccator for 1 h and weighing. Moisture in non-fat substance was calculated.

Fat. Grated cheese curd (9·0 g) was placed in a Babcock bottle and dissolved with 17·5 ml sulphuric acid, added in three or four portions. The test bottles were placed in a water bath at 65 °C for 5 min and then centrifuged at 165 ***g*** for 5 min (New Zealand Ministry of Agriculture and Fisheries, 1979). Fat in dry matter was then calculated.

Salt. Grated curd (~ 2 g) was weighed accurately (to 0·001 g) into a titration sample cup and mixed with 40 ml dilute (20 ml/l) HNO_3 at 65 °C. After 30 min the sample container was attached to the autotitrator measuring electrode and titrated with silver nitrate (New Zealand Ministry of Agriculture and Fisheries, 1979). Salt in moisture was calculated.

Calcium. A sample (~ 0·5 g) was weighed, mixed with 10 ml 0·1 M-HCl and then diluted with 90 ml water. Solid NaOH (0·5 g) was added and stirred until it dissolved.

The solution was titrated with 0·02 M-EDTA using Patton and Reeder's indicator (New Zealand Ministry of Agriculture and Fisheries, 1979).

Sodium. For determination of the sodium content of the centrifuged curd, a sample (~ 0·05 g) was accurately weighed and dispersed in ~ 50 ml of high purity water and 10 ml lithium reference standard was added. The volume was made up to 200 ml and analysed using a flame photometer (New Zealand Ministry of Agriculture and Fisheries, 1979).

Electrophoresis. The mini alkaline urea-PAGE technique described by Creamer (1991) was followed using Bio-Rad mini-Protean II equipment (Bio-Rad Laboratories, Richmond, CA 94804, USA). The cheese curd or serum sample (0·50 g) was mixed with 25 ml sample buffer containing urea, held at 40 °C for 1 h, blended using an Ultra-Turrax T25 (Janke and Künkel, D-79219 Staufen, Germany) at 24000 rev./min for 20 s, and then centrifuged at 13776 *g* and 4 °C for 10 min in a Sorvall RC2 refrigerated centrifuge (Ivan Sorvall Inc., Norwalk, CT 06856, USA). Aqueous supernatant (2 ml) from below the fat layer was treated with 10 μl/ml each of 2-mercaptoethanol and bromophenol blue and held for 18 h. Each slot in the gel slab was loaded with 5 μl of the mixture. Commercial rennet casein (Anchor Products Ltd, Hautapu) was used as the standard. After staining with Coomassie blue and destaining, the gels were scanned on a personal densitometer (Molecular Dynamics, Sunnyvale, CA 94086, USA) and the integrated densities of the major protein bands were determined.

RESULTS

Composition of cheese curd

The composition of cheese curds made at different setting pH values (Trial 2) is given in Table 1. The composition is also typical for samples of the other trials in their corresponding pH range. Standard Cheddar cheese contains 520–560 g fat/kg dry matter, 520–540 g moisture/kg non-fat substance and 47–57 g salt/kg moisture (Lawrence *et al.* 1993). The curds made in the various trials had a higher moisture in non-fat substance because of the higher protein:fat ratio in the cheesemilk, and lower fat in dry matter and salt in moisture than the standard Cheddar cheese.

Trial 1

When the curd was centrifuged, it separated into three layers. The curd compacted on to the side of the bottle and the liquid that separated (the centrifugal serum) had an aqueous lower portion with an upper lipid layer (10–20 g/kg centrifugal serum). The centrifugal serum was decanted out of the bottle and the quantity was measured.

Effect of draining and salting pH values. The results for the cheese curd made with a draining pH of 5·9 are given in Table 2. The quantity of centrifugal serum released from the curd on day 1 decreased with decrease in the pH at salting from 5·6 to 5·3. The average moisture contents of the curds made with salting pH values of 5·6 and 5·3 were 484 and 459 g/kg respectively.

Similarly, the quantities of centrifugal serum on day 1 from the curd made with a draining pH of 5·6 also decreased with a decrease in the pH at salting from 5·3 to 5·0 (Table 2). The average moisture contents of the curds made with salting pH values of 5·3 and 5·0 were 511 and 482 g/kg respectively. A decrease in the quantity of centrifugal serum was thus observed with a decrease in the pH at draining and salting.

Table 1. *Composition of curds made with different setting pH values (Trial 2)*†

	Composition of curds. g/kg		
pH at setting	Fat in dry matter	Moisture in non-fat substance	Salt in moisture
6·0	409	604	45·8
5·7	391	586	41·9
5·4	387	579	38·7
5·1	393	577	37·5

† See text for experimental details.

Samples of centrifuged curd and centrifugal serum of the curd made with a draining pH of 5·9 and a salting pH of 5·6 were analysed by urea-PAGE. The values for the dye bound to the major casein fractions are shown in Fig. 1 (*a*. *c*). The ratio of α_{s1}-I-casein and β-casein to α_{s1}-casein was greater in the centrifugal serum than in the curd. Similar trends were observed for all the samples of centrifuged curd and centrifugal serum studied. Calcium content of the serum was determined to see if it migrated from curd to the serum phase. To assess whether there was a corresponding movement of sodium, its content in the centrifuged curd was also determined. There was an increase in the calcium content of the centrifugal serum with a decrease in the curd pH whereas the sodium content of the centrifuged curd tended to increase in the samples of curd in which the centrifugal serum decreased with time (Table 2). suggesting that salt might have migrated from the serum to the curd phase while calcium migrated in the opposite direction.

Effect of ageing. The quantity of centrifugal serum decreased gradually with time for the curd made with a draining pH of 5·9 and there was no release from centrifugal serum after day 9. whereas there was no centrifugal serum from day 3 onwards from the curd made with a draining pH of 5·6. The calcium content of the centrifugal serum and the sodium content of the centrifuged curd tended to increase with time for the pH values studied (Table 2).

The results of the PAGE analysis of the cheese curd prepared with a draining pH of 5·9 and a salting pH of 5·6 (Fig. 1*a*. *c*) showed that the amounts of β-casein and α_{s1}-casein decreased. indicating that proteolysis was taking place. The quantities of intact caseins in the centrifugal serum tended to increase with time. suggesting that either dissociation of caseins from the curd matrix was taking place or that. as the water was leaving the vacuoles. the casein concentration was increasing.

The inconsistencies in some of the observations made in this initial trial could have been due to differences in the size of the curd pieces in the bag. and the fact that the curd was not pressed during manufacture. These may have contributed to a variation in composition. e.g. in lactose or moisture content.

Trial 2

In this trial. an attempt was made to manufacture cheese curd such that there would be minimum proteolysis during the initial period of ripening so that other factors contributing to the water binding in cheese could be studied. The cheddaring was not as satisfactory as in Trial 1 because of the small quantity of curd. Thus the curd after milling was a mixture of small curd particles and fingers of cheddared curd. The results for the samples of curd made with setting pH values of 6·0 and 5·7 are given in Table 3.

Effect of setting pH. The quantity of centrifugal serum on each day was greater at

Table 2. *Studies on cheese curd made with different draining and salting pH values (Trial 1)*†

Characteristic	No. of days after manufacture						
	1	3	5	7	9	1	3
	Draining pH, 5·9					Draining pH, 5·6	
	Salting pH, 5·6					Salting pH, 5·3	
pH of cheese curd	5·34	5·24	5·31	5·20	5·26	5·12	5·13
Centrifugal serum, g/kg curd‡	66·4 ± 3·21	42·2 ± 4·29	43·3 ± 4·06	31·9 ± 2·46	1·70 ± 0·30	17·3 ± 1·75	—
Calcium, mmol/kg centrifugal serum	86·6	89·7	104	102	—	94·2	—
Sodium, mmol/kg centrifuged curd	344	394	412	431	466	366	430
	Salting pH, 5·3					Salting pH, 5·0	
pH of cheese curd	5·28	5·16	5·19	5·19	5·22	5·08	5·06
Centrifugal serum, g/kg curd‡	18·8 ± 3·17	27·2 ± 2·64	29·7 ± 3·38	47·3 ± 1·24	1·37 ± 0·33	13·5 ± 2·02	—
Calcium, mmol/kg centrifugal serum	92·6	112	99·6	105	—	94·7	—
Sodium, mmol/kg centrifuged curd	439	448	481	492	478	502	507

—, No serum was obtained on centrifugation of the curd.
† See text for experimental details.
‡ Values are means ± SD for $n = 6$.

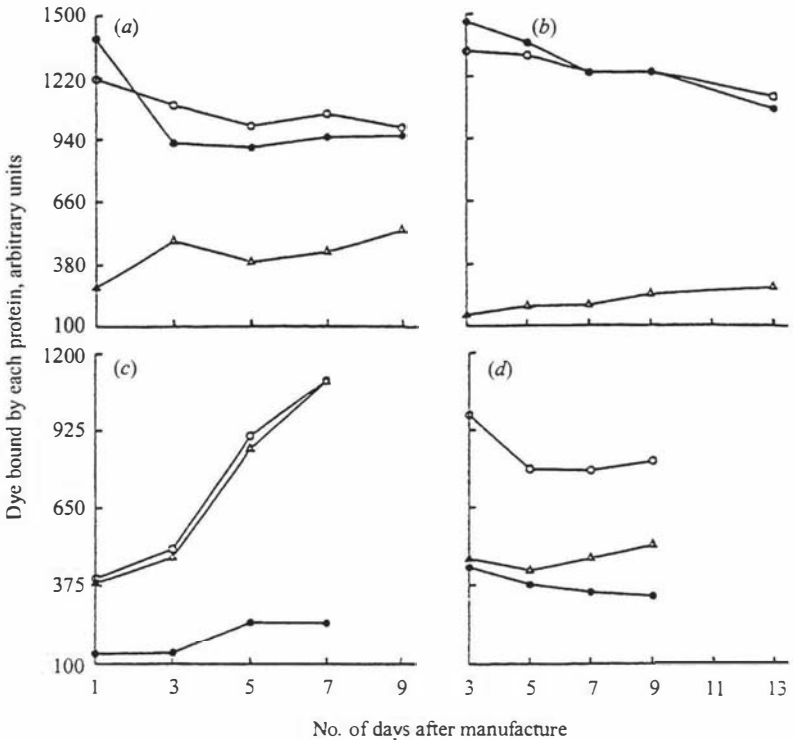

Fig. 1. Quantities of dye bound by casein fractions in urea-PAGE: ○. β-casein: ●. α_{s1}-casein: △. α_{s1}-I-casein. (*a*) Centrifuged curd and (*c*) centrifugal serum of curd made with a draining pH of 5·9 and a salting pH of 5·6 (Trial 1). (*b*) Centrifuged curd and (*d*) centrifugal serum of pH 5·3 curd made by direct acidification and addition of glucono-δ-lactone (Trial 3). Experimental details are given in the text.

Table 3. *Studies on cheese curd made with different setting pH values (Trial 2)*†

Characteristic	No. of days after manufacture					
	1	3	5	7	9	13
Setting pH 6·0						
pH of cheese curd	5·93	5·94	5·96	5·96	5·96	6·00
Centrifugal serum, g/kg curd‡	69·2 ± 4·39	70·4 ± 2·59	82·3 ± 4·28	61·3 ± 1·80	43·8 ± 2·60	79·8 ± 2·51
Calcium, mmol/kg centrifugal serum	31·2	30·6	33·4	33·5	30·6	32·3
Sodium, mmol/kg centrifuged curd	690	656	668	563	717	634
Setting pH 5·7						
pH of cheese curd	5·73	5·72	5·72	5·72	5·72	5·70
Centrifugal serum, g/kg curd‡	37·6 ± 2·43	32·9 ± 2·58	25·0 ± 2·09	33·6 ± 2·72	39·0 ± 2·28	39·0 ± 1·72
Calcium, mmol/kg centrifugal serum	41·7	40·2	44·4	45·8	45·2	46·4
Sodium, mmol/kg centrifuged curd	496	564	499	506	565	552

† See text for experimental details.
‡ Values are means ± SD for $n = 6$.

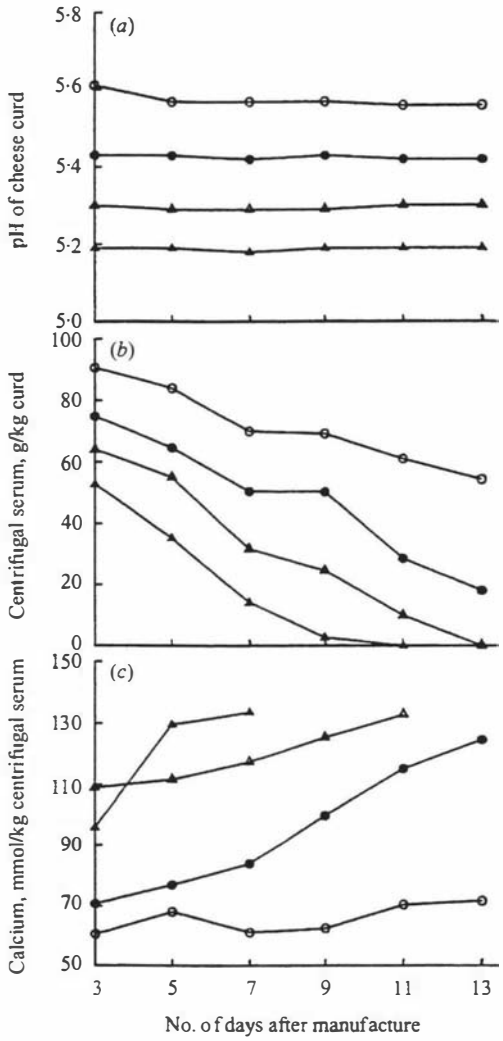

Fig. 2. Variation of (*a*) pH of cheese curd. (*b*) average quantity of centrifugal serum and (*c*) calcium content of the centrifugal serum from samples of cheese curd with time and different levels of added glucono-δ-lactone (g/kg): ○, 7; ●, 11; △, 16; ▲, 20. Experimental details are given in the text.

setting pH 6·0 than at pH 5·7. There was no release of centrifugal serum even on day 1 from the curd samples made with setting pH values of 5·4 and 5·1. The average moisture contents of the curds made with setting pH values of 6·0, 5·7, 5·4 and 5·1 were 475, 464, 457 and 453 g/kg respectively.

The calcium content of the serum was greater at setting pH 5·7 whereas the sodium content of the centrifuged curd was greater at pH 6·0 (Table 3).

Effect of ageing. The pH values of the curd samples remained essentially constant with time and water retention appeared to be dependent on the pH of the curd samples.

Neither the sodium content of the centrifuged curd nor the calcium content of the centrifugal serum varied consistently with time at setting pH 6·0 or pH 5·7 (Table 3).

Trial 3

In this trial, an attempt was made to retain the stable pH and low proteolysis achieved in Trial 2 but to have curd with a more constant initial moisture content and within a narrower pH range. To this end, small quantities of GDL were added to portions of a single batch of curd made by lactic acid acidification of Rennilase-treated milk. A physical examination of the samples of curd showed that the curd at the higher pH was more elastic than that at the low pH, which was crumbly and friable. The moisture contents of all these samples ranged between 481 and 488 g/kg. The results of the centrifugation studies on these samples are shown in Fig. 2.

Effect of curd pH. The initial quantity of centrifugal serum (i.e. on day 3 to allow the GDL to fully hydrolyse) decreased with decrease in curd pH. The calcium content of centrifugal serum was lower for higher curd pH (Fig. 2*c*). These results reflected the decreased binding of calcium to the curd at lower pH.

Effect of ageing. There was a reduction in the quantity of serum with time for each sample, the decrease being more rapid at lower pH values. The quantity of centrifugal serum decreased even though the pH was fairly constant during the period of study for each sample. For the pH 5·30 curd there was no centrifugal serum on day 13. This was also found for the pH 5·19 curd on days 11 and 13.

The quantity of each casein component in the curd and serum was estimated as the value for the dye bound to the major casein fractions after urea-PAGE. The values for the centrifuged curd and centrifugal serum for the pH 5·30 curd are shown in Fig. 1 (*b. d*). On comparison with the values for the curd made in Trial 1 (Fig. 1*a. c*). it is obvious that there was less change with time in the quantities of casein fractions for the curd made with added GDL in Trial 3. The proteolysis still taking place in the curd was probably due to the Rennilase 46L used in the manufacture.

DISCUSSION

These experiments were carried out to study the changes in the water distribution in cheese curd during the first 2 weeks of maturation. The initial trial based on normal Cheddar cheesemaking showed that the quantity of centrifugal serum from cheese curd. an indicator of the extent of apparent water binding, was influenced by the pH of the curd and usually decreased with time (Table 2).

A subsequent trial was therefore carried out using lactic acid in place of starter. in order to minimize the changes in curd pH during storage. and using Rennilase 46L instead of calf rennet for curd making to ensure a more nearly constant retention of the coagulant enzyme in the cheese curd (Creamer *et al.* 1985). The quantity of Rennilase 46L used was reduced in order to have a similar set-to-cut time to that using rennet. Curd proteolysis was less than in Trial 1 and was similar for all curd samples. The quantity of centrifugal serum from samples of curd made with setting pH values of 6·0 and 5·7 showed no consistent trend during the 2 week period of the study (Table 3). The results are also inconsistent with the observations of Guo & Kindstedt (1995). It is possible that at higher pH values ($> 5{\cdot}65$) the moisture content did not alter with time.

In the third trial, the procedure was altered so that a greater quantity of moisture was retained in each curd sample and the pH values of subsamples of curd were adjusted by mixing predetermined quantities of GDL into the curd subsamples. The results (Fig. 2) showed that the pH values of the subsamples did not alter appreciably with time and predetermined pH values could be attained by using

particular ratios of GDL : curd, and that there were pH- and time-dependent changes in the quantity of centrifugal serum and the calcium content of the serum. Thus the system of curd preparation using lactic acid and Rennilase 46L followed by dicing and mixing in GDL gave curd samples with an appropriate range of pH values and similar enzyme and moisture contents. Such a range of samples was useful to study the effect of compositional parameters on the quantity of water retained in cheese curd.

Although the three experimental trials were not replicated and the conclusions are not definitive, our more recent studies have confirmed the results and the preliminary conclusions in this paper.

In a normal commercial cheese, the variation in the quantity of centrifugal serum with time can be attributed to proteolysis, removal of calcium and the dissociation of casein from cheese (Guo & Kindstedt, 1995), all of which contribute to cheese structure. As the pH decreases from that of normal milk ($\approx$ 6·7), the micellar calcium phosphate begins to dissociate (Van Hooydonk *et al.* 1986). The dissociation of calcium in turn can cause the dissociation of individual casein molecules (Holt *et al.* 1986). Dalgleish & Law (1988) also found that the amounts and proportions of dissociated caseins are pH-dependent.

In our final model system (Trial 3), there was some cheddaring, minimal proteolysis by coagulant (Fig. 1) and minimal pH change, as a consequence of low lactic acid bacterial growth (Fig. 2). Nevertheless, the quantity of recoverable serum decreased as the curd aged, indicating that redistribution processes that may have been a consequence of the impact of salting on the system were occurring.

In any system such as cheese curd, the various components will be distributed so that the system tends to attain a minimum energy. Once this state is attained, the system is considered to be in equilibrium and each region within the system will be indistinguishable from any other region. During the formation of the cheese curd from milk, there are a number of severe disruptions to the system, namely micelle destabilization by rennet action, curd formation with consequent separation of a whey phase, pH decrease with a consequent redistribution of minerals between the casein-bound state in the micelle (and gel protein matrix) and the serum phase, further mineral changes with the admixture of solid salt with the curd and physical deformation of the cheese curd during processing such as cheddaring or stretching. The initial milk can be considered an equilibrium system and adapts to addition of salt, acid and so on, or temperature changes, reasonably quickly. However, once there are two phases, curd and whey, then attainment of a new equilibrium state requires movement of material between the phases as well as within each phase. For example, addition of salt to moist curd may not affect the inner regions of the curd particles for some time because of the diffusion time (Guinee & Fox, 1993). Indications of the final equilibrium positions can be obtained using model systems of finely divided curd material. For example, Van Hooydonk *et al.* (1986) reported that the voluminosity of casein in normal skim milk was at a maximum around pH 5·3 and that it decreased after renneting, and Snoeren *et al.* (1984) observed a relationship between the hydration of casein micelles and pH, with a 'peak' in the voluminosity of the casein micelles at pH 5·45. In a similar study on rennet-treated milk, Creamer (1985) found the peak to be near pH 5·2. Creamer (1985) also noted that addition of NaCl increased the water retention by the chymosin-treated micelles (curd) whereas $CaCl_2$ decreased it. At higher salt concentrations, large quantities of water were retained by the renneted micelles at pH 5·2–5·3. In all these studies, the gel or curd was formed under the final environmental conditions and thus the curd

characteristics were more likely to represent the equilibrium condition that curd might attain when placed in an appropriate environment. The rate of attainment of equilibrium is limited by rates of diffusion of various components within the system and this is much slower in a cheese system than in a renneted micelle model system. The earlier study (Creamer, 1985) indicated that water retention by chymosin-treated casein micelles decreased slightly with decreasing pH in the range from 6·7 to 5·6 but increased as the pH decreased from 5·6 to 5·25 and that water retention increased markedly when the salt concentration was increased. The present results (Fig. 2*b*) indicated that when the casein matrix of the curd was at pH 5·6 the centrifugal water in the curd diminished more slowly with time than for curd at pH 5·2. On the basis of the similarity of this result to that of Creamer (1985), which showed that the equilibrium water content of rennet-treated casein micelles was greater at pH 5·2 than at 5·6, it is likely that the behaviour observed in this study (and that reported for Mozzarella cheese by Guo & Kindstedt (1995)) is a consequence of fundamental properties of the casein matrix in cheese and the extent to which it absorbs water in the presence of NaCl.

In conclusion, the changes in curd properties early in the ripening process in a normal commercial Cheddar cheese appear to be a consequence of the changing pH and continuing proteolysis superimposed on the effect of the reabsorption of centrifugal serum (which was generated by the effects of curd manipulation and the impact of salting) into the casein matrix.

We are grateful to the Ministry of Foreign Affairs and Trade for a Commonwealth Scholarship to C. Ramkumar. Useful discussions with Osvaldo Campanella. Craig Honoré and Philip Watkinson and advice on gel electrophoresis techniques and interpretation from Nicola White and Christina Coker were invaluable during the course of this work.

REFERENCES

AKKERMAN, J. C. 1992 *Drainage of Curd*. PhD thesis. Wageningen Agricultural University, The Netherlands

CREAMER, L. K. 1985 Water absorption by renneted casein micelles. *Milchwissenschaft* **40** 589–591

CREAMER, L. K. 1991 Electrophoresis of cheese. *International Dairy Federation Bulletin* no. 261 14–28

CREAMER, L. K., LAWRENCE, R. C. & GILLES, J. 1985 Effect of acidification of cheese milk on the resultant Cheddar cheese. *New Zealand Journal of Dairy Science and Technology* **20** 185–203

DALGLEISH, D. G. & LAW, A. J. R. 1988 pH-Induced dissociation of bovine casein micelles. 1. Analysis of liberated casein. *Journal of Dairy Research* **55** 529–538

GREEN, M. L. & GRANDISON, A. S. 1993 Secondary (non-enzymatic) phase of rennet coagulation and post-coagulation phenomena. In *Cheese: Chemistry, Physics and Microbiology*, vol. 1. *General Aspects*, pp. 101–140 (Ed. P. F. Fox). London: Chapman and Hall

GUINEE, T. P. & FOX, P. F. 1993 Salt in cheese. Physical, chemical and biological aspects. In *Cheese: Chemistry, Physics and Microbiology*, vol. 1. *General Aspects*, pp. 257–302 (Ed. P. F. Fox). London: Chapman and Hall

GUO, M. R. & KINDSTEDT, P. S. 1994 Age-related changes in the water phase of mozzarella cheese. 2. Evaluation of moisture expelled by centrifugation. *Journal of Dairy Science* **77** (Suppl. 1) 41 (Abstr.)

GUO, M. R. & KINDSTEDT, P. S. 1995 Age-related changes in the water phase of Mozzarella cheese. *Journal of Dairy Science* **78** 2099–2107

HOLT, C., DAVIES, D. T. & LAW, A. J. R. 1986 Effects of colloidal calcium phosphate content and free calcium ion concentration in the milk serum on the dissociation of bovine casein micelles. *Journal of Dairy Research* **53** 557–571

KIMBER, A. M., BROOKER, B. E., HOBBS, D. G. & PRENTICE, J. H. 1974 Electron microscope studies of the development of structure in Cheddar cheese. *Journal of Dairy Research* **41** 389–396

LAWRENCE, R. C., GILLES, J. & CREAMER, L. K. 1993 Cheddar cheese and related dry-salted cheese varieties. In *Cheese: Chemistry, Physics and Microbiology*, 2nd edn. vol. 2. *Major Cheese Groups*, pp. 1–38 (Ed. P. F. Fox). London: Chapman and Hall

NEW ZEALAND MINISTRY OF AGRICULTURE AND FISHERIES 1979 *Chemistry: Standard Laboratory Methods* Wellington, NZ: MAF Dairy Division

OBERG, C. J., MCMANUS, W. R. & MCMAHON, D. J. 1993 Microstructure of Mozzarella cheese during manufacture. *Food Structure* **12** 251–258

SNOEREN, T. H. M., KLOK, H. J., VAN HOOYDONK, A. C. M. & DAMMAN, A. J. 1984 The voluminosity of casein micelles. *Milchwissenschaft* **39** 461–463

TANEYA, S., IZUTSU, T., KIMURA, T. & SHIOYA, T. 1992 Structure and rheology of String cheese. *Food Structure* **11** 61–71

TUNICK, M. H., MACKEY, K. L., SHIEH, J. J., SMITH, P. W., COOKE, P. & MALIN, E. L. 1993 Rheology and microstructure of low-fat Mozzarella cheese. *International Dairy Journal* **3** 649–662

VAN HOOYDONK, A. C. M., HAGEDOORN, H. G. & BOERRIGTER, I. J. 1986 pH-induced physico-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physico-chemical properties. *Netherlands Milk and Dairy Journal* **40** 281–296