

DEVELOPMENT OF LOW FAT AND REDUCED FAT MOZZARELLA CHEESE

A thesis submitted for the degree of Doctor of Philosophy

By

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2004

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Development of low fat and

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cheese

DEDICATED

TO

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ABBREVIATIONS

Organizations

ADC = Australian Dairy Corporation

AOAC = Association of Official Analytical Chemist

ASCRC = Australian Starter Culture Research Centre

FAO = Food and Agriculture Organization

FSA = Food Science Australia

IDF = International Dairy Federation

NHMRC = National Health and Medical Research Council

FDA = United States Food and Drug Administration

Chemicals

APS = Ammonium Persulphate (Ammonium peroxodisulphate)

BCP = Bromocresol Purple

BPB = Bromophenol Blue

Ca = Calcium

CaCl₂ = Calcium Chloride

d.H₂O = Deionised Water

DMSO = Dimethyl Sulfoxide

EDTA = Ethylene Diamine Tetra Acetic Acid

H₂O₂ = Hydrogen Peroxide

HCL = Hydrochloric Acid

K = Potassium

Mg = Magnesium

N = Nitrogen

Na = Sodium

$\text{Na}_2\text{H}_2\text{PO}_4$ = Sodium Dihydrogen Orthophosphate

Na_2HPO_4 = Disodium Hydrogen Orthophosphate

NaOH = Sodium Hydroxide

PAGE = Poly Acrylamide Gel Electrophoresis

SDS = Sodium Dodecyl Sulphate

TCA = Tri Chloroacetic Acid

TEMED = N,N,N',N' - Tetra Methyl Ethylene Diamine. A catalyst for polymerization of acrylamide. It is used with ammonium per sulfate as initiator to prepare poly acrylamide gels.

Tris = Tris (hydroxymethyl) Aminomethane

General terminology

AAS = Atomic Absorption Spectroscopy

Acidity = The condition of the milk, whey or cheese curd at various stages of manufacture, expressed as a percentage of lactic acid present in the sample tested.

ANOVA = Analysis of Variance

Bacteriophage = A virus which parasites bacteria by infecting them and reproducing inside them.

CFU = Colony Forming Units

Cheddaring = Treatment of the curd following removal of the whey, in order to produce a sufficiently dry, firm and acid condition for milling.

Cheese-milk = Milk used for cheese making

Conc. = Concentrated

Curd = A solid mass formed when milk is coagulated.

FDM = Fat in Dry Matter

GRAS = Generally Recognised As Safe

HPLC = High Performance Liquid Chromatography

HTST = High Temperature Short Time.

Hunter a-value (a value) = Redness

Hunter b-value (b value) = Yellowness

Hunter L-value (L value) = Whiteness

Instron = Instron universal testing machine

Lactose = Major carbohydrate found in milk which is readily utilized by lactic acid bacteria to produce lactic acid.

M:P = Moisture: Protein ratio

MRS Agar = Man-Rogosa Sharpe agar

Ripening of milk = Development of lactic acid prior to adding rennet.

Running gel = A 20% poly acrylamide gel within which the proteins are separated by applying an electric charge.

S/M = Salt in Moisture content

SDS-PAGE = Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis

SE = Standard Error

SEM= Scanning Electron Microscope

SNF = Solid-Not-Fat

ST Agar = *Streptococcus thermophilus* agar

Stacking gel = A 4% acrylamide gel used to stack all the proteins in the sample to a very narrow band from where all the proteins enter the running gel.

Starter cultures = Lactic acid producing bacteria which are added to milk to promote acid development.

Stretching of curd = In mozzarella cheese manufacture when the cheddared and milled curd has attained the desired pH (usually 5.2) the curd is dipped in hot water and force is applied in opposite directions on the curd mass to form stretched curd. The curd is then re-massed and

further pulled in opposing directions. After several such operations the curd is considered to be stretched.

TPA = Texture Profile Analysis

TS = Total Solids

ULFM = Ultra Low fat Mozzarella

UV/VIS = Ultra Violet and Visible range of the light spectrum

v/v = volume by volume

w/v = weight by volume

w/w = weight by weight

Whey = The serum or watery part of the milk which remains after the separation of the curd by coagulation.

Measurement units

μ = Micro

μg = Microgram

μL = Microlitre

$^{\circ}\text{C}$ = Degree Celsius

cm = Centimeter

g = Gram

h = Hour

IMCV = International Milk Clotting Units

kg = Kilogram

kJ = Kilojoule

L = Litre

M = Molar concentration

mA = Milliampere

mg = Milligram

min = Minute

mL = Millilitre

mM = Millimolar

mm = Millimeter

mm = Millimeter

MOPS = 3-(N- Morpholino) Propane Sulfonic Acid

ppm = Parts Per Million

rpm = Revolutions Per Minute

sec = Second

V = Volts

ABSTRACT

This study broadly aimed to develop an ultra low fat mozzarella (ULFM) cheese by modifying the traditional manufacturing method used by Dairy Farmers and other dairies; examine the effects of method of salting, use of proteolytic strains of starter cultures, effect of pre-acidification of milk using lactic, acetic and citric acids to pH 6.3, 6.1 and 5.9 on the characteristics of ULFM cheeses; effect of rate and type of fat replacer added to pre-acidified cheese milk on the quality of ULFM cheese, and examine the functional characteristics of shredded ULFM cheeses that were sprayed with vegetable oil and baked on pizza bases.

In the first set of experiments on the effects of dry salting (DS) versus traditional brining (TB) of ULFM cheeses (containing 6% fat) we showed that dry salted cheeses retained more moisture and had increased yield, increased starter culture population during storage and a higher salt content. The dry salted and traditionally brined cheeses showed similar textural characteristics and proteolysis, but had poor meltability and pizza bake characteristics, which improved for both cheeses by extended storage (about 75 d). The results showed that increased proteolysis over prolonged storage period improved the functionality of the ULFM cheeses.

In order to reduce the storage time for ULFM cheeses, the effect of using proteolytic *Lactobacillus helveticus* (Lh) instead of *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lb) on the functionality of mozzarella cheeses was investigated. The use of *L. helveticus* increased moisture, FDM and M:P contents in cheeses compared to those made using *L. delbrueckii* ssp. *bulgaricus*. Due to the compositional changes in cheeses made using *L. helveticus*, there was reduced hardness (increased softness), reduced cohesiveness, increased springiness and increased meltability. These cheeses also had better functionality when baked on pizzas with an increased whiteness (L values), reduced redness (a values) and increased yellowness (b

values). The Lb cheeses had poor melt characteristics and did not fuse completely when baked, had excessive blistering and browning. All the ULFM cheeses showed improvement in pizza bake characteristics due to application of hydrophobic material (i.e. oil). Based on observations, modifications in the manufacturing method and addition of fat replacers to cheese-milk were necessary in order to obtain desirable characteristics of melt, flow, stretch and colour (whiteness/glossiness).

For further improvements in the characteristics of ULFM cheeses, it was necessary to reduce the calcium content. The preliminary studies on estimation of calcium showed that the temperature for treating samples had an effect on the absorbance values recorded by the AAS. The skim milk and whey samples showed an increase in absorbance values with increased temperature of warming at all dilutions. In contrast, the absorbance values increased with increased warming of the cheese samples up to 40°C and then decreased at 50°C. Stretch water having higher calcium ion concentrations showed an indefinite trend to an increase in temperature when the sample concentrations produced absorbance values greater than 1.0, while low calcium containing stretch water did not show any significant change in absorbance values due to warming of the samples. The change in measured absorbance values for the same sample at higher temperatures was possibly due to changes in solution characteristics such as surface tension and viscosity, which alter the aspiration rates and to the level of excitation energy available to the calcium ions. Thus it is important to maintain uniform sample temperature and similar dilution levels during their preparation in order to obtain reproducible and comparable results.

Calcium contents measured at several stages of mozzarella cheese manufacture are important to ascertain the amount of calcium that would be present in the stretched cheese. The calcium content of the stretched cheese would affect its characteristics. Online measurement of calcium content during cheese preparation could give a real time control of the quality of cheese. Our

studies showed that an increase in level of pre-acidification decreased the calcium content in cheeses. Control cheeses made without pre-acidification had the highest levels of calcium. Calcium was observed to leach out into the whey at whey draining stage primarily due to acidification and later into stretch water at stretching stage due to replacement of calcium with sodium ions. Also the physical forces of cutting and stretching had an effect on the calcium retained by cheeses.

Pre-acidification studies using lactic, acetic and citric acid showed that cheeses made from milk pre-acidified using citric acid to pH 6.1 (PAC6.1) instead of using either lactic or acetic acids or at the other two levels of pre-acidification namely pH 6.3 and 5.9 had improved pizza bake characteristics without loss in yield. Cheeses made using milk pre-acidified with acetic acid (PAA) became sticky and were difficult to handle especially when the pre-acidification was carried out to pH 5.9. However, during baking pizzas using PAC6.1 cheeses, the cheese shreds melted but did not fuse properly and there was an excessive browning. The pizzas were indicative of the differences in typical baking characteristics of the cheeses made using cheese-milks that were pre-acidified with lactic, acetic or citric acid.

Pilot-scale cheese manufacture was conducted closely simulating commercial scale of manufacture with the modifications of pre-acidification and addition of either maltodextrin Maltrin M100 or Versagel at 0.25% to cheese-milk. The study was conducted over four days using the facilities available at research and development division of Dairy Farmers. These cheese-making trials provided an insight into the difficulties posed at a commercial scale of cheese manufacture such as pre-acidification and addition of fat replacer. The pilot scale cheese manufacture showed that although yield was similar for all cheeses, Versagel based cheeses had higher moisture and FDM contents compared to Maltrin based cheeses while the latter cheeses had higher protein contents. HPLC analysis was performed on Maltrin based cheeses using a new method for sample preparation. Estimation of DP7 (maltoheptaose)

showed these oligosaccharides to be very low in concentration indicating that most of the oligosaccharides having DP7 or lower were lost into whey while DP10 and above oligosaccharides could remain in the cheese. Measurement of expressible serum showed that the moisture was loosely held in Versagel cheeses and such cheeses continued to show higher amounts of expressible serum even after 35 d. The amount of expressible serum decreased with storage for all the cheeses. Versagel based cheeses had reduced proteolysis while Maltrin based cheeses showed higher levels of proteolysis.

Versagel based cheeses were softer compared to control and Maltrin based cheeses throughout storage and the hardness values for the three cheeses decreased with storage. Control cheeses showed significantly higher cohesiveness at 29 and 44 d compared to Maltrin based cheeses, while the latter had similar cohesiveness values as Versagel based cheeses. Cohesiveness increased with storage for the three varieties of cheeses. Springiness values for Versagel based cheeses were higher than those for control and Maltrin based cheeses and Maltrin based cheeses showed the least springiness. Maltrin based cheeses showed greater meltability than control or Versagel based cheeses.

Pizza bake analyses showed Maltrin based cheeses to have better melt, shred fusion and flow characteristics compared to control and Versagel based cheeses. The latter cheeses showed burning and had several brown blisters on their surface at 29 and 44 d. Application of oil improved shred fusion for all cheeses. Application of oil did not produce any defects in the characteristics of unbaked stored cheeses. Prolonged storage to 80 d improved the melt and shred fusion characteristics of Maltrin based cheeses but the cheeses showed browning which was not desirable. Control cheeses did not show sufficient shred fusion and melt and had intact shreds which scorched during baking. Unlike control and Maltrin based cheeses, the Versagel based cheeses that were applied with oil had improved melt and shred fusion. The cheeses had

improved flow and showed increased whiteness after 80 d of storage. Versagel based cheeses required prolonged storage of 80 d along with oil application to show improved cheese characteristics. A new stretch test using Instron Universal Testing Machine that involved measuring distance of stretch, showed that Maltrin based cheeses had excellent stretch at 55 d of storage, which decreased with storage. Control cheeses also showed similar stretchability as Maltrin based cheeses and had the highest stretchability at 55 d of storage, which decreased at 75 d. Versagel based cheeses achieved maximum stretchability earlier (at 40 d) compared to Maltrin and control cheeses.

In order to improve the functionality of PAC6.1 cheeses, addition of fat replacers at a very low rate was considered. Maltodextrin namely M100 (Maltrin cheese) and fat replacer namely Versagel (Versagel cheese) were added to cheese-milk at 0.1 or 0.25% and mozzarella cheeses were prepared. Improvements in moisture retention were observed with the addition of Versagel and pizza bake characteristics of fat replaced cheeses showed cheeses to be completely melted, whiter and less scorched. Versagel based cheeses showed higher expressible serum content and up to 20 d of storage compared to control and Maltodextrin based cheeses.

Further studies were carried out on several native and modified starches using refractive index measurements, rheometric observations and light microscopy to understand the behaviour of starches to various heat treatments. It was observed that heating the N1658 (National Starch Co.) starches to 90°C caused complete gelatinisation of the starch molecules. This native starch showed very good hydration properties and increased moisture retention resulting in enhanced cheese yield by 12- 13%, and when baked on a pizza base showed excellent melt, shred fusion, flow, glossiness and whiteness similar to the characteristics of a full fat mozzarella cheese.

The stretch test developed at Victoria University was tested using commercial cheeses. The stretchability of the cheeses could be expressed in terms of tex values (grams per 1000 m of stretched cheese) and tenacity (peak load divided by tex) and this method of objectively testing stretchability of cheeses was reproducible and can be carried out with great reliability. The test is easy to perform and is not time-consuming.

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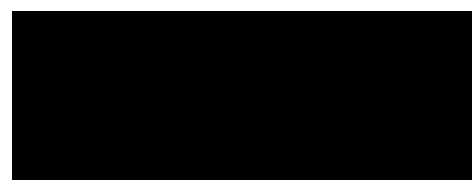
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CERTIFICATE

This is to certify that the thesis entitled "DEVELOPMENT OF LOW FAT AND REDUCED FAT MOZZARELLA CHEESES" submitted by Raman K. Bhaskaracharya in partial fulfilment of the requirements for the award of the Doctor of Philosophy in Food Technology at the Victoria University is a record of bonafide research work carried out by him under my personal guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Werribee, Australia

Date: 8.8.2024



(Dr. N. P. Shah)

Principal Supervisor

ACKNOWLEDGEMENT

I extend my sincere gratitude and appreciation to many people who made this thesis possible. I am deeply indebted to my supervisor Professor, Dr. Nagendra P. Shah for devoting time and efforts in guiding me through the doctoral program. The proverb “Work like you don’t need the money, love like your heart has never been broken, and dance as if no one is watching” explains quite aptly the attitude that has been inculcated and developed during my research activities working under the supervision of Dr. Nagendra Shah. His understanding attitude especially during my frustrating time and his ability to see through problems are highly inspiring. I also wish to thank Mr. Gary Harvey and the Dairy Farmers research and development team including Dr. Mohan Bhargava, Mr. Damien Mead and Mr. Chris Ryan for allowing me to carry out pilot scale trials at Dairy Farmers facilities and enabling me to understand the commercial practices. I shall be ever grateful to Mr. Roderick Williams and Ms. Sandani Udabage of Food Science Australia, Melbourne, Victoria, Australia, for letting me use the Instron universal testing machine. I wish to thank Mr. John Near and Mr. John Crosher of Gilbert Chandler College, Melbourne University, Melbourne, Victoria, Australia, for allowing me to use the cream separator whenever I had to separate milk. I am grateful to Mr. Jeff Mayes and Mrs. Jennifer Favaro of Food Science Australia, Melbourne, Victoria, Australia, for providing me access to use their facilities and also for holding discussions on the most recent research articles on mozzarella cheese.

I wish to thank Mr. Dale Tomlinson and all the laboratory technicians especially Mr. Joe Pelle, Mrs. Iravati Prasathya, Mrs. Stacey Lloyd for being ever ready to provide me with valuable suggestions and assistance in organising, conducting experiments and during conducting sessional demonstration. I wish to acknowledge with much appreciation the crucial role of

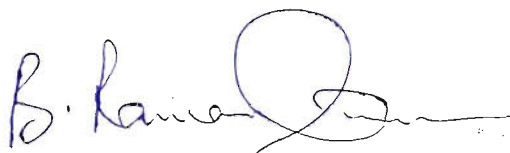
Brian Tyrell and all the library staff at the School of Life Sciences and Technology, Victoria University, Werribee campus, Melbourne, Victoria, Australia.

I also wish to thank my colleagues including, Mr. Ankur Desai, Ms. Alexandra Stevens, Mr. Dimitri Tsangalis, Mr. Frank Bruno, Mr. Bogdan Zisu, and Mr. Thanut Amatayakul, who have been more than a friend to me throughout my doctoral work. There are many people whose names are not mentioned here and I wish to express my gratitude to all of them for making this project a very good experience for me.

Special thanks to Mrs. Maria Lettieri Noto and her family, Mr. Ranjan Sharma and his family for their support and for making me feel at home in Australia. I also appreciate the friendship and brotherly advice from Late Mr. Sanjiv Kumar Das. Last but not the least, I am very grateful to my parents Shri. B. Mohan and Smt. B. Rajeswari for showering me with their love and affection. I cannot conclude without appreciating my younger brother Dr. Madan Bhaskaracharya, families of my elder brothers Mr. Kiran Bhaskaracharya and Mr. Arun Bhaskaracharya for making me feel special everyday.

I feel it was a great privilege for me to have worked on this project and thank the financial support provided by Dr. Nagendra Shah from Victoria University and Mr. Norm Reynolds and Mr. Gary Harvey of Dairy Farmers.

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LIST OF PUBLICATIONS

Refereed Research papers

1. Bhaskaracharya, R.K. and Shah, N.P. 2001. Mozzarella without the fat. *Dairy Industries International* 66 (12): 22- 26.
2. Bhaskaracharya, R.K. and Shah, N.P. 2001. Cultures and Coagulants- Texture and microstructure of mozzarella. *Dairy Industries International* 66 (2): 42- 45.
3. Bhaskaracharya, R.K. and Shah, N.P. 2001. Texture and microstructure of skim milk mozzarella cheeses made using fat replacers. *Australian Journal Dairy Technology* 56 (1): 9- 14.
4. Bhaskaracharya, R.K. 2000. Master of Science thesis entitled “The texture and microstructure of mozzarella cheese as affected by fat content, EPS producing starter culture and fat replacers”, School of Life Sciences and Technology, Victoria University, Melbourne, Australia.
5. Bhaskaracharya, R.K. and Shah, N.P. 2000. Texture Characteristics and microstructure of skim milk mozzarella cheese made using exopolysaccharide or non-exopolysaccharide producing starter cultures. *Australian Journal Dairy Technology* 55 (3): 132- 138.
6. Bhaskaracharya, R.K. and Shah, N.P. 2000. A simplified method for examination of microstructure of mozzarella cheeses with scanning electron microscopy. *Australian Journal Dairy Technology* 55 (1): 28- 32.
7. Bhaskaracharya, R.K. and Shah, N.P. 1999. Texture evaluation of commercial mozzarella cheeses. *Australian Journal Dairy Technology* 54 (1): 36- 40.

Conference Presentations

1. Bhaskaracharya, R.K.* and Shah, N.P. 2002. Fat replacers in low fat mozzarella cheese system. A paper presented at the Young Members Presentation Evening conducted by Australian Institute of Food Science and Tehnology Inc. Victorian Branch, sponsored by Dragoco Australia Pty. Ltd. and held in Melbourne, Australia on 14 October 2002.
2. Bhaskaracharya, R.K.* and Shah, N.P. 2002. Effect of temperature on measurement of calcium concentration in milk, mozzarella cheese and whey. A poster presented (poster no. 0686) at Congrilait 2002 of the 26th IDF World Dairy Congress held in Paris, France, September 24-27, 2002.
3. Bhaskaracharya, R.K.* and Shah, N.P. 2002. Effects of method of salting on yield, starter bacteria population, ad textural and melt characteristics of low-fat mozzarella cheeses. A poster presented at Cheese Science 2002 conference held in Melbourne, Victoria, Australia, July 10-12, 2002. (*Aust. J. Dairy Technol.* 57(2): 169).
4. Bhaskaracharya, R.K.* and Shah, N.P. 2002. A new method of measuring stretchability of low-fat mozzarella cheese. A poster presented at Cheese Science 2002 conference held in Melbourne, Victoria, Australia, July 10-12, 2002. (*Aust. J. Dairy Technol.* 57(2): 168).
5. Bhaskaracharya, R.K. and Shah, N.P.* 2002. Texture and melt characteristics of low-fat mozzarella cheeses made using pre-acidified milk. A poster presented in session 15B-2 at the Institute of Food Technologists Annual meeting and Food Expo of The Society for Food Science and Technology held in Anaheim, California, USA, June 15-19, 2002. (www.am-fe.ift.org/t2p5.php).
6. Bhaskaracharya, R.K. and Shah, N.P.* 2000. Texture and microstructure characteristics of skim milk mozzarella cheeses made using carbohydrate based fat replacers. A paper presented at the International Dairy Federation Symposium on Cheese Ripening and

- Technology held in Banff, Canada, March 13-16, 2000. (*IDF Symposium Abstr.* 2000 VII-03: 38-39).
7. Bhaskaracharya, R.K. and Shah, N.P.* 1999. Ultrastructure of mozzarella cheese made using fat replacers. A poster presented at the 10th World Congress of the International Union of Food Science and Technology held in Sydney, NSW, Australia, October 3-8, 1999. (10th World Congress of Food Science and Technology Abstracts P13/20: 84).
 8. Bhaskaracharya, R.K.* and Shah, N.P. 1999. Microstructure and texture characteristics of low fat mozzarella cheeses made using carbohydrate based fat replacers. A paper presented at the 94th Joint Annual Meeting of the American Dairy Science Association held in Memphis, Tennessee, USA, June 20-24, 1999 (*J. Dairy Sci.* 82(Suppl. 1): 15-16).
 9. Bhaskaracharya, R.K.* and Shah, N.P. 1998. Ultrastructure of a full fat mozzarella cheese made with EPS producing starter culture. A paper presented at the 93rd Joint Annual Meeting of the American Dairy Science Association and American Society of Animal Science, Denver, Colorado, USA, July 27-31, 1998 (*J. Dairy Sci.* 81(Suppl. 1): 12).
 10. Bhaskaracharya, R.K. and Shah, N.P.* 1998. Texture analysis of mozzarella cheese. A paper presented at the 93rd Joint Annual Meeting of the American Dairy Science Association and American Society of Animal Science, Denver, Colorado, USA, July 27-31, 1998 (*J. Dairy Sci.* 81(Suppl. 1): 11).
 11. Bhaskaracharya, R.K.* and Shah, N.P. 1998. Microstructure of full fat mozzarella cheese. A poster presented at the Cheese Science'98 Conference, Melbourne, Victoria, Australia, July 1-3, 1998 (*Aust. J. Dairy Technol.* 53(2): 129).
 12. Bhaskaracharya, R.K.* and Shah, N.P. 1998. Texture evaluation of some commercial mozzarella cheeses. A paper presented at the 31st Australian Institute of Food Science

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(Paper No. A20).

*Presenting author.

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1.0 INTRODUCTION

Consumers are concerned about their consumption of fat from cheese and other dairy products (ADC, 1996; USDA, 1991; Tunick *et al.*, 1993 a, b). In a survey, 23% of the respondents reported that they had stopped eating dairy products because of their high fat content (Barr, 1990). Sales of reduced fat cheeses have reportedly increased by 9% during 1995/96 (ADC, 1996). There is a great deal of emphasis on reduction in fat intake due to research showing significant correlation between fat intake and mortality rates from coronary heart disease (Renaud and deLorgeril, 1992).

The importance relating to reduction in fat intake has led cheese industries to look for ways and means of manufacturing low fat, reduced fat or no fat cheeses. Full fat mozzarella cheeses have a fresh, acidic, delicate whole milk taste and soft body. Such cheeses show a good melt, proper flow and stretch characteristics when heated. These characteristics are mainly related to the fat content of the cheeses (Bhaskaracharya, 2000). Reduction in the fat content of mozzarella causes a decrease in flavour perception, rubbery and hard body depending upon the extent of fat reduced. Low fat mozzarella cheeses have successfully been manufactured containing 18% fat, but any further reductions caused poor textural and functional characteristics. The more butterfat that is skimmed from the cheese the firmer it becomes (Burros, 1992). In mozzarella cheeses, the flavour is of secondary importance whereas stretchability and melting characteristics are of primary importance. There is also a requirement for minimal fat leakage during cooking. Although low fat cheeses are available in the market, their textural qualities have been adversely affected by reduction in fat level or substitution of vegetable oil for milk fat (Malin *et al.*, 1993). A few workers have studied manufacture of imitation cheeses, low fat high moisture mozzarella cheeses and fat substituted mozzarella cheeses without much success. Previous studies using protein based (Simplese

D100 and Dairy lo), and carbohydrate based (Stellar 100X and Novagel RCN-15), fat replacers have achieved some success, especially when protein based fat replacers were used (Fife *et al.*, 1995).

This study broadly aimed to develop ultra low fat mozzarella (ULFM) cheeses by using proteolytic strains of starter cultures and by addition of carbohydrate or protein based fat replacers. The study also aimed to examine the functional characteristics of shredded ULFM cheeses that were sprayed with vegetable oil and baked on pizza bases.

The principal aims of the study were:

- (1) to examine the physical, compositional, textural and functional characteristics of ULFM cheeses made using traditional brining or dry salting methods,
- (2) to assess the characteristics of ULFM cheeses made using the combination starter culture of *S. thermophilus* and *L. delbrueckii* spp. *bulgaricus* or *L. helveticus*,
- (3) to study compositional, textural and functional characteristics of ULFM cheeses made using cheese-milk pre-acidified to pH 6.3 with citric, acetic or lactic acid. Further to study ULFM cheeses made using cheese-milk pre-acidified to pH 6.1 or 5.9 with citric acid or acetic acid for their compositional, textural and functional characteristics,
- (4) to investigate the effects of incorporating carbohydrate or protein based fat replacers on textural and functional characteristics of ULFM cheeses, and
- (5) to examine modifications in manufacturing steps while incorporating fat replacers into pre-acidified cheese milk to develop a ULFM cheese, which has the characteristics of full fat mozzarella.

Chapter 2 of this thesis contains the review of literature, chapter 3 contains materials and methods used in all the experiments, and results are given in chapters 4 to 9. Chapter 4

particularly, contains results from the study of the effects of method of salting on cheese characteristics, chapter 5 shows effects due to addition of proteolytic starter cultures on characteristics of ULFM cheeses, chapter 6 describes quantification of calcium using AAS and role of calcium in mozzarella cheese characteristics. Chapter 7 gives an overview of the effects of pre-acidification to pH 6.3 using lactic, citric or acetic acids on characteristics of ULFM cheeses. Chapter 8 illustrates effects of pre-acidification with citric acid to 3 levels of pH on the ULFM cheeses, chapter 9 focuses on effects of pre-acidification with acetic acid to 3 levels of pH on the ULFM cheeses, and chapter 10 describes effects of addition of two fat replacers to cheese-milk on the characteristics of ULFM cheeses made at a pilot scale, while chapter 11 includes effects of addition of fat replacers to cheese-milk on the characteristics of ULFM cheeses made with laboratory scale facilities. The overall conclusions have been included in chapter 12. Chapter 13 deals with future research directions, chapter 14 lists the references cited, and the appendices are included in chapter 15.

2.0 LITERATURE REVIEW

The aim of cheese making in 'Pasta Filata' is achieving the smooth texture and grain in cheese through a skilful stretching of curd in hot water (Jana and Upadhyay, 1991). Mozzarella cheeses are produced from whole or partly skimmed milk to which small amounts of starter or organic acids are added, followed by a milk coagulator, rennet extract. The curd formed is cut, and allowed to matt. Exposure of the drained curd to warm temperatures permits a mild acid ripening to pH 5.2; at this pH the curd is heated in hot water, stretched or mixed and moulded. The cheese is then slightly salted (Kosikowski and Mistry, 1997).

A typical full fat mozzarella cheese should have desirable characteristics of a fresh to slightly acidic flavour, firm body without excessive firmness, salty taste and white to slightly yellowish colour depending upon the source of milk whether cow or buffalo milk. Moreover, the mozzarella cheese should show the desired functional characteristics of meltability and stretchability upon heating in an oven, although with reduced oiling off. Such characteristics are also important in low fat mozzarella cheese without which the product will not be accepted in the market. An acceptable ULFM cheese with fat content lower than that of the part skim cheeses has the potential of finding an important market among light dairy products (Tunick *et al.*, 1993a, b). The composition of the mozzarella cheeses plays an important role in the functional characteristics especially the fat content. A typical Australian full fat mozzarella cheese contains 45% moisture (equivalent to MNFS of 60%), and 25% fat (equivalent to FDM of 45%).

In Figure 2.1 the domestic cheese production in Australia since 1994 is shown (ADC 2002). There is a distinct increase in the amount of cheese that is produced in Australia. This is

because of the good manufacturing practices and exceptional quality of the cheese produced in this country.

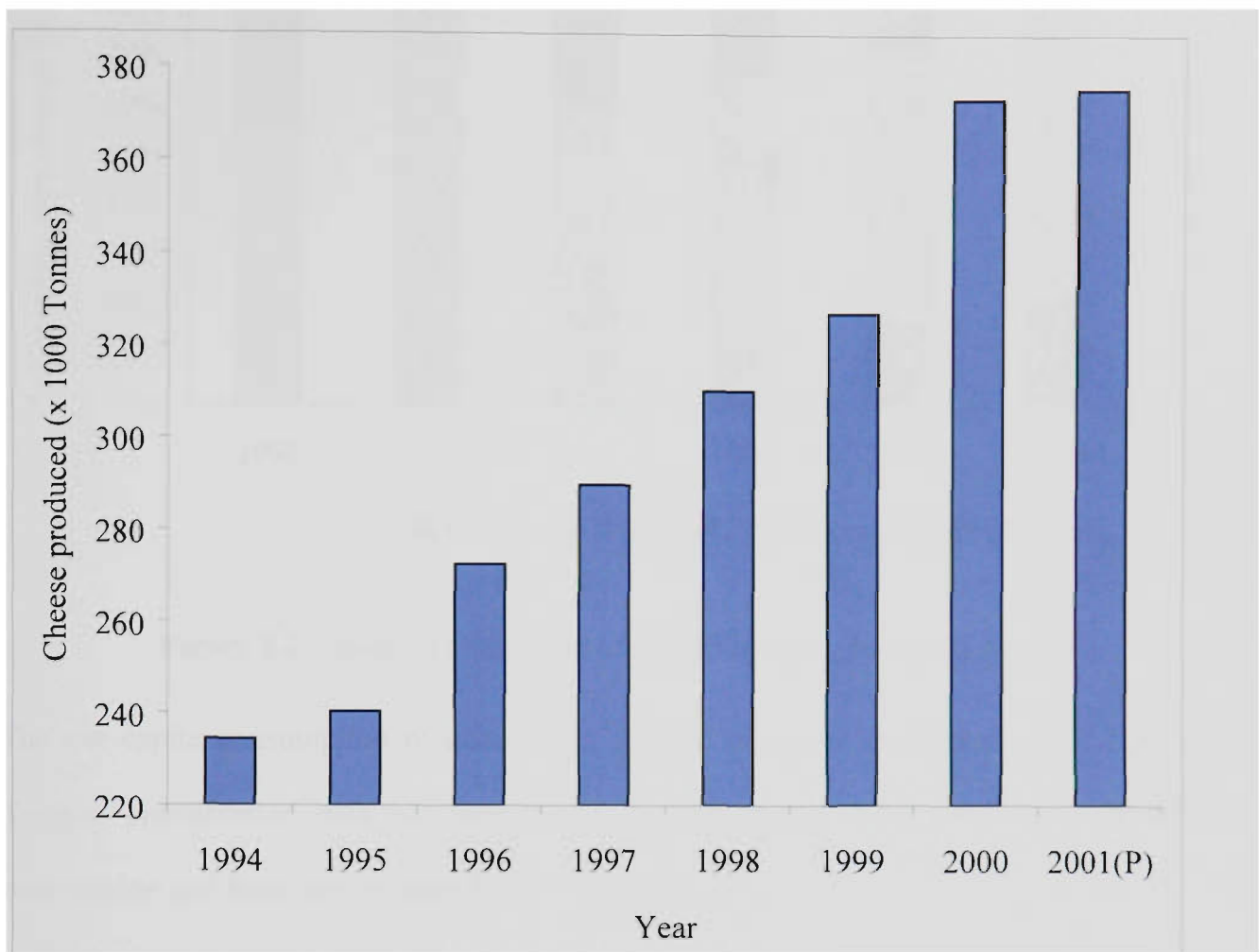


Figure 2.1 Trends in cheese production in Australia since 1994.

Figure 2.2 shows statistical information on the production of mozzarella cheese, pizza cheese and other shredded varieties of cheeses in Australia since 1996 (ADC 2002). Among the three varieties of cheeses, mozzarella cheese variety has been increasing in volumes of cheese produced while the pizza and shredding varieties of cheeses show a decreasing trend in production. This trend is projected to continue in the coming years.

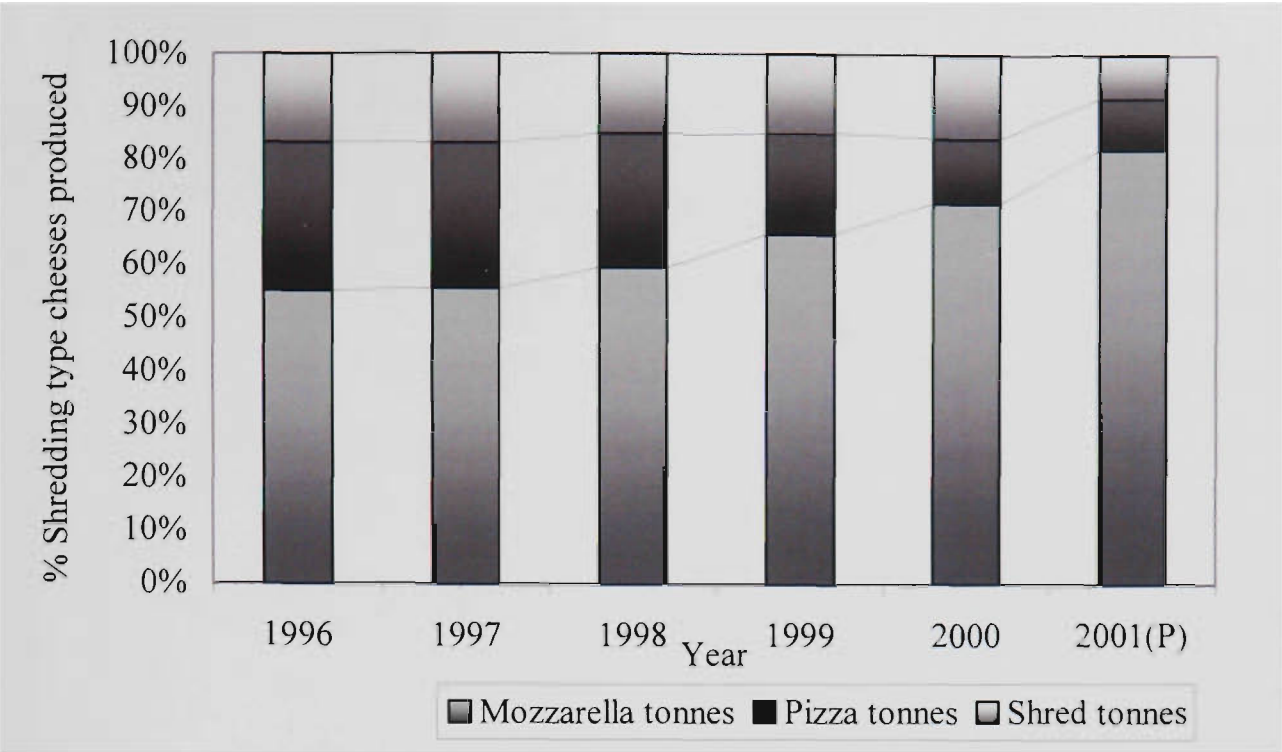


Figure 2.2 Trends in production of shredding type cheeses in Australia.

The per capita consumption of cheese and yoghurt is shown in Figure 2.1.3 (ADC 2002). Yoghurt consumption data has been based on supermarket sales only from 1994/95 and supermarket and food service sales from 1999/2000.

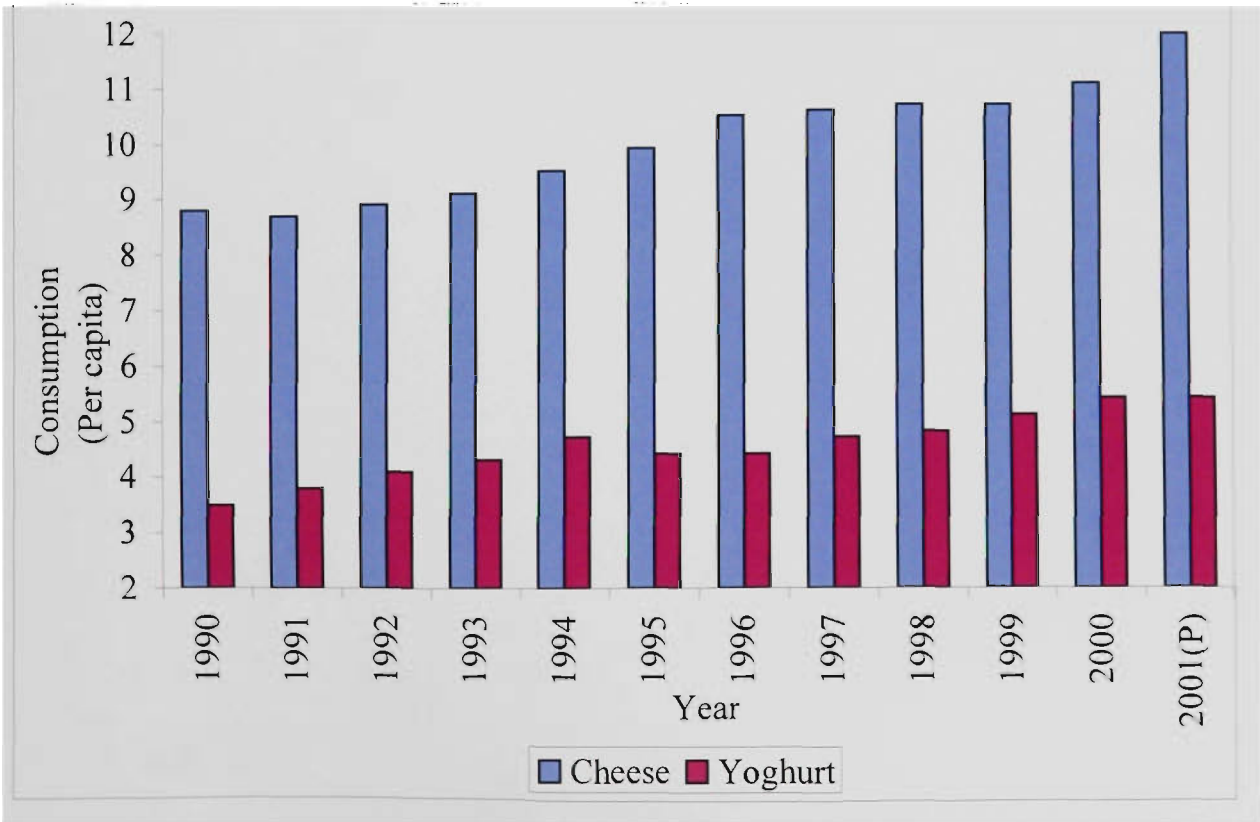


Figure 2.3 The trends in per capita consumption (kg/head) of selected fermented dairy products in Australia.

The above figure shows that among the most commonly consumed fermented dairy products, cheeses seem to be much more preferred compared to yoghurts and this is due to the nature of cheeses as they can be consumed as an ingredient in several foods. The trend depicted in Figure 2.3, clearly shows that without major changes in cheeses such as development of low fat varieties, still, the consumption of cheeses has consistently increased.

A more simplistic approach towards analysing consumption of milk (ADC 2002) is shown in Table 2.1. This table shows the trends in consumption of reduced fat milks and low fat milks in relation to the full fat milk. There has been a trend towards consumption of low fat milk, at the cost of plain full fat milk. This shows that the consumers are switched over to low fat milk products and consider fat in a negative light.

Table 2.1 The changing trends of fat consumption through liquid milk in Australia.

Year	Plain White milk	Reduced fat milk	Low fat milk
1993-1994	1206	329	104
1994-1995	1217	332	111
1995-1996	1195	336	113
1996-1997	1163	352	120
1997-1998	1125	359	130
1998-1999	1111	358	141
1999-2000	1079	354	144
2000-2001(P)	1072	360	139

Source: ADC 2002.

In Figure 2.4 the amount of cheese that is domestically used and exported from Australia is shown (ADC 2002). This figure illustrates that the exports of cheeses have been increasing and

there is a huge market for Australian cheeses. In order to increase the local sales for the health conscious consumers there is a need to develop low fat mozzarella and newer varieties of low fat cheeses.

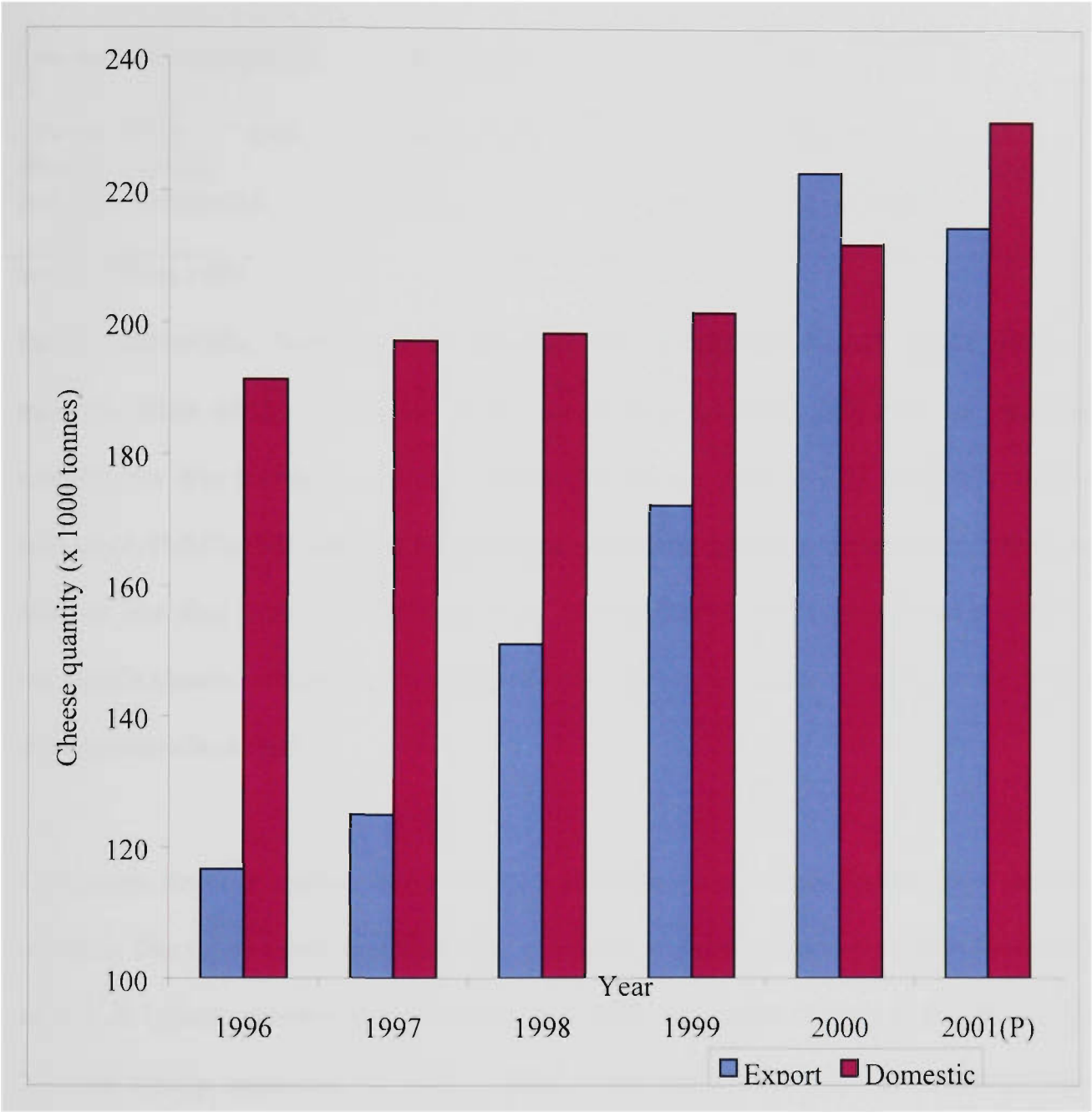


Figure 2.4 Cheese exported and used domestically in Australia.

The regulatory constraints have defined and set standards closely based on the NHMRC food standards code (NHMRC, 1987). The standards for mozzarella, low moisture mozzarella, low moisture part skim mozzarella and park skim mozzarella cheeses in USA are shown in Table 2.2 (FDA, 1989).

Table 2.2 Mozzarella cheese standards for moisture and FDM expressed as percentage.

Type	Moisture (%)	Fat in dry matter (%)
Mozzarella	>52 but ≤60	≥45
Low-moisture mozzarella	>45 but ≤52	≥45
Low-moisture part skim mozzarella	>45 but ≤52	≥30 but <45
Part skim mozzarella	>52 but ≤60	≥30 but <45

Source: FDA, 1989.

Full fat mozzarella cheeses in Australia must have a minimum of 45% FDM and 52-60% moisture. Most of the mozzarella cheese produced in the USA falls into the pizza cheese category, i.e. low moisture part skim mozzarella cheeses with 30-45% FDM and a moisture content of 45-52%. The shreddable type mozzarella must contain a minimum of 50% moisture and not less than 40% FDM (Oberg *et al.*, 1993). Merrill (1994) developed a reduced fat mozzarella cheese with similar stretchability and melting characteristics as low moisture part skim mozzarella cheese.

Consumers desire a product based on their perception and consciousness about health and nutrition. Due to the health consciousness, people have started to recognize fat in food products as bad. A typical comparison of the nutritional value of several cheeses is provided in Table 2.3. The survey conducted by ADC (2000) of cheeses, (Table 2.3) shows that increase in protein at the cost of fat (which is highly desirable) without a major loss in energy is possible but the products turn out to be very expensive if this trend of substitution of protein for fat is continued. Rather, a substitution of carbohydrate in place of fat would be more economical and commercially viable. The latest trend is substitution of fat with fat replacers, which could be fibre (no energy value), carbohydrate (3.0 kj/g), protein (3.0 kj/g) or vegetable fat (low in saturated fatty acids).

Table 2.3 Average nutritional value of mozzarella, reduced fat mozzarella, cheddar and reduced fat cheddar cheeses.

Cheese Type (per 100g)	Protein (%)	Fat (%)	Lactose (%)	Energy (kj)	Calcium (mg)	Sodium (mg)	Cholesterol (mg)
Mozzarella	5.3	4.6	0.04	260	163	73	14
Reduced fat mozzarella	6.2	3.5	0.01	240	190	116	15
Cheddar	5.0	6.7	0.02	336	156	129	20
Reduced fat cheddar	6.0	4.8	0.01	272	122	140	17

Source: ADC, 2000.

Several types of reduced fat mozzarella cheeses are available commercially, but their melt, texture and flavour are generally unacceptable. This has limited their uses. Reduced fat mozzarella cheeses manufactured to date typically have less than 30% of the milk fat removed. Removal of more milk fat usually results in very tough curd and poor melt and stretch properties (Brown, 1989).

2.1 Classification of cheeses

Cheeses can be broadly classified based on their composition as:

Extra hard: Parmesan, Romano, etc.

Hard (with eyes): Emmental, Gruyere, etc.

Hard (no eyes): Cheddar, Cheshire, Provolone, etc.

Semi-hard: Edam, Gouda, etc.

Semi-hard and internally ripened with moulds: Roquefort, Gorgonzola, Stilton, etc.

Semi-hard and surface ripened with bacteria: Monterey, Brick, etc.

Soft and surface ripened with moulds: Brie, Camembert, etc.

Soft and unripened: Cream, cottage, mozzarella, ricotta, etc.

Mozzarella cheeses also fall under the category of pasta filata family of cheeses. These cheeses belong to the Italian origin and are considered to be fresh cheeses.

2.2 Manufacture of mozzarella cheese

The art of cheese making which was practised until the turn of the 19th century was carried out at the farm level as a small-scale operation. Although, Fahrenheit had developed the thermometer in the 1730s, the dairyman/ maid was still estimating temperature using a finger or an elbow dipped in the cheese-milk. Due to lack of proper control measures cheeses turned out to be varying to a degree such that at present there are over a 1000 varieties. The acceptance of cheese making as a science provided better control to the process, reduced incidences of spoilage and produced more uniform cheese of better quality.

The art of manufacturing cheese developed because there was a need to conserve the milk solids without wastage and to manage milk, which becomes available especially during the spring flush season. The milk collected by a dairy factory can be separated into the fat phase widely known as cream and the skim milk remaining is used to manufacture milk powders or is standardised by mixing with cream to make cheeses. Cheeses and their products such as processed cheese can be stored for a very long time and in reality some of the ripened varieties of cheeses such as cheddar, provolone and gouda can be sold at a premium thus increasing the profits for the dairy plant. If the dairy plant is unable to utilise the surplus milk that becomes available during flush season, it is forced to sell the excess milk to another processor resulting in a financial loss. The large variety of cheeses that can be manufactured increase the market value of milk, provide better nutrition because of the partially digested proteins and lactose by the starter cultures, provide variety to the consumer and have a wide choice of applications in diet.

A study was conducted by Nilson (1969) to store pasteurised milk for 10 d and manufacture mozzarella cheese from such stored milk. In this study, the mozzarella cheeses manufactured from stored milk showed similar organoleptic and pizza bake characteristics to that of a cheese made from freshly pasteurised milk. Thus, they concluded that holding milk for up to 10 d does not produce defects in the finished mozzarella cheeses. The study appropriately used mozzarella cheese as a model cheese to establish the effect of storage of milk on cheeses in general because mozzarella cheese is a fresh, unripened cheese, which has a bland flavour except for the lactic acid and salty taste and defects in organoleptic analysis would be easily identified.

The process for manufacture of mozzarella cheese can be broadly divided into the following steps:

1. Preparation of cheese milk

The preparation of milk normally involves standardisation of milk, homogenisation, pasteurisation and storage. The two main aims for standardising milk are (i) to achieve a uniform cheese with maximum yield and of good quality, and (ii) to achieve an economical use of all the milk components (Scott, 1998). Other reasons for standardising milk could be to produce cheeses containing different fat contents namely full fat, half fat or low fat and reduced fat; to compensate for the variations in the milk composition due to seasonal and breed varieties; and to be able to use recombined milk when there is lack of local milk supply. Homogenisation is a process by which fat globules in milk are reduced in size and evenly distributed. This reduces the fat separation during storage and gives a whiter colour to the milk. Also during homogenisation the protein network in milk is broken, which do not fuse easily in the curd structure and hydrophilic links are created enabling such curd to retain more moisture (Green *et al.*, 1985). Pasteurisation or heat treatment is given to the milk to improve the biological quality of milk by destroying spoilage microorganisms and some enzymes. Heat

treatment is achieved under controlled conditions to prevent under or over pasteurisation. Under pasteurisation could lead to problems associated with growth of spoilage organisms during cheese making such as gas formation, uncontrollable fermentations in cheese milk and odour formation. McCammon *et al.* (1933), observed a decrease in calcium retained in cheeses including cottage cheeses with a decrease in pasteurisation temperature accompanied by an increase in time of pasteurisation for milk used for cheese making. Pasteurisation at higher temperature or longer time, could lead to denaturation of whey proteins and complex formation with κ -caseins. Such whey protein and κ -casein complexes would inhibit rennet coagulation and lead to losses in whey. HTST method is normally used for achieving the desired temperature of milk i.e. 72°C and held for 15 sec but present day trends indicate use of higher pasteurisation temperatures for shorter time and alkaline phosphatase may not be suitable as an index of heat treatment and other enzymes such as lacto peroxidase and γ -glutamyl transpeptidase, may need to be considered as reference for measuring effectiveness of pasteurisation (Patel and Wilbey, 1989). Such changes in trends of milk processing and their effects on milk need to be considered during cheese-making.

2. Ripening and enzymatic changes for milk

Cheese milk is inoculated with several species of LAB for cheese manufacture. In mozzarella cheese making homofermentative thermophilic starter organisms namely, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* have been used as a combined starter culture. Both organisms transport lactose as such into the cell via a permease system where it is hydrolysed to glucose and galactose by β -galactosidase. Glucose is then metabolised by the glycolytic pathway while galactose is excreted out of the cell. Another homofermentative thermophilic organism i.e. *Lactobacillus helveticus*, when used as a starter culture, has the ability to metabolise galactose via glucose-6-phosphate using the Leloir

pathway (Walstra *et al.*, 1999). Both *S. thermophilus* and *L. helveticus* produce L(+) lactic acid while *L. delbrueckii* ssp. *bulgaricus* produce D(-) lactic acid.

The cheese-milk does not have sufficient amount of free amino acids for extensive growth of lactic acid bacteria that are inoculated. Hence these bacteria should possess the ability to breakdown proteins through proteolytic activity. Proteolysis by the starter bacteria through exocellular proteinases results in hydrolysis of proteins to oligopeptides, which are further hydrolysed into smaller peptides having low molecular weights and amino acids through cell wall associated amino peptidases (Varnam and Sutherland, 1994). Acidification of milk using starter cultures destabilises the initial stable network of α_{s1} -caseins bound with β -casein. Also the calcium linked to κ -casein micelles through the phosphate bond undergoes changes leading to expulsion of calcium. Further acidification causes disaggregation of β -casein and with increase in H^+ ions at low pH, the α_{s1} -caseins and κ -caseins acquire opposite charges and aggregate leading to shrinkage of the network. β -casein and κ -casein are re-incorporated into the casein network and there is increase in hydration and bonding occurs between the caseins. With lower pH calcium is removed almost completely. This shrinkage causes whey expulsion and is termed as syneresis. The presence of β -casein in the casein network increases the hydrophobic interactions with κ -caseins and this could be the reason for reduced water absorptions by the casein network (Varnam and Sutherland, 1994).

In mozzarella cheese manufacture when the pH decreases by 0.1 unit, rennet is added. Rennet (chymosin) is obtained from the abomasum of a young calf. The lining of the stomach is washed, dried, cut into small pieces and then the rennet is extracted with brine for several days. Rennin or chymosin is the enzyme in rennet, which is a sulphur containing protein and one part of it can clot about 5 million parts of milk. During cheese making this enzyme is usually added after dilution with 40 times water. The enzyme is mostly destroyed at the high temperatures

used during stretching of mozzarella, chemicals that may be present in milk and also loses its clotting ability at temperatures lower than 20°C. It has a working temperature range between 30 to 48°C. This enzyme is inactivated in alkaline conditions and works best in acidic milk. Hence, enzyme is added to milk after acidification with starter culture. The amount of rennet added to milk depends on several factors namely, the quantity of milk, temperature and acidity of milk, composition of milk and purity of the enzyme.

Rennin when added to milk cleaves the κ -casein at the phenylalanine-methionine bond (residues 105 and 106) into a macro peptide and a para- κ -casein. The enzyme itself has a negatively charged site, which attaches to the positively charged site between residues 105 and 106. The macro peptide solubilizes in the serum while the para- κ -casein micelles form a new network. This enzyme also hydrolyses some of the peptide bonds around the Phe-Met bond. Some of the enzyme is adsorbed onto para-casein especially at lower pH. The enzyme reaction is of the first order and is dependent on the diffusion coefficient of the enzyme. At lower pH the affinity of the enzyme increases and causes an increase in reaction rate. When the pH is further decreased the enzyme is adsorbed on to the casein and thus its activity is inhibited. The splitting of κ -casein at the phenylalanine-methionine bond is indicated by flocculation, which is partly due to van der Waals forces and mainly due to calcium ions. The calcium ions neutralise the negative charges on casein micelles and form bridges between the para-casein micelles. Also an effect of temperature is the cross-linking of β -casein and α_{s1} -casein, which increases the network formation and at the same time decreases the diffusion rate of the rennin.

3. Curd formation, Cheddaring and milling of curd

The second phase of curd formation after rennet addition is affected by the temperature and calcium ion concentration as already stated. The firmness of curd, also termed curd tension, is dependent on several factors namely-

- Increase in rennet quantity from 0.006% to 0.03% increases the curd tension and further increases in rennet do not affect this property.
- Increase in curd temperature up to 40°C increases the curd tension while further increases in temperature cause a decrease in curd firmness.
- Composition of milk including casein to fat ratio, total fat content, total calcium content influence the curd properties. Increase in fat content or casein: fat ratio decreases firmness of curd while increase in calcium makes the curd firmer.
- Amount of rennet inhibiting agents such as whey proteins present in the milk could also decrease the curd tension.
- Below pH 5.8 curd tension decreases and hence renneting is usually carried out between pH of 6.5 and 5.9.

During the second phase the milk is not disturbed and the casein micelles are allowed to coalesce. Approximately 6% of the rennin is adsorbed in the casein and remains active causing less specific proteolysis of α -casein and β -casein (Scott, 1998). The curd achieves a desired firmness in about 20- 30 min. The curd firmness is usually measured by the knife test wherein an incision is made in the set-curd and a knife is inserted at a slant into the set curd and tilted upwards to show a clean cut curd with clear whey separation. Tranchant *et al.* (1999) observed a decrease in apparent hydrodynamic diameter of casein particles when exposed to increasingly acidic conditions. The effect was related to a gradual collapse of the surface layers of κ -casein from around the micelles, along with a reduction in particle stability. Further studies are being carried out to ascertain the combined effect of acidification and renneting on the gelation properties of milk using dynamic rheometry (Tranchant *et al.*, 2002).

The curd, which is a gel formed by cross linking of casein micelles behaves like a particle gel and consists of chains of casein micelles about 10 micelles long, with intermittent thicker

nodes. The particle gel is porous with about several micrometers width of pores through which moisture leaks out with increase in acidification. Depending upon the pH of the milk gel the cross-linking is either strong or weak and increase in acidification increased the reactive sites on the caseins micelles. The reactive sites on the micelles would form hydrophobic bonds between the micelles and the network of micelles becomes tighter or closer. This in effect reduces the number of sites available for hydrophilic bonding, and the hydrophilic bonding tends to be the weakest at about pH 5.25. At lower temperature there is stronger hydrophobic bonding between the casein micelles, which could be due to either increase in the number of hydrophobic bonds formed or increased strength of each bond. During syneresis the casein micelles, which have several reactive sites, tend to form new hydrophobic bonds after local hydrophilic bonds (weaker bonds) are broken, which allows syneresis. Largely caseins when bonded are unable to move, are held tightly and are retained in the gel network. The tendency of breaking and forming new bonds continues until the caseins are compacted and strongly held (Walstra *et al.*, 1999). Any further changes in pH or temperature will continue to alter the strength of the hydrophobic bonds and modify the location of each casein particle within the gel leading to making more bonds and stronger ones. The amount of syneresis determines the moisture content in the finished cheese.

The size of curd cubes was found to have a positive effect on the mineral retention. Large sized curd cubes retained more minerals possibly due to reduced surface area from which minerals could leach (Wong *et al.*, 1975). The curd after cutting is allowed to heal during which matting of the curd particles occurs. When the curd is cut it is soft and care needs to be taken in gently stirring the curd avoiding curd shattering until a major portion of the whey is expelled out of the curd particles. This reduces yield losses due to curd fines produced from shattering of curd cubes. Increase in whey expulsion leads to contraction of the curd cubes that is also governed by the temperature, acidity and agitation. The curd cubes slowly form a film on the surface,

which leads to case hardening. High temperatures at the time of cooking causes increased case hardening and leads to soft center and water logging in the curd is observed, which during further processing increases the casein losses, while very low cooking temperatures cause a very thin film formation and the whey is not expelled rapidly from the curd. Once the curd is firm enough, stirring can be increased to uniformly cook the curd with a gradual increase in temperature to 40°C (Kosikowski and Mistry, 1997).

Pitching of the curd is carried out when the cubes are sufficiently firm. This refers to piling the curd at the bottom of the cheese vats before whey is drained (about 25- 30 min from the time coagulum was cut). The pH at whey draining is important to remove calcium from the curd and also for sufficient shrinkage and desirable consistency of the curd cubes. The solid curd also known as junket obtained at the end of whey draining is cheddared. Cheddaring involves packing the curd, turning and piling and re-piling. Packing curd involves forming the curd into heaps with a channel in between for continuous whey separation, which results in two long slabs of curd. These slabs are cut into strips or blocks and left in the cheese vats covered. Every 15 min the blocks of curd are turned upside down and allowed to expel whey. Upon removal of a major portion of the whey from the cheese blocks, the blocks are kept one above the other, called piling and when such piled blocks are turned over it is termed as re-piling.

Cheddaring of curd is important for controlling the moisture content in the finished cheese. The syneresis from the blocks depends on the amount of pressure applied on the surface rather than the endogenous pressure within the coagulum. The latter pressure has been shown to be very small (Walstra *et al.*, 1999). The external pressure due to re-piling increases deformation forces within the curd and breaks some of the bonds between the casein particles. The breaking of bonds as explained earlier causes syneresis and compactness of the casein particles increases and new bonds are again formed between the casein particles. The fat globules remain trapped

within the casein network and only serum along with some serum proteins is expelled. Although fat globules have no direct role in formation of coagulum, it remains interspersed in the casein particle gel and is believed to increase the hydrostatic restriction of the network adjacent to globules (Varnam and Sutherland, 1994).

Completion of cheddaring is indicated by mellowing of the curd, which then has a silky feel, rubbery texture, acidic flavour and pH is about 5.3. The cheddared curd is then milled. Milling is the operation where curd is cut into strips and then further chopped into smaller pieces using a cheese mill or similar devices. This promotes further whey removal and aids in uniform salting if the curd is dry salted. Along with these major advantages milling also helps to cool the curd and allows rinsing the curd (optional). The milled curd is salted by direct sprinkling of common salt on to the curd pieces. Salting helps to further remove moisture and shrink the curd, and if applied at higher concentrations retards growth of starter organisms, although *L. helveticus* is salt tolerant up to about 2% thereby limiting lactic acid formation. Application of salt also gives a salty taste to the curd.

Stretching of the milled and salted curd is carried out in hot water (60-65°C). The time at which the curd is ready for stretching is determined by testing a piece of curd in hot water for string formation. Initially the curd is kneaded in hot water to raise the temperature of the curd to about 55°C. After tempering, the curd is applied with shearing forces, which causes elongation of the protein strands. During this stage there is transfer of serum contents to the stretch water, and the 3-dimensional network of protein matrix is changed into a network of protein fibres that are aligned parallel to each other with the serum trapped between them (McMahon *et al.*, 1993).

High stretching temperatures have been shown to cause an increased loss of fat globules but generally these fat globules remain trapped along with the serum in between the bundles of protein fibres. The temperature and duration of stretching have an impact on the survival of starter bacteria and coagulant. These will affect the rate and extent of proteolysis in the stored cheese. Also some of the native milk proteinases such as plasmin are enhanced when the curd is heated to 50°C while cooking in whey (Farkyne and Fox, 1990). Although the pH at whey draining is definitely higher than during stretching there is a combined effect of temperature, low pH and salt affecting the survival of plasmin, reports have suggested that plasminogen is more readily converted to the active form of plasmin at a higher temperature and there is some residual activity of plasmin in the stored cheese (Creamer, 1976). Moreover, the temperature, auger speed and duration of stretching all play important roles in determining the yield. Barbano *et al.* (1994) observed an increase in fat and moisture losses with higher auger speed at a particular temperature or with higher temperature when keeping the auger speed constant. The alignment of cheese constituents has been suggested to be the reason for the differences in properties of the stretched curd when evaluated parallel vs perpendicular to the fibre orientation (Ak and Gunasekaran, 1997). The stretchability of curd depends on the pH and calcium content. Thus a directly acidified mozzarella can stretch at a higher pH approximately 5.6, while a cultured cheese requires to be acidified to a lower pH of 5.2.

The observations by McMahon *et al.* (1999) of the microstructure of mozzarella cheese indicated that the proteins formed smooth walled fibres when heated and stretched and these fibres were interconnected and separated by channels containing serum, water-soluble cheese components and molten fat. These observations were made for stretched curd while it was hot. The bacteria were also found to be located within the serum channels and were held in the internal walls of the protein fibres (Bhaskaracharya and Shah, 2000a). The curd matrix before stretching has an uneven distribution of serum and fat globules, which during stretching are

more evenly distributed between the protein fibres. Due to the molten state of the fat globules immediately after stretch there is very little resistance to the protein fibres from coalescing, by the fat. But, upon cooling fat globules solidify quickly while the pliable protein fibres tend to fuse together around them. The fusion of protein fibres is probably caused by increased hydrophobic interactions and continues during storage of the mozzarella cheese, only interrupted by solid particles such as bacteria and fat globules, which cause indentations in the protein fibres. Such closely packed indentations have been found to become orientated during extrusion and molding of the cheese prior to packing. McMahon *et al.* (1999) suggested that the size and amount of fat/serum channels is a function of fat content of the mozzarella rather than the moisture content. Any excess moisture located in the fat serum channels would be pushed out of the cheese matrix, which was attributed as the reason for low moisture contents in reduced fat mozzarella cheeses.

4. Storage

During storage there is a redistribution of protein and water and intact fat globules. In the first few days after manufacture the protein matrix has clear, round indentations of fat which due to the physico-chemical changes become deformed and a honey-comb like structure is developed by day 14. Further storage has been shown to reduce the interstitial spaces with the re-adsorption of the serum into the protein matrix (McMahon *et al.*, 1999). Thus the fresh mozzarella cheese shows expressible serum (about 30% of the total moisture) upon centrifugation, which reduces to zero within two weeks of storage. During the first two weeks of storage, Kindstedt and Guo (1997) observed an increase in the concentration of intact α_s -casein and β -casein with no change in whey proteins (α -lactalbumin, β -lactoglobulin and serum albumin) in the expressed serum. They also found an increase in calcium concentrations in the serum and attributed it to phenomena of dynamic interaction of para-casein with the serum phase involving transfer of colloidal calcium and intact caseins into the serum until an

equilibrium was reached. The dynamic interaction of protein matrix and serum phase was affected by the presence or absence of salt with an increase in loss of calcium and intact caseins with the presence of salt in mozzarella cheese.

A lot of research has been carried out to achieve the desired texture and functional characteristics in low fat or reduced fat mozzarella cheese by modifying the manufacturing parameters such as homogenisation, cooking temperature and storage conditions (Mistry and Anderson, 1993a, b; Holsinger *et al.*, 1995; Tunick *et al.*, 1991). Mistry and Anderson (1993 a, b) have reported that homogenisation of milk would help to overcome the problem of hardness in cheese, because the surface area of fat within the cheese matrix would be increased.

Major factors that affect the physical properties of cheese include fat, protein, moisture, minerals including calcium, type of starter cultures used, pH of whey at draining stage, use of fat substitutes, salting and storage conditions.

2.3 Role of fat in mozzarella cheese

A linear decrease in elasticity of mozzarella cheeses was observed as the fat: solids-not-fat ratio increased (Masei and Addeo, 1986). The effect of milk fat on cheese elasticity may be related to the interaction between the fat globule membrane and the cheese protein matrix. Globules that were washed to remove the surface membrane did not contribute to the elasticity of the acid milk gels. They did not react with the protein matrix, and the gels behaved as if the voids were filled with particles possessing the rheological properties of water. Fat globules coated with protein during homogenisation contributed substantially to the elasticity of acid milk gel (Olson and Johnson, 1990).

Chen *et al.* (1979) observed that protein levels play a dominant role affecting elasticity of cheese varieties of varying compositions. However, fat plays a more dominant role in this rheological characteristic. Low fat high moisture cheeses were reported to exhibit stickiness but the effect of fat reduction without change in protein level is unknown (Olson and Johnson, 1990).

The effect of polyunsaturated versus saturated fat has not been studied in much detail but cheddar cheese has been made successfully using Alta milk (milk produced from cows fed protected polyunsaturated fat supplements) but in this process the starter cultures had to be changed (Tunick *et al.*, 1991). The firmness of cheese is affected by the melting point of milk fat (Prentice, 1987). An indirect linear relationship was observed between penetrometer readings of cheese and iodine number of milk fat; the latter is a measurement of unsaturation and an indication of meltability. The impact of fat on temperature induced softening may be questionable because changes in relative firmness were linear and similar between 5°C and 30°C for cheese of varying composition (Prentice, 1987) including low fat (30% fat reduction) cheeses. In Scandinavia, vegetable oils including soybean oil, sunflower oil and cottonseed oil are used to replace milk fat. The typical fat contents of these products are about 50% FDM and the products are being marketed as "Lochol" and "Minichol" (Tunick *et al.*, 1991).

Fecera *et al.* (1995) reported that when the fat content of the mozzarella cheese is decreased, the moisture retention also decreases giving the cheese a poor melt and stretch characteristic. The cheese is said to have a rubbery texture (Fecera *et al.*, 1995) and there is an increase in hardness, springiness and chewiness (Tunick *et al.*, 1991). Tunick *et al.* (1995b), concluded from their study that proteolysis and rheology of cheese are affected more by fat content and storage than by fat globule size or homogenisation of fat. A study by Merrill *et al.* (1994) showed that different casein: fat ratios (1.2; 1.6; 2.0 & 2.4) to make low fat mozzarella cheese

had no effect on the melt and stretch characteristics. It is also well known that low fat high moisture cheeses exhibit stickiness when masticated, especially when their fat content is 15% or less (Olson and Johnson, 1990).

Fewer fat globules in cheese results in more extensive syneresis and denser structural matrix causing firmness, dryness and improper melt. An increase in moisture retention in low fat cheese helps in decreasing hardness (Tunick *et al.*, 1995b). Cheeses tend to be softer when the amount of liquid fat, which is not bound to the protein matrix, increases (Green *et al.*, 1985; Luyten, 1988). Hardness and meltability in mozzarella cheese are affected by the manner in which moisture is distributed in the protein matrix of the cheese (Merrill *et al.*, 1994).

Theoretically, removal of fat, which exists in between the columns of protein fibres, causes narrower columns and makes cheeses harder and less meltable (Merrill *et al.*, 1994). Flavour compounds from fat lipolysis are not produced. Fat-soluble flavour compounds are not dissolved and their later release during consumption may be affected. Although this is not very serious as the other ingredients added during pizza making provide flavour, which masks the blandness of cheese (Tunick *et al.*, 1995a). FDM less than 30% caused an increase in the values of each texture profile analysis (TPA) parameters and decreased meltability values. Reduction in fat or moisture levels and storage time also caused similar changes in the TPA values (Tunick *et al.*, 1995b).

2.4 Role of protein in mozzarella cheeses

Protein plays a major role in the manufacture of cheeses. It is the major component of cheese body and gives the desired firmness, stretch and meltability, which are essential characteristics of mozzarella cheeses. Although cheese firmness is affected by the relative amounts of water, protein and fat, the dominant factor is the amount and ratio of water and protein, which have

the greatest effect (Prentice, 1987; Walstra *et al.*, 1987). The hardness of cheese varieties with diverse composition correlated more closely with protein content than with fat content (Chen *et al.*, 1979). Also when the ratio of fat to SNF increased, there was a decrease in elasticity more so because of decrease in SNF part (Masei and Addeo, 1986). Similarly, an increase in elasticity was reported due to decrease in moisture content (Olson and Johnson, 1990).

2.5 Role of moisture in mozzarella cheeses

The moisture content in cheese is affected by the rate and extent of syneresis which in turn is controlled by milk composition especially the calcium ion concentration, pH of whey, temperature of cooking, rate and duration for which curd is stirred with the whey (Holsinger *et al.*, 1995). Moisture content affects various characteristics of mozzarella cheese such as hardness, springiness, chewiness, meltability and crumbliness. Decrease in moisture content of mozzarella cheese caused decrease in meltability, cohesiveness and an increase in hardness, springiness, chewiness and crumbliness (Tunick *et al.*, 1991). Reduction in moisture levels in mozzarella cheeses resulted in higher values for hardness and springiness (because of increase in elastic modulus and viscous modulus) and lower values for cohesiveness, complex viscosity and meltability (Bhaskaracharya and Shah, 1999, Tunick *et al.*, 1993a). The moisture content in cheese is dependent upon the acidity or pH during curd formation (Oberg *et al.*, 1993). Excessive acid production causes more syneresis and cheeses become crumbly, whereas less acid production gives pasty or sticky cheese due to excess moisture retained. Also by controlling the moisture content the bacterial growth and enzyme activity are regulated which in turn influence the rate and extent of ripening. The studies carried out on commercial cheese (Bhaskaracharya and Shah, 1999) showed that springiness increased with increase in fat content while cohesiveness increased with increasing protein content.

2.6 Role of calcium in mozzarella cheese-making

Calcium that exists in milk in the form of bound calcium phosphate with casein, keeps the casein in a colloidal phase. The removal of calcium causes dissociation of casein, which becomes available for emulsification of fat leading to less oiling off upon melting. Excess of calcium has been linked with excessive curd firmness. In order to reduce curd firmness, part of the calcium is destabilized and expelled along with whey due to acid production by starter culture in milk or direct acidification during manufacture. It has also been reported that a pH of 5.6 obtained by acidification using citric acid gives similar curd as with lowering of pH to 5.2 by other acids because more calcium is removed by greater chelating effect of citric acid in comparison to other acids (Oberg *et al.*, 1993). The decrease in calcium content enhances the susceptibility of casein during ripening to proteolysis and thus the textural and rheological properties of cheese (Holsinger *et al.*, 1995). The total calcium retained in the cheese has been shown (van Slyke, 1928) to decrease with increasing acidity at the time of rennet addition. Thus the lower the pH of milk at the time of rennet addition, the higher is the calcium lost into the whey at the time of whey draining (Kiely *et al.*, 1992).

Differences in the calcium dissolved in the cheese serum and the calcium bound to the protein have been shown (de Jong and de Groot-Mostert, 1977; Noomen, 1978) to cause variations in the extent of proteolysis. The pH at whey draining has been reported (Lawrence *et al.*, 1984) to determine the total calcium content of cheese. The concentration of the calcium ions has been found to increase with a decrease in pH and also with an increase in salt content of cheeses (Lawrence *et al.*, 1987).

2.7 Role of pre-acidification in mozzarella cheese manufacture

The Greek origin of the word chelate signifies the plier-like claws of a crab. A chelate is a water-soluble complex between a metal ion and a complexing agent. Chelating agents yielding soluble metal complexes are also called sequestering agents. A chelating agent has at least two functional groups which donate a pair of electrons to the metal, such as $=O$, $-NH_2$ or $-COO^-$. Furthermore, these groups must be located so as to allow ring formation with the metal. Chelating agents are widely found in living systems and are of importance in cellular metabolism. In milk, citric acid forms complexes with calcium and such complexes are soluble and can be removed from the milk system. Since citric acid is a tricarboxylic acid it has three pK_a values, 3.06, 4.60 and 6.40. Figure 2.5 indicates the predominant species of citric acid in milk represented by the equilibrium pK_a values. The successive pK_a values are related to the successive proton dissociation reactions. When the pH of milk has a value equal to a particular pK_a , the two species of the equilibrium participating in the dissociation described by that pK_a are present in equilibrium eg. $Cit^{2-} = Cit^{3-}$ at pH 6.4. These are the predominant species when the solution pH is near to this particular value.

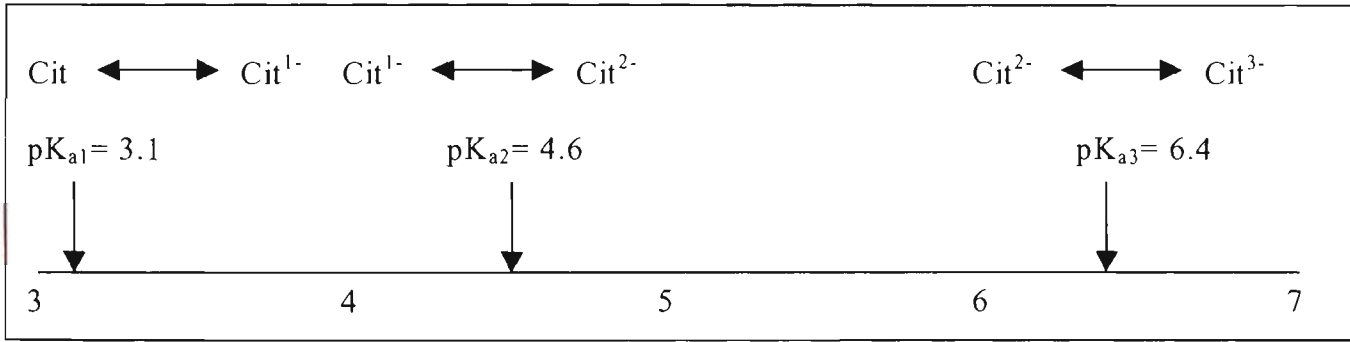


Figure 2.5 Equilibrium state of citric acid and its dissociation state in the range of pH 3 to 7.

Similarly acetic acid has a pK_a value of 4.76 while lactic acid has a pK_a value of 3.86. Citric acid shows a decreasing pK_a value with increase in temperature. Moreover, the three available carboxylic acid groups on citric acid are able to complex with three calcium ions removing

them from casein network during the curd formation. In comparison acetic acid has one carboxylic acid and removes less calcium ions.

Studies conducted by several researchers (Fox and Ernstrom, 1969; Metzger *et al.*, 2000; Kindstedt *et al.*, 2001) have shown that pre-acidification of cheese milk is beneficial in terms of removal of calcium, thereby helping to achieve a softer curd, which stretches easily.

2.8 Role of different types of starter cultures used in cheese-making

The selection of a particular culture is very important to obtain the desired characteristics of the product. The parameters to be considered while selecting cultures are: acidification rate, proteolytic activity, galactose fermentation and salt sensitivity. Normally for pizza cheese *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and/or *Lactobacillus helveticus* are used as starter culture. *S. thermophilus* are mainly used for acid production, whereas *L. delbrueckii* ssp. *bulgaricus* and *L. helveticus* are used for their proteolytic activity. *L. helveticus* has a wider spectrum of proteolysis compared to *L. delbrueckii* ssp. *bulgaricus* and almost all strains of *L. helveticus* are galactose fermenting unlike *L. delbrueckii* ssp. *bulgaricus* (Sigsgaard, 1994). Galactose fermentation is important because most strains of *S. thermophilus* cannot utilise galactose and this may cause Maillard browning in the final cheese when baked on the pizza. To avoid Maillard browning, *L. delbrueckii* ssp. *bulgaricus* or *L. helveticus* are used along with *S. thermophilus* as a combination starter culture. Details of the different types of starter cultures for pizza cheeses and their characteristics are shown in Table 2.4 (Sigsgaard, 1994). ‘

Table 2.4 Cultures program developed by Chr. Hansen's for pizza cheese production.

Thermophilic Lactic cultures	Acid production	Galactose fermentation	Proteolytic activity	Salt sensitivity (50%/100% inhibition)
Type DVS defined strains <i>Streptococcus thermophilus</i>				
TH-3	fast	negative	medium	2.1/4.0
TH-4	fast	negative	Medium	1.9/3.0
St-36	fast	negative	High	2.5/3.0
St-37	fast	negative	High	2.5/3.0
St-75	fast	negative	High	2.5/3.0
St-B 01	slow	negative	Medium	2.0/3.0
Type DVS defined strains <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>				
Lb-9	slow	positive	Medium	2.1/3.0
LB-12	fast	negative	Medium	1.7/3.0
Type DVS defined strains <i>Lactobacillus helveticus</i>				
Lh-B 01	slow	positive	Low	3.1/4.0
Lh-B 02	fast	positive	Low	2.5/3.0
Type DVS multiple strain, yoghurt <i>Streptococcus thermophilus</i> / <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>				
CH- 1	fast	negative	Medium	2.1/3.0
B-3	fast	negative	Medium	1.9/3.0
Type DVS composed cheese cultures <i>Streptococcus thermophilus</i> / <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>				
TCC-1	fast	negative	Medium	2.7/3.5
TCC-2	fast	negative	Medium	3.1/4.0
TCC-3	fast	negative	Medium	2.8/3.5
TCC-4	fast	negative	Medium	2.8/3.5
Type DVS composed cheese cultures <i>Streptococcus thermophilus</i> / <i>Lactobacillus helveticus</i>				
TCC-20	fast	positive	Low	2.8/3.5

Source: Sigsgaard, 1994.

Low fat mozzarella cheeses with improved melting characteristics could be made using exopolysaccharide producing starter cultures wherein the exopolysaccharide increases the moisture retention in the mozzarella cheeses (Fecera *et al.*, 1995; Low *et al.*, 1997; Perry *et al.*, 1997, 1998; Bhaskaracharya, 2000; Bhaskaracharya and Shah, 2000b). Bhaskaracharya and Shah (2001a) observed that the exopolysaccharide produced in the curd caused an increase in moisture content of the mozzarella cheeses and improved the textural characteristics. Such cheeses showed a decrease in hardness, cohesiveness and adhesiveness values while springiness values increased. They also observed an improved porosity in the microstructure of skim milk mozzarella cheeses made using exopolysaccharide producing starter cultures.

Hassan *et al.* (1995a, 1995b) measured the size of capsules formed and their effect on acid production by EPS producing starter bacteria. Hassan *et al.* (1996a) studied viscosity characteristics of yoghurt manufactured using EPS producing starter cultures. Similar studies (Hassan *et al.*, 1996b) on yoghurt made using EPS producing starter cultures including encapsulated non-ropy or un-encapsulated ropy strains of lactobacilli showed an increase in moisture retained in the yoghurt gel. Hassan and Frank (1997) also observed a reduction in curd tension and firmness of rennet curd due to increased open casein network with large pores when made using EPS producing starter cultures compared to non-EPS producing starter cultures.

In the cheese factory whey collected during cheese manufacture is further processed using membrane technology. During membrane filtration of whey isolated from cheese that were made using EPS producing starter culture and increase in membrane fouling may occur. The EPS produced by the starter culture is also expelled out into the whey. This EPS accumulates on the membrane during filtration and increases backpressure. Severe cases of membrane fouling could lead to damage of the membrane. Such membranes need to be cleaned. One of the main disadvantages of using EPS producing starter culture in manufacture of cheese is whey processing and due to this reason use of such culture may not be feasible. Small quantities of citric acid are present in milk and milk products and using micro-organisms such as *Leuconostoc citrovorum* and *Leuconostoc paracitrovorum* further increases these amounts. Mozzarella cheeses made using citric acid also can be measured for the total citric acid using the method described by Babad and Shtrikman (1951). Thus an appropriate starter culture must be used which may be a single strain of *S. thermophilus* or mixed strains of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* and/or *L. helveticus*.

2.9 Importance of controlling pH at whey drain

Acid production is the key to the manufacture of good quality cheeses. Titratable acidity of milk is expressed as lactic acid and during cheese making either titratable acidity or pH is continually monitored. Titratable acidity is a measure of the constituents that react with and neutralise the sodium hydroxide during titration whereas pH is a measure of “free” hydrogen ions (Olson, 1990). Because the titratable acidity is affected by the solids-not-fat (SNF) content of milk including proteins, phosphates, citrates and carbon dioxide, pH was considered to be good measure for monitoring changes during cheese manufacture. It has been observed that at pH 5.15 to 5.2 of cheese curd before hot stretching gives optimum stretchability for starter added cheese manufacture whereas for direct acidification the pH can be 5.4 or higher (Metzger *et al.*, 2000b). At this pH the colloidal calcium phosphate in casein micelles is solubilised and a portion of calcium ions is lost in the whey. In the presence of rennet the native protein, casein is transformed chemically to another form of protein highly sensitive to coagulation in the presence of calcium. This precipitates out at room temperature as dicalcium paracaseinate, entrapping fat, insoluble salts, lactose and some serum. This precipitate is incapable of producing a smooth plastic mass when exposed to hot water or steam (Kosikowski, 1982). The lactic acid dissolves the calcium of dicalcium paracaseinate, and monocalcium paracaseinate is formed. This caseinate has the unique property of forming smooth, pliable and stringy curd when heated to 54°C or higher. Further acid development during ripening causes more calcium to be lost and paracasein is formed which does not retain fat (Kosikowski and Mistry, 1997). During stretching the free casein is available for stabilizing newly created fat surfaces as fat globules are disrupted.

Changes in the composition of mozzarella cheeses made by pre-acidifying cheese milk to pH 5.8 and 6.0 have been studied (Metzger *et al.*, 2000b). When milk was pre-acidified to pH 5.8 using citric acid, a marked decrease in protein recovery was observed. It was also found

(Metzger *et al.*, 2000b) that a lower pH of milk upon pre-acidification caused a decrease in yield efficiency using both citric and acetic acids. Previous reports (Hill *et al.*, 1985 and Yun *et al.*, 1995) have indicated decrease in total calcium content and increase in non-micellar calcium content of cheeses with decrease in pH of curd at draining. Thus pre-acidification of milk has a role in determining the composition and thereby texture and rheological characteristics of the mozzarella cheeses and the pH at whey draining is very important in adjusting the moisture retained, mineral content, texture and functionality of the finished cheese.

2.10 Use of fat substitutes

In recent years consumer demand for healthy and nutritionally balanced products, especially food containing low fat, salt and cholesterol levels, has increased (Anon., 1992, 1995; Shepherd and Stockley, 1987). To meet the increasing demand for reduced fat and low fat cheeses, research work needs to be focused on incorporation of fat substitutes in mozzarella cheese, which have similar functions to fat. The principal requirements for a fresh mozzarella cheese are stretchability and melt characteristics. A wide variety of fat replacers are available which are classified as protein based, carbohydrate based and synthetic.

Summerkamp and Hisser (1990) have listed (see Table 2.4.) fat replacers, which have been cleared by FDA to be of GRAS status whereas a few more are in the various stages of development.

Table 2.5 List of fat replacers available.

Name/Type	Uses/Applications
Simplesse® (protein)	Yoghurt, cheese spread, cream cheese, sour cream, salad dressing, mayonnaise, margarine.
Trail Blazer® (protein)	Frozen desserts.
Olestra® (synthetic-sucrose polyester)	Frozen desserts, table spreads, salad dressings, cheese, bakery items, shortenings, cooking oils.
EPG (synthetic- esterified propoxylated glycerol)	Frozen desserts, table spreads, salad dressings, bakery items.
DDM (synthetic- dialkyl dihexa decymalonate), TATCA (synthetic-trialkoxo tricarbollate)	Mayonnaise, margarine, cooking oils.
Gums (carbohydrate-hydrocolloids)	Salad dressings, formulated foods.
Polydextrose (carbohydrate)	Candy, chewing gum, candy coatings, dry cakes/cookie mixes, frozen dairy products, icings, nutritional bars, puddings, frostings.
Maltrin® M040 and M100 (carbohydrate- maltodextrins)	Frozen desserts, table spreads, salad dressings, margarine, imitation sour cream.
Tapioca Dextrins® (carbohydrate)	Frozen desserts, table spreads, margarine, salad dressings, imitation sour cream, puddings, micro-waveable cheese sauce.
Paselli SA2 (carbohydrate-potato starch/maltodextrin)	Salad dressings, frostings, frozen desserts, dips, bakery products, mayonnaise, table spreads, meat products, confections.
StaSlim® 143 (carbohydrate-modified potato starch)	Pourable and spoonable salad dressings, soups, cheese cakes, imitation cream cheese.
Prolestra® (sucrose polyester), Nutrifat® (hydrolysed starch), Finesse® (piezo-proteins), Colestra® (low calorie Olestra)	Ice-cream, salad oils, mayonnaise, sauces, snacks, table spreads, baked products.

Source: Summerkamp and Hisser (1990).

Various fat replacers have been tried (Fife *et al.*, 1995) in mozzarella cheese and these have resulted in increasing the moisture content to about 56% and a casein-fat ratio of 4.2.

Mozzarella cheese manufactured with Stellar (carbohydrate based fat replacer) showed greater melt, and by 28 d cheeses became sticky and difficult to handle whereas with Simplesse D100 (protein based fat replacer) cheeses showed improved melt without becoming sticky. Mozzarella cheese had more open texture and melted the least when mixed with Novagel RCN-15 (carbohydrate based fat replacer) compared to mozzarella cheeses made with Dairy lo (protein based fat replacer) which showed similar moisture content as control. In the microstructure studies, Stellar and Novagel were observed to be in the pockets of fat and serum between strands of casein matrix while Simplesse and Dairy lo were found embedded in casein matrix as well as in serum pockets. Mann (1992) suggested an addition of 3% gum arabic to give ideal characteristics for the manufacture of high fibre low fat mozzarella cheeses.

Pagliarini and Beatrice (1994) have reported the use of inulin, a vegetable fibre, as a fat replacer. Mozzarella cheeses were made with the polysaccharide substitute (brand name fibrulin) added to make up fat functionally equal to fat in full fat mozzarella cheese using direct acidification to pH 5.8 with citric acid and stretched in brine solution. The product had better moisture retention and showed less hardness, more glossiness, and whiteness similar to the full fat mozzarella cheese. Studies carried out by Bhaskaracharya and Shah (2001b) on skim milk mozzarella cheese made using maltodextrin (carbohydrate based fat replacers) showed decreases in protein content, hardness, cohesiveness, springiness, gumminess and chewiness compared to skim milk mozzarella cheese made without addition of fat replacer. The Maltodextrin based cheese showed decreased textural values of hardness, springiness and chewiness during storage. Thus fat replacers improve the textural characteristics of cheese if added appropriately.

A carbohydrate based fat replacer usually is a starch, gum or fibre. Examples of some starches are maize, corn and rice while those for gums are xanthan, carageenan and alginates and for

fibres are inulin. Table 2.5 shows the classification of carbohydrates. The degree of polymerisation (DP) depending upon the type of starch is also indicated in brackets (FAO, 1997).

Table 2.2 Classification of carbohydrates.

Class (DP [*])	Sub-group	Components
Sugars (1-2)	Monosaccharides	Glucose, galactose, fructose
	Disaccharides	Sucrose, lactose, trehalose
	Polyols	Sorbitol, mannitol
Oligosaccharides (3-9)	Malto-oligosaccharides	Maltodextrins
	Other oligosaccharides	Raffinose, stachyose, fructo-oligosaccharides
Polysaccharides (>9)	Starch	Amylose, amylopectin, modified starches
	Non-starch polysaccharides	Cellulose, hemicellulose, pectins, hydrocolloids

^{*}DP = degree of polymerisation.

Starch is obtained from grains such as rice, wheat, and corn and from tubers such as potato, tapioca starch (cassava) and sweet potato. The starch has the ability to gel, thicken, retain moisture, and provide various textural and functional properties to the food in which they are added. Starch is composed of glucose units, which form the building block. The D-glucose molecules in starch are linked by α-1, 4 and α-1, 6 glycosidic bonds and thus the polymer has one reducing end and the other non-reducing end. The glycosidic linkages are in the α-configuration in starch while cellulose has a β-configuration i.e. the glucose molecule in cellulose is linked by β-1, 4 bonds. This difference in configuration of starch and cellulose molecules leads to major differences in properties of the two molecules such as physico-chemical properties and susceptibility to enzymes. The α-configuration of glucose molecules

provides the polymer in starch a helical structure and is susceptible to amylase enzyme, which is commonly present in herbivores.

In a broad way, the glucose polymers in starch can be divided into two varieties namely, amylose and amylopectin. Amylose is a linear polymer of α -1, 4 linked D-glucopyranose molecules with very few α -1, 6 linkages whereas amylopectin is a largely branched polymer consisting of both α -1, 4 and α -1, 6 linkages. The α -1, 6 linkage gives the amylopectins branched structure. It is important to note that the differences in α -1, 4 or α -1, 6 bonds gives amylose and amylopectin their properties. Amylose typically has molecular weights less than 500,000, forms strong films and firm gels and reacts with iodine to give blue colour (iodine test) whereas amylopectin has molecular weights greater than 10,000,000, form weak films and are non-gelling and react with iodine to give reddish-brown colour (Thomas and Atwell, 1999; Vogel, 1989). The degree of polymerisation (DP) of the starch indicates the length of polymer chain i.e. due to α -1, 4 bonds. Thus amylose tends to have a low DP number (ranges from 1500 to 6000) while the amylopectin has a higher DP number (ranges from 300,000 to 3,000,000).

Gelatinization of starch is brought about by heat, acid or alkali treatment and causes a breaking up of the polymers in the starch granule leading to irreversible changes in properties such as swelling, loss of bi-refringence and starch solubilization. Gelatinisation is thus aptly defined as the disruption of molecules within the starch granule, which causes irreversible changes to the properties of starch such as granular swelling, melting of the semi-crystalline structures, loss of birefringence and solubilization of starch (Thomas and Atwell, 1999). Pasting is an occurrence, which overlaps the gelatinisation phenomenon and has been defined as the phenomenon following gelatinisation in the dissolution of starch and involves granular swelling, exudation of molecular components from the granule and eventually leads to total disruption of the starch granules.

Retrogradation occurs after gelatinising of starch granules whereby the broken polymers in starch begin to re-associate to form an ordered structure. Heat treatment beyond the gelatinisation point (temperature) causes molecular components to exudate out of the starch granule and results in starch paste. The tumbleweed like structure of amylopectin due to the large number of branches (α -1, 6 bonds) retards the rate of retrogradation in amylopectin (Thomas and Atwell, 1999). On the contrary amylose in the purer form retrogrades rapidly after gelatinisation to form thick gels (Pomerenza, 1982).

2.11 Importance of salting

Salt is incorporated into mozzarella cheeses by either brining alone or with dry salting. This step is also necessary to cool the hot stretched cheese. Moisture loss during salting depends on the temperature of brine; with greater loss as brine temperature increases. But the salt uptake is affected by the brine temperature and brine strength (Kindstedt, 1993). This step also has positive effects on the product. Salting improves emulsification of fat because of calcium exchange in the casein and is independent of the moisture content of the product. So there are less chances of oiling-off. This effect has been proven to be better in the case of dry salting of cheese curd before it goes to the stretcher (Oberg *et al.*, 1993; Tunick, 1994). Salting inhibits non-starter organisms from growing and causing spoilage during storage. Excessive salting may also inhibit the activity of starter bacteria, which are required during ripening (for cheddar type ripened cheeses) and grow during storage. So more unfermented sugars remain in cheese leading to browning during pizza making (Tunick *et al.*, 1991). Also a study by Tunick *et al.* (1995c) has shown that salting can cause a decrease in melt.

2.12 Physico-chemical and proteolytic changes during storage of mozzarella cheeses

During storage various biochemical changes occur in cheese including glycolysis, lipolysis and proteolysis and secondary catabolic changes such as deamination, decarboxylation, β -oxidation and ester formation in cheddar cheese (Holsinger *et al.*, 1995). Malin *et al.*, (1993) indicated a specific ripening pathway that occurs during storage of mozzarella cheese. In mozzarella cheese, the principal biochemical change is due to proteolysis. Although there is very limited proteolysis in mozzarella cheese compared to ripened cheese varieties, nevertheless it affects its functional and rheological characteristics.

Various methods of assessing proteolysis in cheeses have been reported (Fox *et al.*, 1995). Kindstedt and Guo (1997) suggested a model for physico-chemical and microstructural changes in pizza cheese during short time refrigerated ageing. They believed that after manufacture of the cheese, the paracasein fibres encompass the serum and fat globules. During storage the paracasein fibres tend to hydrate thereby decreasing the serum cavities and the fat globules are left trapped in between such hydrated paracasein fibres. These changes have been attributed to the physical adsorption phenomenon and some chemical changes creating new bonds between the paracasein and water molecules.

Guo *et al.* (1998) found the casein solvation was accompanied by a microstructural swelling of the protein along with formation of internal gradients of protein density which could be the cause of increased water holding capacity. Guo *et al.* (1998) showed that the physiochemical changes are the driving forces for the structural and functional alterations in mozzarella cheese during storage. Calcium, zinc and crude protein were found to increase in the expressed serum while the sodium and potassium contents remained unchanged when mozzarella cheeses were stored for 10 days (Guo *et al.* 1998).

Tunick *et al.* (1991, 1993a, 1993b, 1995c) reported that during refrigerated storage meltability increased with proteolysis, whereas hardness, springiness, gumminess and chewiness decreased. Tunick *et al.* (1991) observed frozen mozzarella cheeses of 48 d to show poor cohesiveness and meltability when evaluated after thawing compared to fresh cheese samples. Thawing of frozen cheeses at 4.4°C for 21 d produced optimum values of cohesiveness and meltability. Oberg *et al.* (1993), observed an increase in free oil formation after two weeks of storage. It was observed that during storage proteolysis by plasmin and residual coagulant caused cheese softening (Diefes *et al.*, 1993). Reports (Tunick *et al.*, 1991, 1995b; Anonymous, 1995; Dryer, 1994) suggest that 6-wk-old low fat high moisture mozzarella cheese had similar textural properties as fresh 1-wk-old high fat low moisture mozzarella cheese. Thus literature shows that it is possible to manufacture low fat (18%) mozzarella of acceptable quality but any further decrease in the fat content requires modifications in the manufacturing methods, including methods for increasing the moisture content of the cheeses significantly and controlled storage conditions thereby obtaining an ultra low fat mozzarella cheese with functional properties of a full fat mozzarella.

In order to achieve high moisture content two main avenues were considered, namely, use of EPS producing starter cultures and use of fat replacers. The former way has more innate problems and studies carried out in our laboratory (Bhaskaracharya and Shah, 2001a) showed that 1.5 to 2.0% increase in moisture was not sufficient to obtain the desired characteristics in ULFM cheese. Thus it was concluded that using fat replacers along with making well conceived changes in the manufacture protocol to obtain ULFM cheese with characteristics of a full fat mozzarella cheese.

2.13 Effect of baking on mozzarella cheese characteristics

Mozzarella cheeses undergo significant changes in temperature during baking on a pizza. Pizza manufacturers use a variety of cheeses including cheddar, mozzarella cheeses and analogue pizza cheeses as ingredients. A study conducted on several commercial cheeses (Guinee *et al.*, 1998) showed a lot of variation in the characteristics of the cheeses of each variety. They also observed that low moisture mozzarella cheeses had a higher pH, higher expressible serum, reduced proteolysis and reduced calcium concentrations in expressed serum in comparison to the cheddar cheese while the analogue cheeses had a lower protein content, higher pH, increased FDM and MNFS contents along with a lower calcium concentration when compared to the mozzarella cheeses. Based on these findings, Guinee *et al.* (1998) suggested increasing substitution of low moisture mozzarella cheeses with aged cheddar cheese in order to improve the meltability and stretchability on pizzas. Thus blending of cheeses of different varieties would alter the characteristics of the pizza topping. Such methods of pizza making using low-fat mozzarella cheese along with other varieties may be used to improve the functionality of low-fat mozzarella cheese.

Under typical conditions of baking of pizzas at 232°C for 5 min in a commercial forced-air pizza oven, the cheese added as a topping achieves a temperature of 60-70°C. After baking, the cheese cools and is normally consumed at 40-50°C (Kindstedt *et al.*, 1989). The wide temperature range between baking and subsequent cooling has an impact on the appearance of the mozzarella cheese (Metzger *et al.*, 2000a). The whiteness change (L-value) during heating and cooling of pizza indicates a reversible, heat-induced interaction occurring in cheeses. The reported decreases in whiteness of unmelted (fresh), reduced fat mozzarella cheese during refrigerated storage have been correlated with the changes in the composition of the serum phase of the cheese (Rudan *et al.*, 1998). The changes in the serum phase of the cheese have also been reported to be the possible cause for the colour change in low fat mozzarella cheeses

during heating and subsequent cooling on pizzas (Metzger *et al.*, 2000a), which is referred to as translucency.

2.13.1 Effect of application of oil to shredded low fat mozzarella cheese

Generally, proper functionality during pizza baking includes complete melting and shred fusion without the melted cheeses becoming too soupy; some free oil release is also desirable, which gives the surface of melted cheese a shiny appearance without producing a burnt appearance (Kindstedt, 1991). Rudan and Barbano (1998) showed that fat-free and low fat mozzarella cheeses have limited melt and fusion and become scorched during pizza baking in commercial pizza ovens. In full fat mozzarella cheeses due to the release of fat along with the melting of the casein part of the cheese shreds there is very little browning. This has been suggested to provide whiteness to the melted mozzarella with free oil on the pizza surface. During baking, because of the heating of mozzarella cheese, blisters may be formed. The top or peak of the blisters quickly lose fat, which flows down the sides of the blister and further heating causes browning of the blister. This phenomenon was suggested to happen if the blister stays for long without collapsing.

Unlike full fat mozzarella cheese in low fat or fat free mozzarella cheese there is case hardening of the shred surface of the cheese. During heating, the small amount of fat on the shred surface of a low fat mozzarella cheese does not prevent a rapid evaporation of its moisture and the shred dehydrates. This causes case hardening and browning. Once the case hardening sets in, even though the inside of the shred is properly melted the hardened surface, which is brown and crisp, does not allow the melted casein to flow (Rudan and Barbano, 1998). Dehydration of the shred surface and subsequent skin formation are the critical events that limit melting and could cause scorching of fat free and low fat mozzarella cheeses during pizza baking. In low fat mozzarella cheeses the protein content is proportionally higher than

full fat mozzarella and hence when baked they do not have any fat released outside of the shreds. During baking, shreds of such cheeses dehydrate more rapidly than those of full fat cheese due to lack of protective free oil and blisters form on such low fat cheeses would be constituted mainly by proteins which increase rigidity and do not collapse easily. These blisters then become burnt.

Application of oil to the surface of shredded low fat cheese would prevent dehydration during baking and assist in lubricating the surface of the shreds thereby preventing case hardening. Rudan and Barbano (1998) suggested application of 0.9 g vegetable oil/100g cheese to prevent skin formation and to help the fat free and low fat mozzarella cheese to melt and perform similar to a full fat mozzarella cheese.

2.14 Estimation of minerals in milk and cheese

Calcium was estimated from milk using ethylene diamine tetra acetate (EDTA) as titrating reagent or using the photometric method developed by Murthy and Whitney (1955). In both methods the preparation of samples required either ashing or wet digestion to remove interfering material. During the determination of calcium using the flame photometer, sodium ions caused interference, and Murthy and Whitney (1956) developed formulae to calculate the apparent calcium content in the presence of sodium ions. Similarly they found that the Mg content also was affected due to interference by calcium ions. In case of potassium estimation it was found that the method of preparation of the sample influenced the readings obtained, but errors due to this were suggested to be overcome by following proper methods and preparation of sample for single analysis. Estimation of trace ions such as copper, iron, manganese in milk by AAS was found to be difficult due to the small signal from the low concentration of ions which was comparable to the background noise signals.

3.0 MATERIALS AND METHODS

3.1 Sources of chemicals, reagents and microbiological media

3.1.1 Chemicals and reagents

Lactic acid (85% FCC) is described in appendices and all chemicals used in manufacture and analyses of cheeses were obtained from Sigma-Aldrich Pty. Ltd. (Castle Hill, New South Wales, Australia), while the reagents were obtained from Roche Diagnostics Australia Pty. Ltd. (Castle Hill, New South Wales, Australia).

3.1.2 Microbiological media and enzyme assay kits

All the microbiological media were obtained from Oxoid (West Heidelberg, Victoria, Australia). Rennet (Chymosin) was obtained from Chr. Hansen Pty Ltd. (brand Naturen 145, Bayswater, Victoria, Australia) and details of this enzyme are provided in appendices.

3.1.2.1 Maintenance of bacterial cultures

Strains of lactic acid bacteria namely *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lb2515, Victoria University Culture Collection) and *Streptococcus thermophilus* (TS2000) and *Lactobacillus helveticus* (LB1, Australian Starter Culture Research Centre, Werribee, Victoria, Australia) were maintained at -80°C (ultra-low temperature freezer as described in section 3.1.3.21) in 10% reconstituted skim milk (RSM) to which 20% glycerol was added. Working cultures were propagated using frozen mother cultures by transferring 1% to 10 mL McCartney bottles containing sterile RSM (sterilized as described in section 3.1.3.10) and incubated for 18 h at 37°C (for *S. thermophilus*) and 42°C (for *Lactobacillus* cultures). The cultures were

maintained active by transferring once a day for a maximum of 2 wk after which new frozen stock cultures were reactivated and used.

3.1.2.2 Preparation of bulk starter culture for cheese making

The active starter bacteria were separately transferred into 10 mL RSM at 1.6% (for Streptococci) and 0.8% (for Lactobacilli) and then incubated as described in section 3.1.2.1. Such transfers were carried out at least thrice followed by ascertaining purity of the starter bacteria by conducting Gram staining, which will be described in section 3.1.2.4. The active starter culture was finally transferred at the same rate to a Schott bottle containing 400 mL RSM and incubated. All the transfers were carried out under aseptic conditions, using a laminar flow workstation described in section 3.1.3.22.

3.1.2.3 Investigation of purity of starter cultures using Gram staining

A drop of medium containing starter bacteria was placed on a clean glass slide, spread evenly and heat dried. The smear was dipped in crystal violet staining solution for 60 seconds, followed by washing under tap water, then dipped in gram iodine solution for 60 seconds, again washed under running tap water, followed by dipping in safranine solution for 60 seconds and final rinsing in tap water. The stained smear was then air dried, applied with a drop of oil and observed on the oil immersion lens (100x magnification). The bacteria that stained blue were gram positive while those that stained red were considered gram negative. Also the morphological characteristics of the starter bacteria could be observed to determine the purity of the starter bacteria.

3.1.2.4 Microbiological enumeration of milk, whey, curd and cheese samples

Milk and whey samples were drawn during manufacture of cheeses and 1.0 mL transferred aseptically to a 9.0 mL of 1.5% peptone water diluent. The sample and peptone water diluent were vortexed for 10 sec and 1.0 mL of the first dilution was transferred to another sterile 9.0 mL peptone water diluent under aseptic conditions. One gram of curd or cheese sample was macerated in 9.0mL peptone water in sterile bags using a stomacher and the resulting colloidal suspension was considered as 10^{-1} dilution. Further, a series of ten fold dilutions were consecutively prepared and enumeration of the starter cultures was carried out using appropriate dilutions. *Streptococcus thermophilus* was enumerated on ST Agar (Composition: 15.0 g of bacteriological agar, 10.0 g of tryptone, 10.0 g of sucrose, 5.0 g of yeast extract and 2.0 g of K_2HPO_4 dissolved in d.H₂O, adjusted to pH 6.8 ± 0.1 , mixed with 6.0 mL of 0.5% bromocresol purple and final volume adjusted to 1000 mL with d.H₂O). The *Lactobacillus delbrueckii* ssp. *bulgaricus* were enumerated on MRS agar (Oxoid), which was adjusted to pH 5.2 ± 0.1 .

The pour plate technique was used. One millilitre of diluted sample was placed in a sterile petri-dish and melted agar medium at $\sim 45^\circ\text{C}$ was poured into the plate followed by gentle mixing by swirling the contents of the plate and then agar was allowed to solidify. The plates were incubated (incubators described in section 3.1.3.18) at 37°C for 24 h for *Streptococcus thermophilus* and 42°C for 72 h in anaerobic jars (described in section 3.1.3.1) for *Lactobacillus* cultures (Dave and Shah, 1996). Plates containing 25 to 250 colonies were enumerated and counted as colony forming units per gram (CFU/g) of culture or product. All the experiments and analyses were replicated twice.

3.1.3 Analytical equipment and instrumentation

3.1.3.1 Anaerobic jars

Anaerobic jars with 16 and 48 plates holding capacities, catalysts and carbon dioxide generating sachets were obtained from Oxoid (West Heidelberg, Victoria, Australia). The anaerobic jars were used to grow lactobacilli in plates at 42°C.

3.1.3.2 Melt testing tubes

The glass tubes for measuring meltability of mozzarella cheeses were custom made (R. B. Instruments, Mt. Eliza, Victoria, Australia). They measured 24 mm internal diameter x 250 mm length x 3 mm thickness.

3.1.3.3 Stretchability testing spindles

Spindles were custom made (R. B. Instruments, Mt. Eliza, Victoria, Australia). The cross bar measured 35 mm in length of each bar and was shaped in the form of a cross. This cross bar was welded to a spindle of 150 mm length and 1.5 mm diameter. The spindle was shaped into a hook at the opposite end of the cross bar. The glass beakers used for this test measured 48 mm internal diameter x 70 mm height x 1.5 mm thickness. A thermostatic water bath was used to melt the cheese as described in section 3.1.3.18. A typical stretch test is shown in Figure 10.4.11.

3.1.3.4 Instron Universal Testing Machine

Cylindrical specimens were drawn from cheese samples using a cheese corer (25 mm length x 20 mm diameter). The Instron Universal Testing Machine (model 5564; Instron Ltd., London, England) was used to analyse the texture characteristics of mozzarella cheeses. A 500 N load

cell attached to a flat plunger was used to measure hardness, cohesiveness and springiness values for the cheese, while a 100 N load cell was used attached to a crossbar spindle for measuring the stretchability of the cheeses. Merlin software was used to collect and process data to obtain the texture values and tenacity values.

3.1.3.5 Atomic absorption spectroscopy

Lanthanum oxide (minimum 99.9% purity), Magnesium Atomic Spectroscopy standard, Calcium Atomic Spectroscopy standard (1.005 density, prepared in water), Potassium Atomic Spectroscopy standard (1.02 density, prepared in nitric acid) and Sodium Atomic Spectroscopy standard (1005 µg/mL of Sodium in 1% (w/w) hydrochloric acid) were obtained from Sigma-Aldrich (Castle Hill, New South Wales, Australia). Atomic absorption spectrophotometer (Spectra AA400, Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia) was used for analysing calcium, sodium, magnesium and potassium content of cheese-milk, whey, stretch water, curd and cheese samples at several stages of manufacture. The method parameters for calcium, sodium, magnesium and potassium measurements set-up on the AAS are shown in Table 3.1. The background correction was not used in any of the methods as it was felt that it was not required.

Table 3.1 Method parameters set-up on AAS for estimation of calcium, sodium, magnesium and potassium contents in milk and milk products.

Parameters	Magnesium	Potassium	Sodium	Calcium
Instrument mode	Absorbance	Absorbance	Absorbance	Absorbance
Sampling mode	Manual	Manual	Manual	Manual
Calibration mode	Concentration	Concentration	Concentration	Concentration
Measurement mode	Integrate	Integrate	Integrate	Integrate
Replicates standard	3	3	3	3
Replicates sample	3	3	3	3
Expansion factor	1	1	1	1
Minimum reading	0.0	0.0	0.0	0.0
Smoothing	5 point	5 point	5 point	5 point
Concentration decimal places	4	4	4	4
Wavelength	285.2 nm	769.9 nm	589.6 nm	422.7 nm
Slit width	0.5 nm	1.0 nm	0.5 nm	0.5 nm
EHT	317 V	357 V	282 V	282 V
Lamp current	7.5 mA	10.5 mA	10.5 mA	7.0 mA
Lamp position	2	3	3	2
Background correction	BC off	BC off	BC off	BC off
Standard 1	0.5 mg/L	1.0 mg/L	2.0 mg/L	2.0 mg/L
Standard 2	1.0 mg/L	2.0 mg/L	4.0 mg/L	4.0 mg/L
Standard 3	1.5 mg/L	3.0 mg/L	5.0 mg/L	6.0 mg/L
Standard 4	2.0 mg/L	5.0 mg/L	8.0 mg/L	8.0 mg/L
Standard 5	4.0 mg/L	6.0 mg/L	10.0 mg/L	10.0 mg/L
Standard 6	6.0 mg/L	8.0 mg/L	12.0 mg/L	12.0 mg/L
Standard 7	8.0 mg/L	10.0 mg/L	14.0 mg/L	14.0 mg/L
Standard 8	10.0 mg/L	12.0 mg/L	16.0 mg/L	16.0 mg/L
Standard 9	14.0 mg/L	14.0 mg/L	18.0 mg/L	18.0 mg/L
Standard 10	16.0 mg/L	-	-	20.0 mg/L
Reslope rate	50	50	50	50
Reslope standard no.	2	2	2	2
Reslope lower limit	75.0%	75.0%	75.0%	75.0%
Reslope upper limit	125.0%	125.0%	125.0%	125.0%
Recalibration rate	100	100	100	100
Calibration algorithm	New rational	New rational	New rational	New rational
Calibration lower limit	20.0%	20.0%	20.0%	20.0%
Calibration upper limit	150.0%	150.0%	150.0%	150.0%
SIPS	Off	Off	Off	Off
Measurement time	5.0 sec	5.0 sec	5.0 sec	5.0 sec
Pre-read delay	10 sec	10 sec	10 sec	10 sec
Flame type	Air/acetylene	Air/acetylene	Air/acetylene	Nitrous oxide/acetylene
Air flow	13.50 L/min	13.50 L/min	13.50 L/min	N ₂ O: 11.00 L/min
Acetylene flow	2.00 L/min	2.00 L/min	2.00 L/min	4.50 L/min
Burner height	13.5 mm	13.5 mm	13.5 mm	17.2 mm

3.1.3.6 High performance liquid chromatography

Ortho phosphoric acid (85%, Hipersolv for HPLC) was obtained from Merck Pty. Ltd. (Kilsyth, Victoria, Australia). Supelcogel C610-H, H-Supelguard guard column and oligosaccharide kits were obtained from Sigma-Aldrich. The HPLC instruments consisted of a Varian 9100 auto sampler unit, Varian 9012 solvent delivery system and a Varian RI-4 analogue refractive index detector (Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia). A sample concentrator (Techne Inc., Princeton, New Jersey, USA) was used to concentrate and remove traces of hexane from the HPLC samples where required before aspirating into the HPLC column. An HPLC column heater with thermostatic control (Alltech Associates Australia Pty Ltd., Baulkham Hills, NSW, Australia) was used to maintain a constant column temperature.

3.1.3.7 Centrifuges

3.1.3.7.1 Beckman-Coulter Centrifuge

A Beckman-Coulter centrifuge (model J2-HS; Beckman-Coulter Instruments Inc., Palo Alto, California, USA) was used to separate the serum from the cheeses.

3.1.3.7.2 Sorvall centrifuge

A Sorvall RC28S centrifuge (Dupont Co. Biotechnology Systems, BRML, Chandler, Wilmington, Delaware, USA) with an SS34 rotor was used to centrifuge the protein samples for SDS-PAGE at 18000rpm for 1.0 h at 4-5°C.

3.1.3.7.3 Eppendorf centrifuge

A small laboratory scale Eppendorf centrifuge (model 5415C; Eppendorf Netheler-Hinz GmbH, Hamburg, Germany) was used to centrifuge the final stage protein samples prior to loading on an SDS-PAGE gel.

3.1.3.7.4 Bench top centrifuge

A medium sized Hettich Universal centrifuge (HD Scientific Supplies Pty Ltd., Mountain Hwy, Bayswater, Victoria, Australia) was used for general purpose centrifugation.

3.1.3.8 Digital camera

All still images from the various melt, stretch and pizza bake experiments were recorded using a digital Mavica still camera (MVC-FD85, Japan Sony Marketing Co, Tsujidoshinmachi, Fujisawashi, Kanagawaken, Japan). The images were recorded as JPEG files.

3.1.3.9 Conventional pizza oven

A conventional pizza oven (Lincoln Impinger model 1304-4, Lincoln Foodservice Products Inc., Fort Wayne, Indiana, USA) was used to prepare pizzas. The oven temperature was set at 260°C and the conveyor speed was adjusted such that a pizza would take 7 min transit time from start to finish.

3.1.3.10 Autoclave

A Getinge Autoclave GE150 (Getinge Australia Pty. Ltd., Bulimba, Brisbane, Queensland, Australia) was used to sterilize all the microbial media and also to sterilize the microbiological waste before disposing appropriately. The autoclave was set at 121°C for 15 min for sterilising media and 121°C for 30 min to sterilise waste under wet steam conditions.

3.1.3.11 Protein estimation equipment

Kjeltabs (Foss Tecator AB, Hoganas, Sweden) containing 3.5 g of potassium sulphate and 0.4 g of copper sulphate were used as mixed catalyst for digestion of samples in the Kjeldahl

method. Kjeldahl tubes were custom made (Victoria University, Werribee, Victoria, Australia) and measured 38.5 mm int. diameter x 330 mm length (42 mm ext. diameter, 47 mm ext. diameter of lips, pyrex glass to withstand $>400^{\circ}\text{C}$ repeated heating and holding for 1-1.5 h). Digestion system 20 (model 1015 digester; Foss Tecator AB, Hoganas, Sweden) was used to digest the milk, whey, cheese samples by heating to $>400^{\circ}\text{C}$ and holding till the samples were completed digested. Kjeltex system 1002 distilling unit (Foss Tecator AB, Hoganas, Sweden) was used to distil the digests of the samples and collect the ammonium sulphate produced by distillation into saturated boric acid.

3.1.3.12 Fat testing equipment

The Babcock centrifuge (Garver Electrifuge model 108; Garver Manufacturing Inc., Union City, Indiana, USA) was used for fat testing. The centrifuge is designed with heating coils fitted on the inside of the lid to obtain the desired temperature of $55\text{-}60^{\circ}\text{C}$ and has an operating speed of 740 ± 25 rpm. It has 8 bottle holding cups (capacity) and has a diameter of 21 inches. The centrifuge is prepared for fat analysis of samples by turning on the heating coils and closing its lid for about 10 min with the motor running. When the internal temperature is $55\text{-}60^{\circ}\text{C}$ the heater and motor are stopped. Fat testing bottles are loaded in the centrifuge cups such that the centrifuge is balanced.

3.1.3.13 Ultra Turrax homogeniser

An Ultra Turrax homogeniser (type T25; Janke & Kunkel GmbH & CoKG, IKA Labortechnik, Staufen, Germany) was used to prepare the curd and cheese samples for SDS-PAGE electrophoresis and also for the mineral content estimation.

3.1.3.14 Gel electrophoresis

Gel Air Cellophane Support, Mini-Protean[®] II Dual slab Cell for conducting SDS-PAGE gel electrophoresis, pre-stained broad range marker (containing approximately 625 µg total protein in 33% (v/v) glycerol, 3% SDS ($\text{CH}_3\text{-(CH}_2\text{)}_{10}\text{-CH}_2\text{OSO}_3\text{Na}^-$), 10 mM Tris pH 7.0, 10 mM DTT, 2 mM EDTA, 0.01% NaN_3), a Power Pac 300 for electricity supply to the gel system, and a gel air drying system were obtained from Bio-Rad Laboratories Pty. Ltd. (North Ryde, New South Wales, Australia). Bovine serum albumin (BSA Fraction V) and heat shock protein were obtained from Roche Diagnostics Australia Pty. Ltd.. Dithiothreitol (DTT) was obtained from Progen Industries Ltd. (Darra, Queensland, Australia). An orbital mixer (model EOM5; Ratek Instruments Pty. Ltd., Boronia, Victoria, Australia) was used to stain and de-stain the protein gels for several hours at low speed. The various reagents prepared and used for SDS-PAGE electrophoresis are listed below along with their constituents.

Solution 1. 1.25 M Tris HCl pH 6.8 buffer

This reagent was made using 37.8 g Tris-HCl, 150mL distilled water, adjusted to pH 6.8 with 5 M HCl and volume made-up with distilled water to 250 mL.

Solution 2. Double strength loading buffer

Solution 1 (2.5 mL) was added to 1.0 g of SDS, 5.8 mL of 87% glycerol, 5.0 mg bromophenol blue and 10 mL of distilled water. Once all the ingredients were dissolved 2.5 mL of 2-mercaptoethanol was added and volume made up with distilled water to 25 mL.

Solution 3. Single strength loading buffer

Solution 2 (9.0 mL) was added to 10 mL of distilled water and mixed.

Solution 4. 0.166 mM Tris, 1.0 mM EDTA, pH 8.0 buffer

Preparation of the above buffer solution was carried out by weighing 0.002 g Tris, 0.037 g EDTA into 25 mL distilled water, adjusting the pH to 8.0 using sodium hydroxide or hydrochloric acid and making up the final volume to 100 mL with distilled water.

Solution 5. 7% SDS buffer

7 g of sodium dodecylsulphate (SDS) was dissolved in 100 mL of d.H₂O. Similarly 10% SDS solution was also made by dissolving 10.0 g of SDS in 100 mL of d.H₂O.

Solution 6. 10 mM Dithiothreitol (DTT) in buffer

0.154 g DTT was dissolved into 100 mL of solution 4. Dithiothreitol is a reducing agent, which reduces the disulphide bonds on the proteins.

Preparation of standard broad range marker (stained)

1 µL of stained marker was measured into 19 µL of Solution 3. The diluted marker was loaded onto a polyacrylamide gel.

Preparation of 30% stock acrylamide solution

300 g of acrylamide and 8 g of bis-acrylamide were dissolved into 500 mL of d.H₂O and final volume was made up to 1000 mL.

Preparation of running gel buffer or 1.875 M Tris-HCl buffer (pH 8.8)

Tris (56.8 g) was dissolved in 150 mL of d.H₂O and the pH adjusted to 8.8 with 5 N HCl. The final volume was made up to 250 mL with d.H₂O.

Preparation of 10% ammonium persulphate solution

Ammonium persulphate (0.1 g) was dissolved in 1.0 mL of d.H₂O. This solution was prepared and used immediately.

Preparation of polyacrylamide running gel and 4% acrylamide stacking gel

The recipe (Hoefer applications guide) for making a 20% SDS polyacrylamide gel (0.75 mm thick gel) is shown below.

Table 3.2 Recipe for 20% polyacrylamide gel (0.75 mm thick).

Solution	Quantity (mL)
4X Running gel buffer (pH 8.8)	7.5
d.H ₂ O	2.1
Stock acrylamide (30%)	20.0
10% SDS solution	0.3
Ammonium persulphate (10%)	150 µL
TEMED	10 µL

The recipe (Hoefer applications guide) for making a 4% acrylamide stacking gel is shown below.

Table 3.3 Recipe for making 4% acrylamide stacking gel.

Solution	Quantity (mL)
Stock acrylamide (30%)	1.33
4X stacking gel buffer (pH 6.8)	2.5
10% SDS solution	0.1
d.H ₂ O	6.0
10% ammonium persulphate	50 µL

Preparation of Coomassie blue staining solution

Coomassie blue staining solution was prepared fresh by mixing 800 mL of 50% (v/v) methanol, 140 mL of 10% (v/v) acetic acid, 0.5 g of 0.25% (w/v) coomassie blue R250 to d.H₂O and final volume was made up to 2000 mL with d.H₂O.

Preparation of destaining solution

Destaining was carried out for the stained polyacrylamide gels using a solution made up of 800 mL methanol, 200 mL glacial acetic acid diluted and volume made up with d.H₂O to 2000 mL.

Preparation of electrode Tris-glycine buffer (pH 8.3)

Concentrated Tris-glycine buffer (10X) was prepared by mixing 30.3 g of Tris (FW 121.1), 144.2 g of glycine, 5 g of SDS in d.H₂O and final volume made up with d.H₂O to 1000 mL. The 10X electrode buffer was diluted by mixing 40 mL of above solution in 360 mL of d.H₂O.

3.1.3.15 Light microscope

A Light microscope (model Ch-2; Olympus Australia Pty. Ltd., Oakleigh, Victoria, Australia) was used to observe the gram stained starter bacteria to check for purity.

3.1.3.16 Rheometer

A Physica MCR300 rheometer (Physica Messtechnik GmbH, Vor dem Lauch, Stuttgart, Germany) attached with a cup and bob spindle (CC27; measuring bob radii 13.33 mm; cup radii 14.46 mm; ratio of radii (r_i/r_e indicating ratio of internal and external cylinder radius). δ 1.0847; gap length, L 40 mm; cone angle 120°; measuring gap 1.13 mm; end effect correction

factor, C_L 1.10; active length 119.2 mm; check length 119.2 mm; length for ISO position 71.5 mm; sample volume 19.35 mL) and attached with a Universal-software US200-0 + R/N was used to study the starch gelatinisation.

3.1.3.17 Hand held mixer/grater

A Braun (MR 555 M CA, multi quick/minipimer control plus vario; 300 watt; Braun Australia Pty Ltd., North Sydney, NSW, Australia) hand held mixer/grater was used to grate the cheeses into finely ground samples for compositional and meltability testing.

3.1.3.18 Water baths and incubators

An Ultrasonic water bath with timer (type FX14PH; Unisonics Pty. Ltd., Sydney, Australia) was used to prepare samples of milk, cheese and byproducts for carbohydrate estimation using HPLC and calcium content estimation using AAS.

A Laboratory water bath with digital temperature control (Thermoline Scientific Equipment Pty. Ltd., Smithfield, New South Wales, Australia) was used for general purpose heat treatment in most of the experiments where the samples required some form of heating and holding at a specific temperature.

A Thermostatic water bath with circulator (Grant Instruments Ltd., Barrington, Cambridge, England) was used for samples of cheeses, which required warming and holding at 60°C for 1 h before being analysed for stretchability using spindles (described in section 3.1.3.3). This type of water bath was used for warming samples such as cheese, which are solid and are difficult to be uniformly heated. The circulation in these water baths helped in maintaining uniform temperature for the samples. Such water baths were used for general purpose heating or warming of different samples.

Incubators (Thermoline Scientific equipment Pty. Ltd., Wetherill Park, New South Wales, Australia) were used to grow starter cultures at their optimum growth temperature and under controlled growth conditions. Also these incubators were used to incubate samples such as those of starch for undergoing enzymatic degradation at different temperatures.

3.1.3.19 Weighing balance

3.1.3.19.1 Analytical balance

All the weighing of chemicals up to four decimal places accuracy was carried out on the Phoenix analytical balance (model AA-200; Phoenix Equipment Inc., Rochester, New York, USA).

3.1.3.19.2 Laboratory weighing balance

Weighing of most samples with two decimal places accuracy was carried out using a laboratory weighing balance (DE series, model 3000D; Denver Instrument Company, Arvada, Colorado, USA).

3.1.3.19.3 Milk weighing balance

A weighing balance (model HP-12K; A & D Co. Ltd., Higashi Ikebukuro, Toshima-ku, Tokyo, Japan) was used to weigh milk for standardising cheese-milk.

3.1.3.20 Electric boiler

A Simons electric boiler (model VS 580, Series automatic electric steam boiler; Simons Electric Boiler Co., Paramatta Road, Camperdown, Australia) was used to generate steam, which was used for steam sterilisation and hot water requirements for cleaning purposes during cheese-making.

3.1.3.21 Ultra-low temperature freezer

An ultra-low temperature freezer (Sanyo, at -80°C temperature: Distributed by Laboratory Supply Pty. Ltd., Mulgrave North, Victoria, Australia) was used for long term storage of starter cultures that were used for cheese making.

3.1.3.22 Laminar flow work station

A laminar flow work station (HWS series; GelmanSciences Pty Ltd., Lane Cove, New South Wales, Australia) was used to perform all the microbiological transfers and enumeration, where sterile conditions were required, except for transfer of bulk starter to the cheese vats.

3.1.3.23 Fume cupboard and fume hood

Fume cupboard and fume hood (E & I Lewis Plastics Pty. Ltd., Bayswater, Victoria, Australia) was used to store and work with concentrated acids and alkalis for safety reasons.

3.1.3.24 Spectrophotometer

A spectrophotometer (Novaspec II, Pharmacia LKB Biochrom Ltd., Science Park, Cambridge, England), was used to analyse the protein concentration in the cheese samples prepared using the Biuret method of protein analyses. PMMA cuvettes (#1939) for UV spectroscopy obtained from Kartell SPA (Richmond, Victoria, Australia) were used to measure the absorbance values on the spectrophotometer.

3.1.3.25 Chroma meter

A Minolta chroma meter (CR-300; Minolta Corporation, Ramsey, New Jersey, USA) a compact tri-stimulus colour analyser for measuring reflected colours of surfaces consisting of a measuring head and a data processor, DP-301 was used to analyse the pizza bake characteristics of mozzarella cheeses. The measuring head had an 8 mm diameter and used diffused illumination at 0° viewing angle to measure the colour of cheese samples. A pulsed xenon arc lamp provided illumination (D₆₅ lighting conditions) on the sample surface for colour measurements in the range of 380 to 780 nm. Before measurements, the chroma meter was calibrated with a white reference tile. The L, a, and b values for the cheeses, which correspond to whiteness, red-green and blue-yellow were measured in triplicate.

3.1.3.26 Hot plate with magnetic stirrer

The general heating and stirring was carried out using a hot plate with magnetic stirrer (Industrial Equipment and Control Pty Ltd., Thornbury, Victoria, Australia) on the work bench in laboratory.

3.1.3.27 Multitube vortex

A multitube vortexer was used to mix samples for the Biuret test of protein content in the cheese samples prior to loading the samples on the SDS-PAGE gel. Also such vortexer was used for general purpose mixing of various solutions in different experiments.

3.1.3.28 Multivac vacuum packaging/gas flush machine

A multivac vacuum packaging machine (type A300/16; Multivac Sepp Haggenmuller KG, Wolfertschwenden, Germany) was used to pack cheeses into barrier bags (Cryovac Australia Pty Ltd., Fawkner, Victoria, Australia) after applying gas flush.

3.2 Cheese making raw materials, equipment, starter cultures and enzyme

3.2.1 General outline of experiments carried out as part of this project

The outline of the project is represented in Figure 3.1. In this study raw milk was received and cream was separated. The resulting skim milk and raw milk were mixed in appropriate proportions (described in section 3.2.6.1) to obtain standardised milk for manufacture of 6% fat containing mozzarella cheeses. Modifications in the method of salting and type of starter culture used, were made to the manufacturing method used by Dairy Farmers, which was part of the preliminary experiments (exp 1, exp 2, exp 3, exp 4) as shown in Figure 3.1.

Mozzarella cheeses were made as per the experimental modifications and analysed for chemical composition, physical, textural, rheological, proteolysis and functional properties. In exp 1 ULFM cheeses were made using two methods of salting. In exp 2 the effects of *L. helveticus* were observed against that for *L. delbrueckii* spp. *bulgaricus* on the characteristics of ULFM cheeses. In exp 3, 6% fat mozzarella cheeses were manufactured using pre-acidification techniques with citric, acetic and lactic acids. Further, in exp 4, pre-acidified milk was mixed with each fat replacer (trials were conducted with two rates of addition of each fat replacer) and cheeses made were analysed for their characteristics of composition, texture and functionality. In exp 5, the results from exp 1, exp 2, exp 3 and exp 4 were used to develop a method for manufacture of ULFM cheeses using pilot scale facilities.

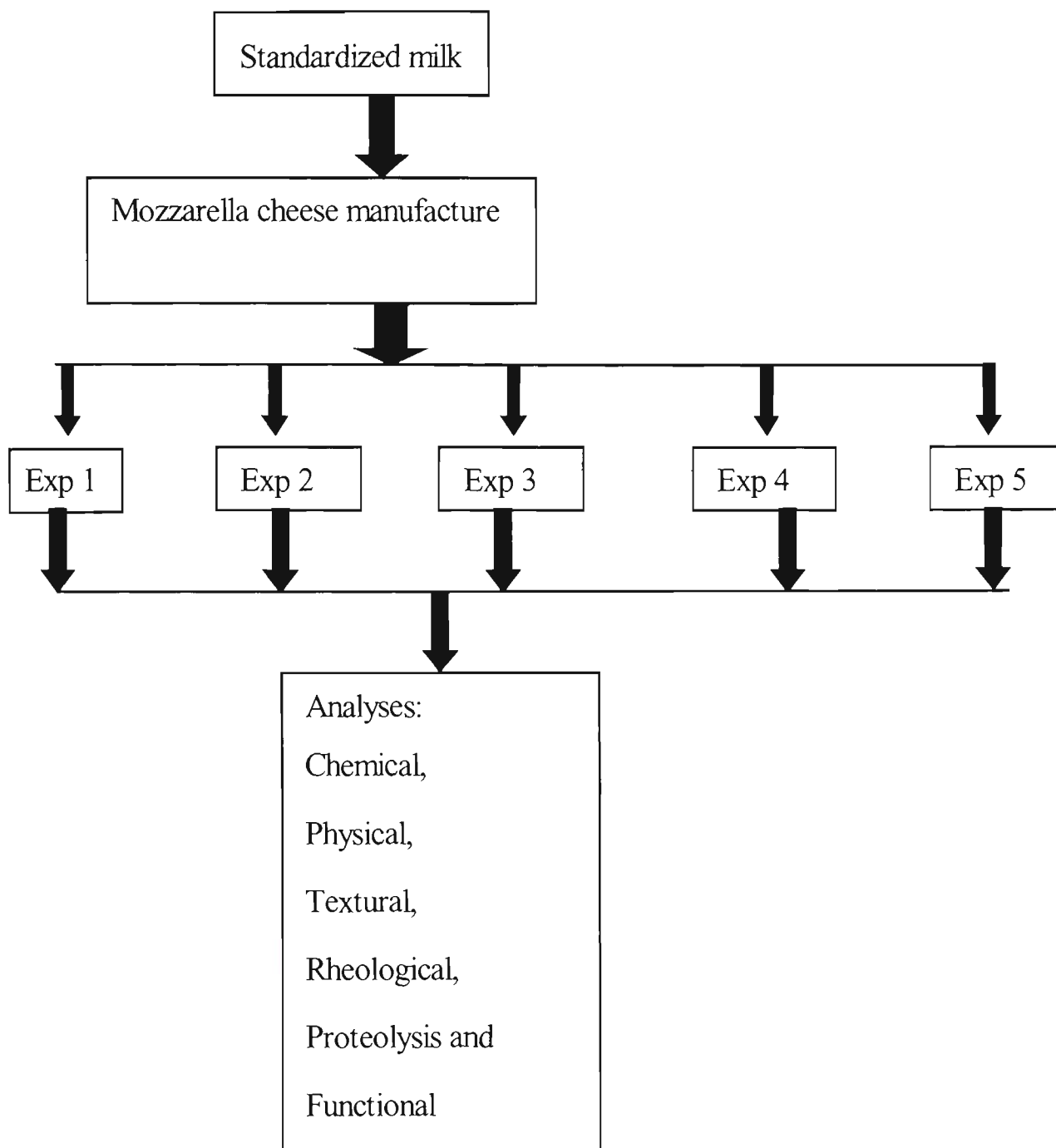


Figure 3.1 General outline of the experiments and type of analyses carried out in this project.

Mozzarella cheeses were made using manufacturing protocol similar to that used at the Dairy Farmers factory (Figure 3.2) after making the necessary adjustments. Throughout the study un-homogenised standardised milk (casein: fat ratio of 5.6, fat content of 0.5%) was used for cheese manufacture.

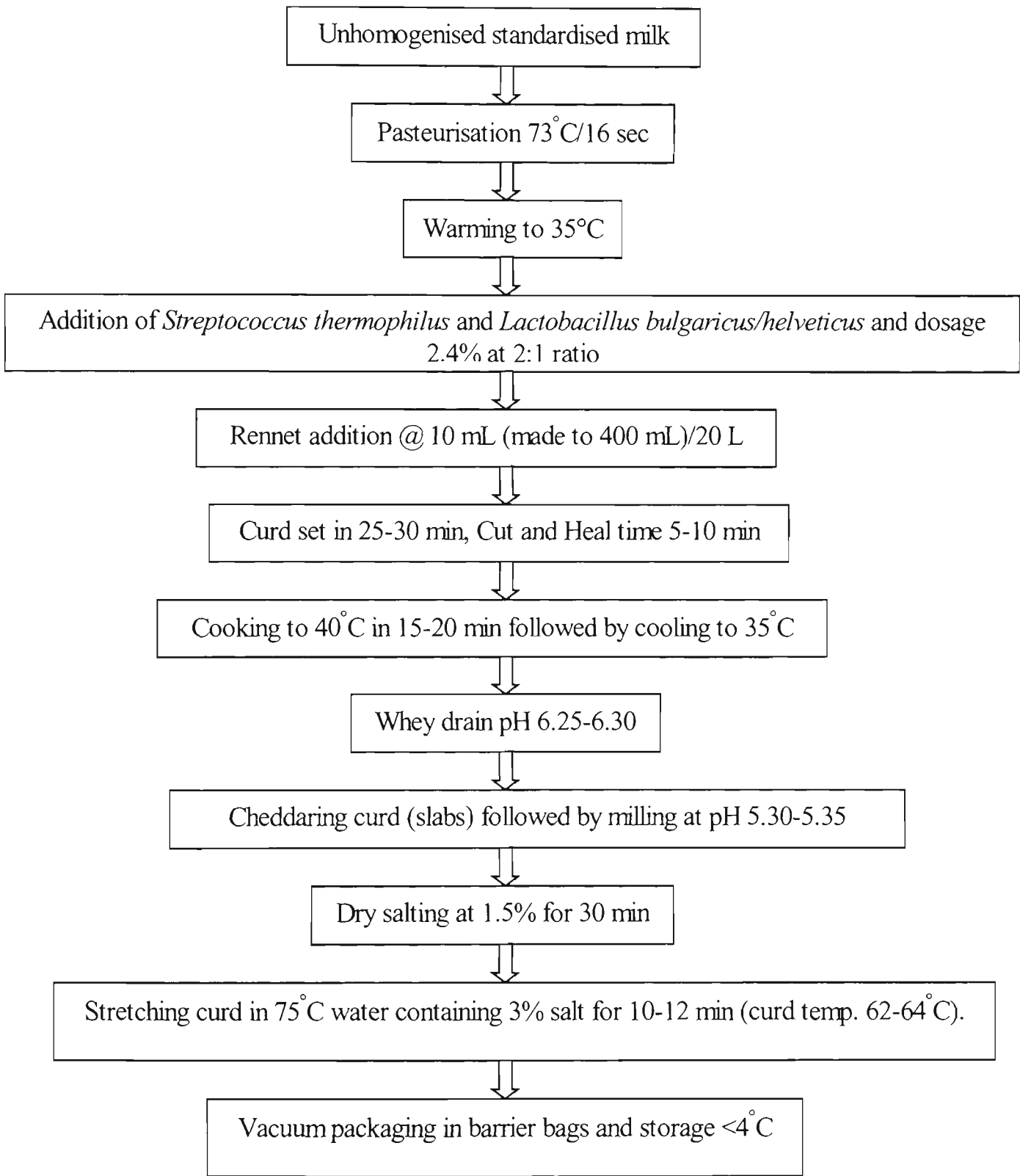


Figure 3.2 Flow chart for manufacture of mozzarella cheeses.

3.2.2 Milk supply

Raw whole milk was obtained from Mamma Lucia Cheese/Puglia Cheese a division of Fresh Cheese Co. (Aust) Pty. Ltd. (Brunswick, Victoria, Australia). The milk was transported in 60 L plastic drums (Nuplas; Mentone, Victoria, Australia) to Victoria University (Werribee, Victoria, Australia) and further processing was carried out.

3.2.3 Fat replacers

Maltrin[®]M040, Maltrin[®]M100 (Grain Processing Corporation, Muscatine, Iowa, USA) are maltodextrins having a Dextrose Equivalent (DE) less than 20. These are spray-dried, non-sweet carbohydrates made by hydrolysing corn starch and are characterised by bland flavour, smooth mouth feel and short texture. Pure Gel B990, a corn starch, which was recommended for processed low-fat and low-temperature products was also tried along with B994, a corn starch which was recommended for low pH, moderate to high shear, high heat processes. These corn starches were propylated to form distarch phosphate, 2-hydroxypropyl ether. Pure Set B950 and B965 are flash dried, acid modified native dent corn starches, which have a low hot viscosity and their solutions form strong gels upon cooling. Pure Gel B990, Pure Gel B994, Pure Set B950 and Pure Set B965 were obtained from Grain Processing Corporation (Muscatine, Iowa, USA).

Versagel (old name X-Tendagel S1) was obtained from Gelled Food International (Wantima South, Victoria, Australia). It is a mixture of β -lactoglobulin, carageenan and xanthan gum. Sta-Slim 143 (A.E. Staley Manufacturing Co., Decatur, Illinois, USA) is labelled as Food Starch modified and is a product derived from potato starch.

National[®]1658 (National Starch and Chemical Pty. Ltd., Seven Hills, Sydney, New South Wales, Australia) is a white to off-white powder having approximately pH of 6.0 and labelled

as Food Starch modified. It is a high viscosity modified corn starch, which is stated to impart heavy-bodied, smooth, short texture when hot and yields a slightly firm texture upon cooling. All the fat replacers are commercially available and have been certified to be generally recognised as safe (GRAS) food ingredients.

3.2.4 Milk processing and cheese making equipment

Full cream milk was warmed to 45-50°C in hot water bath and cream was separated using a batch type cream separator (Model 107 AE, Alfa Laval, Sweden). The skim milk contained less than 0.1% fat. Some of the raw milk was kept aside for re-standardisation of the skim milk to the desired fat content. The raw skim milk and raw whole milk were separately pasteurised at 72°C for 15 sec using a laboratory scale HTST pasteuriser (Alfa Laval Type P20 HRB. APV Australia, Clayton, Victoria, Australia) or a pilot scale HTST pasteuriser shown in Figure 3.3. The milk was standardised (casein: fat ratio of 5.6, fat content of 0.5%) in the cheese-vats before cheese making.



Figure 3.3 A pilot scale HTST pasteuriser used for pasteurising milk.

Cheese-vats of 30 L capacity (Figure 3.4) were custom made (Victoria University, Werribee, Victoria, Australia) from stainless steel, fitted with temperature regulators (model E5CW, Omron Corporation, Toranomon Minato-ku, Tokyo, Japan) and varidrive DC motors with speed control (model KBWM-240; KB Electronics Inc., Coral Springs, Florida, USA) to drive the agitators in the vats. Two such cheese-vats were mounted on a single stand to the appropriate height for ease in handling milk, cheese and byproducts as well as for cheese making. The cheese- vats were fitted with electrical heaters such that the jackets of the vats contained water, which was heated to controlled temperature using electrical coils in the jacket. The heating was controlled using a programming switch, which could be adjusted to the desired temperature and the temperature was maintained using a thermostatic control.



Figure 3.4 Two laboratory scale cheese vats used for making mozzarella cheese.

Mozzarella cheeses were made using 200 L pilot scale cheese making facilities at the Dairy Farmers Research and Development centre, (Brisbane, Queensland, Australia). The cheese vats are shown in Figure 3.5. These vats were heated using steam injected into the water filled jackets with separate thermostatic temperature control for each vat.



Figure 3.5 Pilot scale cheese vats used for manufacture of mozzarella cheeses available at Dairy Farmers facilities.

Cheeses were packed in a vacuum packaging machine (Cryovac Australia Pty. Ltd., Fawkner, Victoria, Australia), which was attached to a gas bottle (containing nitrogen and carbon dioxide in the ratio 70:30) obtained from BOC Gases Australia (North Ryde, New South Wales, Australia).

3.2.5 Starter cultures and enzymes for cheese making

The starter cultures consisting of *L. delbrueckii* ssp. *bulgaricus* (Lb2515), obtained from Victoria University Culture Collection (Victoria University, Werribee, Victoria, Australia) and *S. thermophilus* (372 strain no. TS2000) and *L. helveticus* (474 strain number, LB1) in the form of frozen concentrated starter cultures, obtained from Australian Starter Culture Research Centre (ASCRC, Princes Highway, Werribee, Australia) were used for cheese manufacture. All the starter cultures were non-EPS producing and were stored in 10% RSM mixed with 20% glycerol. The cultures were propagated in 10% sterile RSM several times before being used for cheese-making as explained in sections 3.1.2.1, 3.1.2.2 and 3.1.2.3. Rennet enzyme (described in section 3.1.2) was used for coagulating the cheese-milk. *Streptococcus thermophilus* was added at 1.6% while *Lactobacillus delbrueckii* ssp *bulgaricus* or *Lactobacillus helveticus* at 0.8% to cheese-milk. Rennet (Halal Veal origin as shown in appendices) was added at 50 ppm to milk.

3.2.6 Cheese making

3.2.6.1 Standardisation of cheese milk

Weight whole milk and skim milk (previously pasteurised as described in section 3.2.4) into the cheese vats at a proportion to obtain a casein fat ratio of 5.6 and fat content of 0.5% in the standardised milk. The standardised milk was mixed at 50 rpm using the agitators for 15 min and warmed to 35°C.

3.2.6.2 Preparation of cheese from standardised milk

Standardised milk in the cheese vats (described in section 3.2.6.1) were inoculated with 2.4% (w/v) starter cultures consisting of 2:1 ratio of TS2000 and either Lb2515 or LB1) followed by addition of rennet at 5 mL (diluted 40 times in d.H₂O)/10 L milk. Curd was allowed to set in

25-30 min (curd firmness was manually checked using a knife), cut and allowed to heal for 10 min, cooked in whey at 40°C/20 min, cooled to 35°C and at pH 6.30 curd was pitched and whey was drained. The curd obtained is shown in Figure 3.6.



Figure 3.6 Curd obtained after draining whey. The pH of the curd was about 6.3.

Curd was piled into two slabs and the slabs were turned every 15 min with continuous whey drainage. The fused curd obtained after completion of cheddaring is shown in Figure 3.7.



Figure 3.7 Fused curd obtained after cheddaring operations were completed. The pH of the curd was about 5.3.

Curd was milled at pH 5.1-5.15, salted @ 2.5% (w/w) of curd as shown in Figure 3.8, held for 20 min and stretched in hot saturated brine (if dry salted) or directly after milling the curd was dipped into hot water at 75°C and stretched for 10-12 min as shown in Figure 3.9 followed by dipping in saturated brine solution at 4°C for 2 h (if traditional brining method was followed). The finished cheese was allowed to cool overnight in barrier bags and vacuum packed with gas flush in the vacuum packing machine as described in sections 3.1.3.28, 3.2.4.



Figure 3.8 Application of dry salt to milled curd. The pH of the curd was about 5.2.



Figure 3.9 Curd being kneaded and stretched to a smooth and pliable texture.

3.2.6.3 Preparation of cheese from pre-acidified milk

The standardised (as described in section 3.2.6.1) cheese-milk was pre-acidified with diluted acids namely- food grade citric acid (50% w/v) or edible grade lactic acid (50% v/v) or acetic acid (50% w/v) non food grade). The acids were added into the standardized milk drop-wise to lower the milk pH to 6.10, prior to addition of starter culture. The rest of the steps for cheese making were the same as mentioned in section 3.2.6.2 except that no cooling was carried out for the curd after cooking at 40°C similar to the method suggested by Dairy Farmers (thus all steps following cooking were carried out at 40°C). Also only dry salting was carried out for all the cheeses made from pre-acidified milk.

3.2.6.4 Preparation of pre-acidified, fat-replaced cheeses

Milk for cheese making was standardised as mentioned in section 3.2.6.1. The standardised milk was mixed with 0.10%, 0.25% or 0.5% (w/w) of one of the fat replacers followed by addition of an appropriate amount of citric acid, drop-wise to obtain the desired pH of 6.10. The cheese making was carried out similar to that for cheeses made from pre-acidified milk as described in section 3.2.6.3.

3.2.7 Preparation of mozzarella cheeses for pizza bake analysis

Mozzarella cheeses (3 d old) were manually shredded into long strips (5 mm wide x 20-30 mm long x 0.3-0.5 mm thick) using a shredder. The shredded cheeses were appropriately labelled and packed into barrier bags flushed with mixed gas consisting of carbon dioxide and nitrogen (30: 70) and vacuum packaged using a vacuum packaging machine as described in sections 3.1.3.28, 3.2.4. An equal number of samples were sprayed with 3 g vegetable oil/150 g shredded cheese (sample size for cheese was 150 g), mixed manually, gas flushed and vacuum

packaged. The samples had Nitrogen/ Carbon dioxide gas blanket in the bags and not completely vacuumed before sealing bags. All the sample bags were stored at 3-4°C.

3.2.8 Preparation of mozzarella cheeses for texture analysis

The gas flushed and vacuum packaged mozzarella cheese blocks were unwrapped and cylindrical specimens (20 mm x 25 mm) were cut randomly from several areas of the cheese-block using a cheese corer. The remaining block of cheese was immediately gas flushed, vacuum packaged and stored at 3-4°C. Specimens were obtained in triplicate from each block.

3.2.9 Preparation of mozzarella cheeses for compositional analysis, melt test, stretch test and for measuring expressible serum content

Mozzarella cheeses were shredded manually using a shredder and then grated in a mixer/grater (Braun) to a fine powder (<0.5 mm mesh size). The grated samples were immediately used for compositional analysis, melt test, stretch test or for estimating the expressible serum content. Each of the above mentioned tests were carried out after freshly grating from a shredded cheese block.

3.2.10 Determination of proteolysis in cheeses

Proteolysis in cheese samples was measured using SDS-PAGE. Electrophoresis is a technique to separate compounds on the basis of their electrical charge by placing in an electrical field whereby, positively charged cations move towards the cathodes (negatively charged electrode) and negatively charged anions move towards the anode (positively charged electrode). Due to bacterial, rennet and the native milk enzymes acting on casein and whey proteins, break down products of proteolysis such as amino acids and peptides are produced. These amino acids, peptides and other breakdown proteins possess ionisable groups and therefore can be made into

solution using suitable buffers. Polyacrylamide gel electrophoresis (PAGE) is a technique, which is used to separate molecules based on their size and charge characteristics. A polyacrylamide gel is a porous matrix obtained by polymerisation of acrylamide monomer and cross linking with N, N'-methylene-bis-acrylamide. The structures of acrylamide monomer and N, N'-methylene-bis-acrylamide are shown below:

Acrylamide monomer: $\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$

N, N'-methylene-bis-acrylamide: $\text{CH}_2=\text{CH}-\text{CO}-\text{NH}-\text{CO}-\text{CH}=\text{CH}_2$

When the monomer and cross linker concentrations are varied the degree of polymerisation (length of monomer chain) and the size of the pores in gel matrix are varied. The two parameters used to describe a polyacrylamide gel are: the total acrylamide percentage (%T) and the cross linker percentage (%C), which are shown below.

$$\%T = (\text{g of acrylamide} + \text{g of bis-acrylamide}) \times 100 / 100 \text{ mL H}_2\text{O}$$

$$\%C = \text{g of bis-acrylamide} \times 100 / (\text{g of acrylamide} + \text{g of bis-acrylamide})$$

The %T values depend on the recipe used for preparing the gel and ranges normally between 8 and 18%. The %C is usually kept constant at 3%, which indicates stock gel solution.

The protein samples when applied through the gel matrix (cathode end) migrate towards the anode (bottom of the gel) and the distance of their migration depends on their charge, mass, shape and molecular weight for a given polyacrylamide gel. The charge: mass ratio varies from protein to protein and at neutral pH some proteins have a negative charge while others have a net positive charge. The milk proteins also vary in shape and could be fibrous or globular. In order to have similar shape and charge: mass ratio for the proteins the protein solution is treated with SDS. SDS binds to the proteins and gives them a rod shape and a net negative

charge. Thus when the SDS treated proteins are subjected to PAGE, the rate of migration of proteins is determined by their mass. This means that the smaller proteins having less mass migrate across the polyacrylamide gel faster than do the larger proteins with greater mass. The distance that the proteins migrate in a given time is inversely proportional to their molecular weight. The migrated proteins form bands and stabilise once the electrical charge is removed. Such bands are made visible by soaking the gel in a coomassie brilliant blue, which stains the proteins only.

Samples of protein solutions were prepared as described below:

Cheese samples were grated and 5 g of representative sample was mixed with 10 mL of solution 4 (section 3.1.3.14) and centrifuged using an ultra turrax homogeniser (described in section 3.1.3.13) at 9500 rpm for 60 sec. The centrifuged sample was mixed with 10 mL of solution 5 (section 3.1.3.14), using the ultra turrax homogeniser at 8000 rpm for 30 sec and mixed with 4 mL of solution 6 (section 3.1.3.14). The sample was then mixed properly by inverting 5-6 times and held in an ice bath for 30 min. The sample was centrifuged in a Sorvall RC-28S using rotor SS34 (described in section 3.1.3.7.2) at 18000 rpm for 1 h at 4°C. The supernatant was filtered using a Whatman 1 filter paper and the resultant extract centrifuged in an Eppendorf centrifuge (described in section 3.1.3.7.3) and stored at -20°C. When needed the stored extract was diluted 1:1 with single strength loading buffer (prepared as described in section 3.1.3.14), placed in a boiling water bath for 5 min, analysed for protein content by the Biuret method (described in section 3.2.21), appropriately diluted with single strength loading buffer (approximately 1:20 dilution) such that the loaded sample had 10-15 µg protein/5 µL. A SDS-PAGE gel (20% polyacrylamide gel with SDS as described in section 3.1.3.14) in mini-vertical electrophoresis system was prepared and the stacking gel (described in section 3.1.3.14) was loaded with 5 µL of sample and a similar quantity of standard broad-range marker. The gel was run at 300 V and 30 mA for about 1 h. The gels were taken out of the mini

vertical electrophoresis system, stained with coomassie blue R250 (described in section 3.1.3.14) on the orbital mixer, destained, stored in Gel Air cellophane support and dried in the Gel Air drying system. Details of orbital mixer, Gel Air cellophane support and Gel Air drying system are described in section 3.1.3.14. The above method is similar to that reported by Tunick *et al.* (1995).

3.2.11 Estimation of calcium, sodium, potassium and magnesium in milk, whey, curd, cheese and stretch water samples

3.2.11.1 Preparation of calcium standards

Calcium nitrate (AAS grade as described in section 3.1.3.5) of 10000 ppm concentration was prepared. 1 mL of this standard was added to 25 mL of 24% TCA solution, 25 mL of d.H₂O and 49 mL of 1% lanthanum oxide solution made up in concentrated nitric acid to obtain 100 mL of 100 ppm calcium standard. Further, the 100 ppm calcium standard was diluted using TCA, deionised water and lanthanum oxide in the above mentioned ratio to obtain 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ppm calcium standards as shown in Table 3.1. The method is similar to that reported by Metzger *et al.* (2000). The standards were stored in clean glass bottles, which were placed overnight in dilute nitric acid and then washed in deionised water.

Table 3.4 Calcium standards prepared using 12% TCA, deionised water and 1% lanthanum oxide in nitric acid.

100 ppm calcium standard (mL)	12% TCA (mL)	d.H ₂ O (mL)	1% lanthanum oxide in conc. nitric acid (mL)	Calcium ppm
0 (blank)	25	24.8	0.2	0
1	25	23.8	0.2	2
2	25	22.8	0.2	4
3	25	21.8	0.2	6
4	25	20.8	0.2	8
5	25	19.8	0.2	10
6	25	18.8	0.2	12
7	25	17.8	0.2	14
8	25	16.8	0.2	16
9	25	15.8	0.2	18
10	25	14.8	0.2	20

Standards for sodium, potassium and magnesium were prepared in a similar manner to the calcium standards

3.2.11.2 Preparation of milk samples for AAS

Milk was warmed to approximately 20°C, mixed and 0.75 g of representative sample was weighed, mixed with 14.25 g of 12% (w/w) TCA solution, 0.60 g of 5% (w/v) lanthanum oxide prepared in concentrated nitric acid and 14.40 g of d.H₂O, mixed by inverting several times and filtering through Whatman 541 filter paper. Filtrate (2.5 g) was mixed with 12.37 g of 12% (w/w) TCA solution, mixed with 0.21 g of 5% (w/v) lanthanum oxide prepared in concentrated nitric acid and 4.92 g of d.H₂O. The method used is similar to that reported by Metzger *et al.* (2000). The resultant diluted sample was aspirated into the AAS for calcium estimation. Similarly, dilutions of the filtrate were made for sodium, potassium and magnesium estimation on the AAS as described in section 3.1.3.5.

3.2.11.3 Preparation of whey samples for AAS

Whey at room temperature was mixed and 0.75 g of representative sample was weighed and added to 14.25 g of 12% (w/w) TCA solution, 0.60 g of 5% (w/v) lanthanum oxide prepared in concentrated nitric acid and 14.40 g of d.H₂O, mixed by inverting several times and filtering through Whatman 541 filter paper. Filtrate (5.0 g) was mixed with 9.87 g of 12% (w/w) TCA solution, 0.21 g of 5% (w/v) lanthanum oxide prepared in concentrated nitric acid and 4.92 g of d.H₂O. The method used is similar to that reported by Metzger *et al.* (2000). The resultant diluted sample was aspirated into the AAS for calcium estimation. Similarly, dilutions of the filtrate were made for sodium, potassium and magnesium estimation on the AAS as described in section 3.1.3.5.

3.2.11.4 Preparation of curd and cheese samples for AAS

Curd and cheese samples were grated, 7.50 g of representative sample was weighed and mixed with 22.50 g of 12% (w/w) TCA solution, using an ultra turrax homogeniser, placed in an ultrasonic water bath (described in section 3.1.3.18) for 90 min and filtered through Whatman 541 filter paper. Filtrate (1.0 g) was mixed with 19.0 g of 12% (w/w) TCA solution, 0.8 g of 5% (w/v) lanthanum oxide prepared in concentrated nitric acid and 19.2 g of d.H₂O, The method used is similar to that reported by Metzger *et al.* (2000) and the resultant diluted sample was aspirated into the AAS for calcium estimation. Similarly, dilutions of the filtrate were made for sodium, potassium and magnesium estimation on the AAS as described in section 3.1.3.5.

3.2.11.5 Preparation of stretch water samples for AAS

Stretch water at room temperature was mixed, 2.50 g of representative sample was weighed, mixed with 5.0 g d.H₂O and 7.50 g of 24% (w/w) TCA solution, by inverting several times and

filtered through Whatman 541 filter paper. Filtrate (2.0 g) was mixed with 12.87 g of 12% (w/w) TCA solution, 0.21 g of 5% (w/v) lanthanum oxide prepared in concentrated nitric acid and 4.92 g of d.H₂O. The method used is similar to that reported by Metzger *et al.* (2000). The resultant diluted sample was aspirated into the AAS for calcium estimation. Similarly, dilutions of the filtrate were made for sodium, potassium and magnesium estimation on the AAS as described in section 3.1.3.5.

3.2.12 Determination of casein nitrogen content of cheese-milk

Well mixed cheese-milk sample (5.0 g) was weighed into a 100 mL beaker, mixed with 50 mL of warm (40°C) d.H₂O, 0.5 mL of 10% (w/v) acetic acid solution, and held for 10 min. Further, 0.5 mL of 1M sodium acetate was added, mixed, cooled and filtered through a 9 cm pleated Whatman filter paper. The precipitate was washed thrice from the beaker and twice on the filter paper with d.H₂O. The washed filter paper along with the precipitate was dropped into a digestion tube and protein estimation was performed using Kjeldahl method, described in section 3.2.25. The resultant nitrogen content was measured and converted to casein content by multiplying by a factor of 6.38 (Case *et al.*, 1985; Barbano *et al.*, 1991).

3.2.13 Estimation of salt in mozzarella cheeses (standard Volhard method)

2-3 g of cheese sample was added to 10.0 mL of d.H₂O, mixed with 25.0 mL of 0.05 M silver nitrate solution and heated to 75-80°C. 10.0 mL of concentrated nitric acid was added to the contents of the beaker and boiling was continued for 10 min. The contents were cooled, and mixed with iron alum indicator, 50 mL of d.H₂O and were titrated against 0.05 M potassium thiocyanate until brick red precipitate persisted for 15 sec. A blank solution (without salt) was prepared and analysed in a similar manner.

3.2.14 Determination of the expressible serum content in mozzarella cheeses

Cheese was grated using a hand held mixer/grater (as described in section 3.1.3.17) and 120.0 g of sample was weighed into a centrifuge tube. The tubes were tightly closed and centrifuged at 12500 g at 25°C for 75 min using a Beckman-Coulter centrifuge (described in section 3.1.3.7.1). The expressed serum was decanted into a pre weighed aluminium tray and weighed. The separated serum was denoted as expressible serum.

3.2.15 Determination of meltability of mozzarella cheeses

Mozzarella cheeses were prepared as described in section 3.2.9 and 10.0 g of finely grated cheese was transferred into a glass tube (details in section 3.1.3.2). The cheese was firmly packed using a plunger and the glass tube was stoppered using a rubber stopper (having a hole in the middle so that during heating the hot air inside the tube can escape). The stoppered tubes containing the cheese plug were placed vertically overnight in a refrigerator (<4°C). The length of the cheese plug was measured in each of the tubes using a vernier calliper. The glass tubes containing the cheese plugs were placed horizontally in a preheated hot air oven and the cheese plugs were allowed to melt at $110 \pm 1^\circ\text{C}$ for 100 min. The method is similar to that used by Poduval and Mistry (1999). The glass tubes were air cooled for 10 min and the length of the melted cheese plugs was measured again with a vernier calliper. The difference in initial and final lengths of the cheese plugs was represented as the melt distance.

3.2.16 Determination of stretchability of mozzarella cheeses

A simple test was developed which uses a custom made cross bar spindle (described in section 3.1.3.3) with a hook at one end to attach to an Instron (described in section 3.1.3.4) to conduct the test. The cheese samples were prepared as described in section 3.2.9 for conducting the stretch test. An aliquot of 50 g of cheese was weighed into a glass beaker containing a spindle

placed in the middle of the beaker without touching the sides and perpendicular to the bottom of the beaker. Care was taken to raise the spindle approximately 5 mm from the bottom of the beaker before conducting the stretch test. This would avoid lifting of the cheese plus from beaker. The beaker was covered with an aluminium foil and dipped in a water bath at 60°C for 1.0 h. The melted samples were placed under the load-cell of the Instron and the spindle was attached to the 100 N load cell using a U-clip as shown in Figure 3.10, and stretched to 300 mm/min. The force exerted by the cheese on the spindle was recorded along with the instantaneous distance of load cell movement. Merlin software was used to collect data such as extension at which the strands broke, peak load before breaking and break point during the stretch tests. The weight of a 100 mm cheese strand (cut from the middle of the stretched strand) was taken using an analytical balance and compared among the four sample cheeses.



Figure 3.10 Set-up of an experimental mozzarella cheese sample tempered to 60°C for conducting the stretch test.

3.2.17 Determination of TCA soluble nitrogen content of cheeses

A well mixed grated cheese sample (1.50 g) prepared as described in section 3.2.9 was mixed with 25.0 mL of 12% (w/v) TCA solution, blended for 30 sec using a ultra turrax homogeniser and filtered through a Whatman # 42 filter paper. The blender and filter paper were rinsed with 20.0 mL of 12% (w/v) TCA solution and the rinse was added to the filtrate. The filtrate and rinsings were quantitatively transferred to a Kjeldahl tube and protein estimation was carried out by the Kjeldahl method (described in section 3.2.25). The amount of nitrogen distilled was titrimetrically measured and the protein content was calculated by multiplying the nitrogen content by a factor of 6.38 as suggested by Bynum and Barbano (1985).

3.2.18 Preparation of milk, whey, cheese and stretch water samples for HPLC analysis

3.2.18.1 Preparation of milk and whey samples for HPLC analysis

Well mixed sample (1.0 g) was weighed accurately into a 50 mL falcon tube, mixed with 8.0 mL of 0.1% (v/v) ortho phosphoric acid by inverting several times. The contents were ultra sonicated (description provided in section 3.1.3.18) for 15 min at $60 \pm 1^{\circ}\text{C}$. The samples were cooled to refrigeration temperature ($<5^{\circ}\text{C}$) and held for 30 min, filtered through a Whatman #541 filter paper into a 50 mL volumetric flask. The falcon tube and filtrate were washed twice with 4.0 mL of cold ($<5^{\circ}\text{C}$) 0.1% (v/v) orthophosphoric acid and the washings were added to the flask. To extract fat, 5.0 mL of hexane was added to the volumetric flask, mixed well by inverting several times and the top layer was decanted. Fat extraction using hexane was repeated twice. Traces of hexane were removed under nitrogen using a sample concentrator (Techne) as described in section 3.1.3.6. The contents of the flask were neutralised with 4 M sodium hydroxide to pH 6.6 (approximately 150 μL), mixed with 0.2 mL of lactase enzyme and incubated for 5 h (for milk samples) or 6 h (for whey samples) at $20\text{--}25^{\circ}\text{C}$. The contents of

the flask were made up to a final volume of 50 mL with 0.1% (v/v) orthophosphoric acid (except for whey where the contents were made up to a final volume of 25 mL only).

3.2.18.2 Preparation of cheese samples for HPLC analysis

A representative cheese sample (1.0 g) was weighed accurately into a 50 mL falcon tube, mixed with 8.0 mL of 0.1% (v/v) ortho phosphoric acid using a ultra turrax homogeniser at 9500 rpm for 30 sec. The blender connected to the ultra turrax homogeniser was given three washings with 0.1% orthophosphoric acid (10.0 mL each time) and the washings were added to the falcon tube. The contents were ultra sonicated (description provided in section 3.1.3.18) for 15 min at $60 \pm 1^{\circ}\text{C}$. The samples were cooled to refrigeration temperature ($< 5^{\circ}\text{C}$) and held for 30 min, filtered through a Whatman # 541 filter paper into a 50 mL volumetric flask. The falcon tube and filtrate were washed with 4.0 mL of cold ($< 5^{\circ}\text{C}$) 0.1% (v/v) orthophosphoric acid and the washings were added to the flask. To extract fat, 5.0 mL of hexane was added to the volumetric flask, mixed well by inverting several times and the top layer was decanted. Fat extraction using hexane was repeated twice. Traces of hexane were removed under nitrogen using a sample concentrator (Techne) as described in section 3.1.3.6. The contents of the flask were made up to a final volume of 50 mL with 0.1% (v/v) orthophosphoric acid.

3.2.18.3 Preparation of stretch water samples for HPLC analysis

Well mixed sample (1.0 g) was weighed accurately into a 50 mL falcon tube, mixed with 8.0 mL of 0.1% (v/v) ortho phosphoric acid by inverting several times. The contents were ultra sonicated (description provided in section 3.1.3.18) for 15 min at $60 \pm 1^{\circ}\text{C}$. The samples were cooled to refrigeration temperature ($< 5^{\circ}\text{C}$) and held for 30 min, filtered through a Whatman # 541 filter paper into a 50 mL volumetric flask. The falcon tube and filtrate were washed with 4.0 mL of cold ($< 5^{\circ}\text{C}$) 0.1% (v/v) orthophosphoric acid and the washings were added to the

flask. The contents of the flask were made up to a final volume of 50 mL with 0.1% (v/v) orthophosphoric acid.

The prepared samples of milk, whey, cheese and stretch water were loaded into Varian 9100 auto sampler and analysed for individual peaks of DP1 to DP10 (components of starch) using Supelcogel C610-H using Supelguard as guard column. Details of equipment used are provided in section 3.1.3.6. A loop-size of 80 μ L was used to inject 80 μ L of sample and solvent was delivered at 0.6 mL/min. A thermostatically controlled column heater was used to maintain the column temperature at $30 \pm 1^\circ\text{C}$.

3.2.19 Preparation of fat replacer sample for light microscopy, gel formation study and refractive index measurement

Lite milk containing 1.5% fat was obtained from the local supermarket and adjusted to pH 6.1 using 50% (w/w) citric acid at 4°C . The milk was portioned into 100 mL beakers and was mixed with 0.25% of fat replacer, mixed using a magnetic stirrer. Each 100 mL of pH-adjusted and fat-replaced Lite milk was then sub divided into 20 mL falcon tubes. The tubes were either subjected to no heat treatment or heated to 50°C , 75°C or 90°C and held for 30 min.

3.2.19.1 Analysis of fat replacer sample using rheometry

A rheometer attached with a cup and bob spindle was used to profile the gelatinisation behaviour of fat replacer solution. A fresh 0.25% solution of fat replacer was made in citric acid pre-acidified (pH 6.1) 'Lite' milk as described in section 3.2.19. This solution was taken into the rheometer cup and the spindle was lowered into the cup. A strain of 1 and 0.5 Hz oscillations was set and the solution was heated from 40 to 90°C in 20 mins, followed by holding at 90°C for 20 min and then cooled to 20°C in 20 min. The shear stress ' τ ', torque ' M ', strain ' γ ', deflection angle ' Φ ', shear rate ' $\dot{\gamma}$ ', speed ' n ' and angular velocity ' ω ' were

measured. The gelatinisation profile was plotted using the universal software as described in section 3.1.3.16. The method developed is similar to that reported by Gunasekaran and Ak-Mehmet (2000).

3.2.19.2 Physical examination and refractive index measurement of heated and cooled fat-replaced milk

The pre-acidified, fat-replaced Lite milk samples which were heated to 50, 75 or 90°C and held for 30 min (section 3.2.19) were allowed to cool to room temperature, left overnight in a refrigerator(<4°C) for 20 h and observed for physical characteristics such as gel formation and moisture separation. Their refractive index was measured using a hand held refractometer (0-20 brix range).

3.2.19.3 Preparation of fat replacer sample for light microscopy study

The pre-acidified, fat-replaced Lite milk was heat treated as described in section 3.2.19, cooled to room temperature and subjected to microscopic examination. A sample of 2-3 drops was taken on a glass slide and covered with a cover slip taking care to avoid air bubbles without applying pressure. Observations were made at 10, 40 and 100 x magnifications under a light microscope (described in section 3.1.3.15). Pictures were taken of typical fields using a digital still camera (described in section 3.1.3.8).

3.2.20 Determination of moisture/total solids content

The standard gravimetric method was used in which a known weight of sample was placed in a hot air oven at 100°C overnight and was reweighed to determine loss in weight of the sample. The loss in weight of sample was calculated in relation to original sample weight and was

expressed as per cent moisture in sample. Similarly the weight of residue in relation to the initial sample weight was expressed as per cent total solids (TS).

3.2.21 Protein estimation of cheeses using Biuret method

Reagents used for Biuret estimation of protein in samples are shown below:

Preparation of Biuret reagent

9.0 g of cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 36.0 g of sodium potassium tartarate ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$, Rochelle salt) were dissolved in 500 mL of distilled water in a 1000 mL volumetric flask. 300 mL of freshly prepared carbonate free 10% NaOH solution was added into the 1000 mL volumetric flask with constant stirring and final volume was made up with d. H_2O . The prepared Biuret reagent was stored in a brown bottle.

Preparation of BSA standards

Bovine serum albumin (obtained as described in section 3.1.3.14) of 10 mg/mL concentration was prepared using the standard formula mentioned below.

$$\text{BSA concentration (mg/mL)} = (\text{Absorbance}_{280} - (\text{Absorbance}_{320} \times 1.7)) / 0.66$$

A standard curve was plotted by measuring dilutions of the known BSA standard against the absorbance measured at 540 nm. The Biuret assay works best for protein concentrations around 200-1000 μg . To carry out the test, 1.0 mL of sample (containing 1-15 mg of protein) was mixed with 4.0 mL of the biuret reagent, mixed using a multitube vortexer (details provided in section 3.1.3.27) and allowed to stand for 30 min at room temperature and the absorbance read at 540-650 nm.

3.2.22 Determination of fat content of milk and cheeses using Babcock method

Fat testing in milk and cheese was carried out using Babcock method. In this method 17.5 g freshly prepared milk was poured into the Babcock bottle, mixed with 1.0 mL isoamyl alcohol and 25.0 mL Gerber sulphuric acid (37% concentration). The contents were mixed properly and the volume increased to the top of the neck with more Gerber sulphuric acid and the bottle was placed in a preheated Gerber centrifuge (details in section 3.1.3.12), centrifuged for 10 min followed by immersing in 60°C water bath for 30 min. The top and bottom meniscus of the fat column was read off and recorded. Cheese samples (9.0 g) were weighed into cheese Babcock bottles and mixed with 2-3 mL of (~60°C) hot water, mixed thoroughly, mixed with isoamyl alcohol and Gerber sulphuric acid and fat determination was carried out similar to that for milk samples. The procedure is based on the recommendations of the supplier and standard method of fat testing.

3.2.23 Texture profile analysis of mozzarella cheeses

The cylindrical samples of cheeses obtained as described in section 3.2.8 were placed under a flat plunger of the Instron Universal Testing Machine (details in section 3.1.3.4) and compressed to 50% of their height. Data was collected and hardness, cohesiveness and springiness values were calculated. Cheese samples were analysed for the texture characteristics at several storage times. Compression curves for mozzarella cheeses of force plotted against distance were used to analyse the textural characteristics of the cheeses. The hardness and springiness were directly obtained from the curves while cohesiveness was calculated as a ratio. Hardness is the peak value of force exerted by the sample during the first compression for the given set of conditions of compression and can be measured using a single compression. Springiness is the length by which the sample recovers after the first compression. Cohesiveness is measured as the ratio of area A2 to area A1 (Figure 3.3).

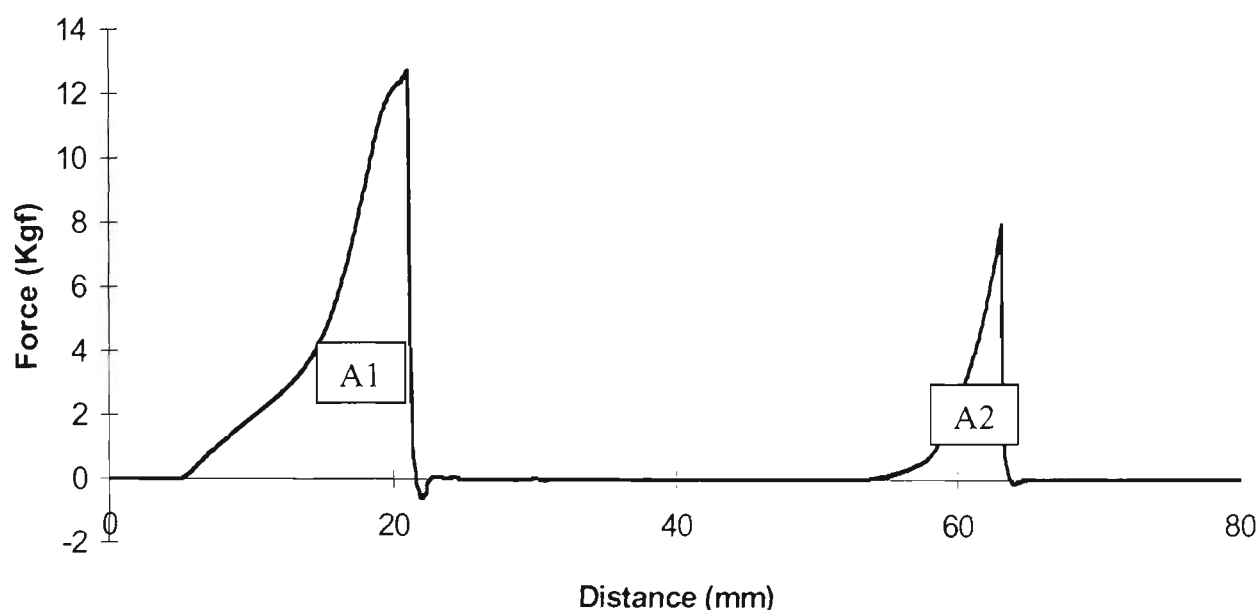


Figure 3.11 A typical texture profile curve obtained by double compression of mozzarella cheese specimen and plotting force (Y-axis) against distance (X-axis). A1 and A2 are the areas of curves obtained by the first and second compression, respectively.

3.2.24 Analysis of functionality of mozzarella cheeses using the pizza bake method

The experimental and commercial cheeses were shredded and prepared as explained in section 3.2.7. The pizza bake test was conducted by applying a table spoon of tomato paste (Leggos, Simplot Australia Pty Ltd., Nepean Hwy, Cheltenham, Victoria, Australia) to a pre-made commercial pizza base (Don Emilio's pizza bases, Freshwell Foods, Coolaroo, Victoria, Australia) followed by spreading the stored cheese (samples without oil application were spread first) on one-half of the pizza base and spreading similar cheese (applied with oil) on the other half. Care was taken not to turn the cheese on the base so as to avoid coloration due to tomato paste and colour measurements were carried out for the fresh cheeses in triplicate using a chroma meter described in section 3.1.3.25. The prepared bases with the cheese were placed in an impinger oven (described in section 3.1.3.9) and upon completion of baking were immediately measured for Hunter L, a, and b values using a Minolta chroma meter (section

3.1.3.25). Also digital images were recorded using the digital still camera (details in section 3.1.3.8). The pizzas were allowed to cool for 25 min and again the Hunter L, a, b readings were taken. The Hunter L, a, and b values were recorded for representative white regions on the pizza as well as for representative dark regions. All the Minolta chroma meter readings were manually entered into an Excel[®] spreadsheet and analysed.

3.2.25 Determination of the protein content of milk, curd, cheese and stretch water using Kjeldahl method

The protein content of samples (milk, curd, cheese, stretch water, 12% TCA soluble nitrogen content of cheeses) were determined by the Kjeldahl method. A known quantity of the sample was weighed into the digestion tube, mixed with two Kjeldahl catalyst tablets, a few glass beads and appropriate amount (10-15 mL) of sulphuric acid using a dispenser. The digestion tubes with their contents were placed in a pre-heated digestion block, preset at 420°C with maximum air-flow through the exhaust for 3-5 min, and covered with a cooling head/manifold. The digestion was continued until all samples were completely digested (approximately 45-55 min) as indicated by a lack of black residue and clarity of the digestion mixture (sky blue or green). The digestion tubes with the mixture were then taken out of the digestion block along with the covering manifold and allowed to air cool to room temperature. The cooled mixtures were mixed with nitrogen free d.H₂O by swirling the tubes and washing the inside surfaces of the tubes so that any sample wetting the top of the tube should not be lost during distillation. Each tube was then placed in the distillation unit and distillates were collected in 25 mL of saturated (4%) boric acid (previously mixed with mixed indicators consisting of 0.7% methyl red and 1% bromocresol green). The total volume collected in the boric acid was kept constant and the distillates were titrated against 0.05 M sulphuric acid until the solution in the receiver flask returned to its original colour (pink). The amount of burette solution (0.05 M H₂SO₄) added was recorded and used to calculate the total nitrogen obtained per gram of sample. The

nitrogen content was then multiplied by a factor of 6.38 to calculate the protein content, which was expressed as per cent protein in the original sample. The method is similar to that reported by Barbano *et al.* (1991).

4.0 EFFECTS OF METHOD OF SALTING ON YIELD, STARTER BACTERIA POPULATION, AND TEXTURAL, AND MELT CHARACTERISTICS OF ULTRA LOW FAT MOZZARELLA CHEESES

4.1 Introduction

Salt added to mozzarella cheeses helps to improve palatability and fat emulsification (Kindstedt *et al.*, 1992), and inhibits the starter and non-starter bacteria during storage and thus improves the overall functionality of cheeses. Brining is also necessary to cool the hot stretched cheese. Salting as an intermediary step during cheese making (salt added to curd after whey draining) has been found to enhance the fermentation capability of some mesophilic starter cultures including *Streptococcus lactis*, *Streptococcus cremoris* and *Streptococcus lactis* ssp. *diacetylactis* strains (Ramazanov *et al.*, 1982). Non-starter organisms including coliforms and aerobic spore formers were inhibited in Domiati type cheeses while *Staphylococcus aureus* were found to be inhibited in Sudanese cheeses (Khalid and Harrigan, 1984) due to addition of salt (Shehata *et al.*, 1984).

Traditional brining where the finished cheese (no salt added during its manufacture) is dipped in a salt solution, has been associated with several disadvantages such as:

1. Contamination with microorganisms
2. Cheese composition within the block varies due to salt and moisture gradients and takes 7 to 14 d to reach equilibrium (Lee *et al.*, 1980; Farkyne *et al.*, 1991)
3. Soft rind formation (McMahon *et al.*, 1993)
4. Disposal of brine solution that has been contaminated with cheese solids
5. Capital cost, time and floor space wasted for carrying out salting

In order to overcome these disadvantages several methods have been developed to perform salting in mozzarella cheeses including:

- 1) brine dipping using higher salt concentration and shorter time (similar to traditional brining),
- 2) dry salting (where dry salt is added to the milled curd before stretching the curd in hot water),
- 3) stretching the unsalted milled curd in hot brine solution, and
- 4) combination of one or more of the above.

In addition to the above, some of the *pasta filata* varieties of cheeses such as bocconcini are packed in containers filled with a dilute brine. This enhances the functionality of the cheese for its end use.

Salt was suggested to act as a regulator in the production of free amino acids and volatile fatty acids (Ramanauskas and Pesetskis, 1976), influence water distribution and the extent of proteolysis in Dutch type cheeses (Ramanauskas, 1971). Ramanauskas and Pesetskis (1976) also concluded that brine salting was better for Dutch cheeses than dry salting. In a study by Furtado *et al.*, (1982), an adverse effect on proteolysis was found to be due to a change in pH with high salt concentration rather than salt content itself. Addition of salt to the cheese-milk prior to cheese making has been reported (Moneib *et al.*, 1970) to increase the yield of cheese. Kindstedt *et al.* (1996) studied the salt and moisture distribution in 9.1 kg blocks of mozzarella. The results indicated that the salt content increased, while moisture content decreased from centre to the surface of the cheeses. Moreover, they found that the salt content was higher at the surface of the blocks.

Studies carried out by Kaya *et al.* (1999) and Kaya (2002) on Gaziantep cheese which is formed without fermentation but enzymatic clotting (Turkish variety) showed that diffusion of salt into the cheese dipped in salt solution takes about 3-6 d and depends on the strength of brine solution. This study also concluded that cheese stored in lower salt concentration brine was softer and had increased L-value. In a study by Kristiansen *et al.* (1999), increasing salting time was found to decrease the moisture content and increase salt-in-moisture content in cheeses. A similar trend in moisture and salt uptake was found in Feta cheese (Prasad and Alvarez, 1999).

The salt distribution in cheese affects the concentration of sodium ions and thus has a localised impact on the micro-constituents and ionic balance among several cations within the cheese body. Addition of sodium chloride (salt) causes an increase in sodium ions, which replace the calcium bound to the protein as colloidal calcium phosphate. The exchange of calcium with sodium ions has an indirect effect on emulsification of fat (Kindstedt, 1993). The ionic exchange was not found to affect the total moisture uptake into the cheese although there could be interchange in the bound versus free moisture contents of the cheese. The amount of moisture held in the cheese depends on the brine temperature and a greater loss in moisture has been reported with an increase in the brine temperature (Kindstedt, 1993). The salt uptake was reported to be only slightly affected by an increase in brine temperature.

Salting was carried out in different varieties including Manchego type cheeses using salting techniques such as a salting gun (Nilson and LaClair, 1975), mineral-based ionic integrator (Innocente and Sensidoni, 1996), high pressure brining (Pavia *et al.*, 2000), vacuum impregnation and pulsed vacuum impregnation (Gonzalez-Martinez *et al.*, 2002) and the salt diffusion rates were studied. These studies suggest that the vacuum pressure driven brining promoted hydrodynamic transport along with the diffusion kinetics thereby improving the salt

penetration into the cheese. Also they found that the curd porosity and moisture content increased the salt uptake dynamics. In a study by Nilson (1969), moisture losses were found to increase whereas salt penetration was unaffected by an increase in brine temperature. The rate of salt penetration into the cheese is thus affected by several factors namely, coefficient of diffusion, cheese geometry, duration of salting, method of salting, brine concentration and amount of salt used, type of cheese and its method of manufacture, compositional factors including fat and moisture contents, distribution of moisture and temperature of storage (Georgakis, 1973; Guinee and Fox, 1986; Morris *et al.*, 1985).

4.2 Aims

The aims of this study were:

1. to examine the characteristics of ULFM cheeses made by traditional brining (TB),
2. to examine the characteristics of ULFM cheeses made by dry salting and stretching in hot brine solution (DS), and
3. to compare the characteristics of TB and DS cheeses and ascertain the most appropriate method for salting ULF mozzarella cheeses.

4.3 Materials and methods

4.3.1 Cheese making

Low-moisture, part skim (LMPS) *pasta filata* mozzarella cheeses containing 5.8% fat were manufactured using starter cultures, namely *Streptococcus thermophilus* (TS2000) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lb2515). Three batches of cheeses per method were made using the TB and DS methods as described in sections 3.2.6.1 and 3.2.6.2 and stored at 4°C.

4.3.2 Analysis of cheeses

The yield of cheeses was measured and the cheese samples were prepared as described in section 3.2.9 for estimation of composition, starter bacteria counts, melt, proteolysis, texture characteristics (section 3.2.8) and for pizza bake characteristics (sections 3.2.7 and 3.2.24). The composition including moisture/total solids was estimated using the method described in section 3.2.20, and fat as in section 3.2.22, protein as in section 3.2.25 and salt as in section 3.2.13. Enumeration of starter bacteria was carried out at various stages of cheese manufacture and during storage as explained in section 3.1.2 (subsections 3.1.2.1 and 3.1.2.4). Proteolysis in cheese samples was studied at weekly intervals using SDS-PAGE (section 3.2.21 and 3.2.10). Textural characteristics including hardness, cohesiveness and springiness were measured by compressing cylindrical cheese samples to 50% height as described in section 3.2.23 using an Instron according to the method of Bhaskaracharya (2000). Meltability test was carried out as described in section 3.2.15 at weekly intervals during storage using 10 g of grated cheese sample heated in glass tube at 110°C for 100 min similar to the method described by Poduval and Mistry (1999). Pizza bake test was carried out as described in section 3.2.24 at 30, 44 and 75 d of storage and the colour, size of blisters and their distribution upon baking were assessed. The fresh, baked and cooled cheese on pizzas were measured for their Hunter L, a, and b values.

4.3.3 Statistical analysis

Statistical analysis of the results was carried out using StatPro[®] software on Microsoft[®] excel. The sample sizes, sample means, sample standard deviations and standard error were calculated. The means from at least two populations were compared. The analysis of variance table was prepared and two sources of variation: the variation *within* each population and variation *among* sample means from the different populations was compared. If the latter variation was large relative to the former, as was measured using an F test, this was deemed to

be evidence of differences between the population means. The p-value obtained from this table at 95% confidence level for all the differences between pairs of means were considered for describing the statistical differences and their significance in the results tables. A small p-value was a result of large differences in population means and the confidence intervals that did not include zero were taken to indicate that the means that were compared were not equal.

4.4 Results and discussion

4.4.1 Yield and composition

The yield and composition of TB and DS cheeses are shown in Table 4.4.1. The yield of DS cheeses was greater ($p<0.05$) than TB cheeses. The DS mozzarella cheeses had similar moisture (Pappas *et al.*, 1996), FDM, M:P, and lower protein (Ahmad *et al.*, 1978), higher salt content ($p<0.03$) and higher salt-in-moisture (S/M) content compared to TB cheeses. The TB cheeses were slow in salt uptake and would have probably achieved higher salt content if longer contact time had been provided for the cheeses in brine solution. Ideally a low-fat mozzarella cheese with 58% moisture content and 1.5% salt content would have a S/M of 2.58%. The increase in amount of salt in DS cheeses would be caused due to the method of salting similar to the results obtained by Gonzalez *et al.*, (2000). Thus the DS cheeses although having lower moisture content, showed 2% S/M content, which could be increased to obtain the ideal S/M content. However, a lower S/M content would be more suitable as suggested by Turner and Thomas (1980) for lactose fermentation to L-lactate by the starter bacteria during storage rather than a cheese with high S/M content where lactose is fermented to the D-lactate by the non-starter bacteria.

4.4.2 Texture characteristics

The mean and standard error for hardness measured of TB and DS cheeses are shown in Table 4.4.2. The hardness values were similar for TB and DS cheeses on 2, 16, 23, 34 and 44 d of storage. The cheeses showed significant differences in hardness values on 9 d and 37 d of storage with the TB cheeses showing lower hardness. TB and DS cheeses showed a significant ($p<0.01$) decrease in hardness during storage from 2 d to 44 d. Although the decrease in hardness was not uniform over the storage period, both cheeses showed the highest values in the first 9 d of storage (Table 4.4.2). Also the DS cheeses had slightly higher (statistically non-significant) hardness values compared to TB cheeses at any storage time, which could be due to higher salt content in DS cheeses. The microstructure observations of unsalted cheeses showed increased open structures compared to salted cheeses (Paulson *et al.*, 1998), which indicates that lower salt containing cheeses would have an open cheese matrix and thus such cheeses may show reduced hardness.

Mean and standard error values of cohesiveness for TB and DS cheeses are shown in Table 4.4.3 along with the significance of storage, calculated using one-way ANOVA. Cohesiveness values for DS cheeses were significantly different to those of TB cheeses on 9 d and 37 d of storage while all other measurements taken on 2, 16, 23, 34 and 44 d were similar for both the cheeses. The effect of storage on cohesiveness was significant for DS cheeses that were stored for 44 d but no clear trend could be established. DS cheeses showed an increase in cohesiveness values with storage to 23 d and then the values decreased thereafter. This seems to point out that the mozzarella cheese system which takes about 21 d to stabilise wherein most of the moisture is absorbed into the protein strands is reflected in terms of changes in cohesiveness values which were found to be lower before and after 16 to 23 d of storage. The cohesiveness values for DS cheeses were the least, soon after manufacture and highest towards

the end of storage period at which measurements were made. TB cheeses did not show a clear trend.

Mean and standard error values of springiness for TB and DS cheeses are shown in Table 4.4.4 along with the significance of storage calculated using one-way ANOVA analysis. TB and DS cheeses showed similar springiness values at 2, 9, 16, 23, 34, 37 and 44 d of storage ($p>0.05$). There was a significant change in springiness values for both the cheeses due to storage. The springiness values were the lowest at 16 d and 9 d for TB and DS cheeses respectively and the highest at 44 d. Thus with storage mozzarella cheeses showed increase in springiness values probably due to expansion of protein strands in the cheese body and decrease in the size and number of voids within the protein matrix (Kindstedt and Guo, 1997). The effect of method of salting was not apparent and both cheeses showed similar springiness characteristic at any storage period.

4.4.3 Functional characteristics

4.4.3.1 Meltability

Meltability measured as the distance of horizontal flow (mm) under no external stress conditions in an enclosed system is shown in Table 4.4.5. The mean and standard error were calculated using the one-way ANOVA analysis and effect of storage on melt distance was also analysed. TB and DS cheeses melted to the same extent on 9 d and 16 d of storage. TB cheeses showed significantly greater meltability ($p<0.01$) at 23 and 34 d compared to DS cheeses. At the end of storage (44 d), both cheeses showed similar meltability. The DS cheeses as shown in Figure 4.4.1 had poorer meltability throughout storage while TB cheeses had significantly increased ($p<0.05$) meltability with storage similar to that reported by Paulson *et al.* (1998). Reports suggest that meltability and free oil in cheeses increase with an increase in salt content but within limits (Apostolopoulos *et al.*, 1994). Both the salt content and the period of brining

were found to affect meltability and meltability was reported to decrease with increase in brining time (Ghosh and Singh, 1991).

4.4.3.2 Pizza bake results at 30 d of storage

4.4.3.2.1 Effects on physical properties as observed with the naked eye on low fat TB and DS mozzarella cheeses (coated or uncoated with oil) due to baking in oven

DS cheeses shown in Figure 4.4.2a, b, c and TB cheeses shown in Figure 4.4.2d, e, f were excessively brown after baking. TB and DS cheeses, which were applied with oil showed excessive blister formation and browning although the cheese shreds were almost completely melted and fused together. TB (no oil) and DS (no oil) cheese samples did not completely melt, had reduced shred fusion, and shreds were burnt without any blisters. All cheeses with or without oil application showed small white regions, but most of the pizza surfaces were scorched and brown.

The representative white and brown regions on the pizzas were measured for the Hunter L, a and b values to obtain an understanding of the changes occurring during baking of experimental low fat mozzarella cheeses on pizza-bases. The mean and standard error for Hunter L, a and b values of 30 d old TB and DS mozzarella cheeses are shown in Table 4.4.6. The table shows the effects on L, a and b values due to-

1. method of salting
2. application of a hydrophobic material (canola oil) to the shredded cheeses
3. baking to 272°C in an impinger oven
4. cooling of baked pizzas for 0.5 h to room temperature.

Table 4.4.6 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for white regions on baked cheeses; L^4 , a^4 , b^4 values for brown regions on baked cheeses; L^5 , a^5 , b^5

values for white regions on cooled cheeses and L^6 , a^6 , b^6 values for brown regions on cooled cheeses.

4.4.3.2.2 Effects on Hunter L, a and b values due to method of salting employed during cheese making

The Hunter L, a and b values for fresh TB and DS cheese were not different indicating that the fresh cheeses were similar in colour. The L, a and b values of baked and cooled TB and DS (with oil) cheeses were not significantly different. Similarly the L, a and b values for baked and cooled TB and DS (no oil) cheese and stored for 30d were similar. The L^4 values (Table 4.4.6) of TB (with oil) cheeses were significantly higher than the L^4 values of DS (no oil) cheeses showing that a combined effect of dry salting without the application of hydrophobic material, could be the cause for increased browning.

4.4.3.2.3 Effects on Hunter L, a and b values due to application of hydrophobic material

The Hunter L, a and b values (Table 4.4.6) for fresh TB and DS cheese (with/no oil) were similar indicating that the fresh cheeses were similar in colour and the hydrophobic material did not affect the colour as measured by Minolta chromameter. The L, a and b values of baked and cooled TB and DS cheeses (with/no oil) were not significantly different. Similarly the L, a and b values for baked and cooled TB and DS cheese (no oil) and stored for 30 d were similar. Between the two treatments of application of oil (with oil) and no application of oil (no oil) for both TB and DS cheeses, there were no marked differences in L, a and b values.

4.4.3.2.4 Effects of baking on Hunter L, a and b values

The L values of TB (no oil) cheeses showed significant changes during baking. The L values as shown by L^3 in Table 4.4.6 decreased with baking and L^4 also showed significant ($p < 0.01$) browning. The TB (with oil) cheeses showed a slight decrease in L values (not significant) during baking although L^3 values were significantly higher than L^4 values of the same cheese.

The DS (with/no oil) cheeses showed a similar trend upon baking. DS (with/no oil) cheeses showed significantly lower L^3 values upon baking compared to their L^2 values. These cheeses were also significantly more brown (L^4 values) compared to fresh and baked white regions of respective pizzas. The Hunter a values significantly increased upon baking for both TB (with/no oil) and DS (with/no oil) cheeses. The a^3 and a^4 values were similar for TB (no oil), TB (with oil) and DS (with oil) cheeses but DS (no oil) cheeses showed a significantly decreased a^4 values.

The Hunter b values for all cheeses increased significantly by baking compared to fresh cheeses. The b^4 values of baked brown regions on pizzas made using TB (with/no oil) and DS (with/no oil) cheeses were significantly lower compared to their respective b^2 values for fresh cheeses and b^3 values (for baked white regions on pizzas).

4.4.3.2.5 *Effects of cooling of pizzas on Hunter L , a and b values of TB and DS cheeses*

Cooling of pizzas to room temperature decreased the gloss/shine of the mozzarella cheeses that were melted on the pizzas. TB (with/no oil) and DS (with/no oil) cheeses showed a significant decrease in L^5 values compared to L^2 values (fresh cheeses). Similarly the L^6 values were the least for each of the TB (with/no oil) and DS (with/no oil) cheeses. The L^3 and L^5 values for TB (with/no oil) and DS (with/no oil) cheeses were similar indicating that although upon physical examination there was a change in the aesthetic appeal of the pizzas after cooling, the L values do not reflect these changes in terms of any marked decrease in L values as was expected. The L^4 and L^5 values although not significantly different showed a further lowering of L values or increase in brown colouration upon cooling. The Hunter a^5 and a^6 values for cooled TB (with/no oil) and DS (with/no oil) cheeses were significantly higher compared to respective a^2 values for the same cheeses. The a^5 values for cooled cheeses were slightly lower (not significant) compared to a^3 values for TB (with/no oil) and DS (with/no oil) cheeses. The

a^6 values for cooled cheeses were similar to a^3 and a^4 values for baked TB (no oil) and DS (with oil) cheeses, similar to a^4 values only for baked TB (with oil) cheeses and were significantly lower than the a^3 and a^4 values for baked DS (no oil) cheeses. Also the a^6 values were similar to a^5 values for TB (with/no oil) and DS (with oil) cheeses whereas they were significantly lower for DS (no oil) cheeses. The Hunter b values i.e. b^5 were significantly higher than b^2 , b^4 and b^6 values and were similar to b^3 values for TB (with/no oil) and DS (with/no oil) cheeses. Similarly the b^6 values were significantly lower than b^2 , b^3 and b^5 values and were similar to b^4 values for TB (no oil) cheeses. The b^6 values were significantly lower than b^3 and b^5 values and similar to b^2 and b^4 values for TB (with oil) cheeses. The b^6 values were significantly lower than b^2 , b^3 and b^5 values but similar to b^4 values for DS (no oil) cheeses. The b^6 values were significantly lower than b^2 , b^3 and b^5 values and similar to b^4 values for DS (with oil) cheeses.

4.4.3.2.6 Overall conclusions from pizza bake test for TB and DS cheeses stored for 30 d

The functionality of TB (with/no oil) and DS (with/no oil) cheeses were similar after baking and after cooling. Each cheese behaved differently due to the temperature treatments during baking and cooling. This effect of heating and cooling of low fat cheeses was observed but the Hunter L, a and b measures were unable to identify these differences between TB and DS cheeses or within each cheese between oil coated (with oil) and no oil coated (no oil) TB or DS cheese samples.

4.4.3.3 Pizza bake results at 44 d of storage

4.4.3.3.1 Effects on physical properties as observed with the naked eye on low fat TB and DS mozzarella cheeses (coated or uncoated with oil) due to baking in oven

Figure 4.4.3 shows pizzas a and b made using DS cheeses while c and d were made using TB cheeses. Both varieties of cheeses showed poor melt and shreds were not completely fused together. The TB and DS cheese shreds, which were either applied with or without oil were

similarly brown and had burnt. Representative white and brown regions on the pizzas were measured for Hunter L, a and b values to understand the changes occurring during baking and cooling.

The mean and standard error for Hunter L, a and b values of 44 d old TB and DS cheeses are shown in Table 4.4.7, where L^2 , a^2 , b^2 values are for fresh cheeses; L^3 , a^3 , b^3 values for white regions on baked cheeses; L^4 , a^4 , b^4 values for brown regions on baked cheeses; L^5 , a^5 , b^5 values for white regions on cooled cheeses and L^6 , a^6 , b^6 values are for brown regions on cooled cheeses.

4.4.3.3.2 *Effects on Hunter L, a and b values due to method of salting employed during cheese making*

The Hunter L, a and b values for fresh TB (no/with oil) and DS (no/with oil) cheeses were similar. Thus there was no effect on colour of the shredded cheeses due to method of salting. The L^2 , L^3 , L^4 and L^6 values were similar for both cheeses indicating that there were no significant differences in the L values caused by the two methods used for salting the cheeses. Between the TB (no oil) and DS (no oil) cheeses, L^5 values for the latter were significantly higher indicating that upon cooling the DS cheeses were whiter. The Hunter a^2 values for TB (no oil) and DS (no oil) cheeses were similar while that for TB (with oil) cheeses were significantly lower than for DS (with oil) cheeses. The a^3 , a^4 , a^5 and a^6 values for TB and DS cheeses were similar and no effect due to the method of salting could be seen. The Hunter b^2 , b^3 , b^4 and b^6 values for TB and DS cheeses were similar. Only b^5 values for TB (no oil) cheeses were significantly lower than for DS (no oil) cheeses while the b^5 values for TB (with oil) and DS (with oil) cheeses were similar.

4.4.3.3.3 Effects on Hunter L, a and b values due to coating with hydrophobic material

Application of hydrophobic material to TB cheeses caused an increase in L^3 , L^4 and L^5 values indicating increased whiteness. The L^2 values for TB (no oil) and TB (with oil) cheeses were similar. In case of DS (with oil) cheeses, the L^2 and L^3 values were similar to those of DS (no oil) cheeses while L^4 , L^5 and L^6 values were significantly higher. The a^2 , a^3 , a^4 , a^5 and a^6 values were similar for TB (with oil) and TB (no oil) cheeses whereas for DS (with oil) cheeses the a^3 and a^4 values were significantly higher while a^2 , a^5 and a^6 values were similar to those for DS (no oil) cheeses. The Hunter b^2 , b^4 and b^6 values for TB (with oil) and TB (no oil) cheeses were similar while b^3 and b^5 values seemed higher for the former cheeses. In case of DS cheeses only b^5 and b^6 values were higher for the ones that were applied with oil when compared to those not applied with oil while b^2 , b^3 and b^4 values were not significantly different.

4.4.3.3.4 Effects of baking on Hunter L, a and b values

The TB (no/with oil) and DS (no/with oil) cheeses showed a significant decrease in L^3 values compared to their L^2 values upon baking. The L^3 values for the white regions on the pizza were significantly higher than their L^4 values (for brown areas on the baked pizzas) for all the cheeses. The a^3 values were significantly higher than the a^4 and a^2 values for TB (no/with oil) and DS (no/with oil) cheeses. The b^3 values for TB (no oil) and DS (no oil) cheeses were similar to their respective b^2 values but were higher than b^2 values for TB (with oil) and DS (with oil) cheeses. The pizzas showed significantly lower b^4 values compared to their b^3 values but the b^4 values were significantly higher than b^2 values of the respective TB (no/with oil) or DS (no/with oil) cheeses.

4.4.3.3.5 Effects of cooling of pizzas on Hunter L, a and b values

The L^5 , a^5 and b^5 values of TB (no/with oil) and DS (no/with oil) cheeses were similar to L^3 , a^3 and b^3 values of the respective cheeses. Similarly the L^6 , a^6 and b^6 values of TB (no/with oil) and DS (no/with oil) cheeses were similar to L^4 , a^4 and b^4 values of the respective cheeses.

These results show that after 44d of storage the cheeses when baked on pizza retain their colour even after cooling to room temperature.

4.4.3.3.6 Overall effects on 44 d old TB and DS cheeses when baked and after cooling

There were no marked differences between TB and DS cheeses. Pizzas made using TB and DS cheeses were burnt and showed excessive scorching which were reflected in their lower L values after baking and after cooling. Application of oil improved the whiteness of both TB and DS cheeses. Baking also increased the whiteness of TB and DS cheeses compared to respective fresh cheeses.

4.4.3.4 Pizza bake results at 75 d of storage

4.4.3.4.1 Effects on physical properties as observed with the naked eye on low fat TB and DS mozzarella cheeses (coated or uncoated with oil) due to baking in oven

Figure 4.4.4 shows typical pizza bake results for TB and DS cheeses. TB cheeses (with/ no oil) had excessive browning and were unappealing. Similarly, DS cheeses also were unappealing but were not as dark as TB cheeses. Pizza made using TB (no oil) cheese showed that the cheese shreds were not completely melted and were not fused together. There were a few blisters formed and the surface of the cheese was burnt. The pizzas made using TB (with oil) cheeses showed better melting of cheese shreds, had improved shred fusion and numerous, larger blisters compared to TB (no oil) cheeses. The DS (no oil) cheeses were less dark than the TB cheeses but still showed poor melt, flow and shred fusion while DS (with oil) cheeses had almost completely melted with numerous large blisters.

Representative white and brown regions on the pizzas were measured for Hunter L, a and b values to understand the changes taking place during baking and cooling. The mean and standard error for Hunter L, a and b values of 75 d old TB and DS cheeses are shown in Table 4.4.8 where L^2 , a^2 , b^2 values are for fresh cheeses; L^3 , a^3 , b^3 values are for white regions on

baked cheeses; L^4 , a^4 , b^4 values are for brown regions on baked cheeses; L^5 , a^5 , b^5 values are for white regions on cooled cheeses and L^6 , a^6 , b^6 values are for brown regions on cooled cheeses.

4.4.3.4.2 *Effects on Hunter L, a and b values due to method of salting employed during cheese making*

The Hunter L^2 , L^3 , L^4 , L^5 and L^6 values for TB (no/with oil) cheeses were not significantly different to DS (no/with oil) cheeses except L^4 values of TB (no oil) cheeses were significantly higher than DS (no oil) cheeses. The Hunter a^2 , a^3 , a^4 , a^5 and a^6 values for TB (no/with oil) cheeses were similar to DS (no/with oil) cheeses. The Hunter b^2 , b^5 and b^6 values for TB (no/with oil) cheeses were not significantly different to those of DS (no/with oil) cheeses. The b^3 and b^4 values of TB (with oil) cheeses were significantly greater than the respective b^3 and b^4 values of DS (with oil) cheeses. The b^3 values of TB (no oil) cheeses were similar to b^3 values of DS (no oil) cheeses. The b^4 values for TB (no oil) cheeses were significantly greater than the b^4 values of DS (no oil) cheeses.

4.4.3.4.3 *Effects on Hunter L, a and b values due to coating with hydrophobic material*

There were no significant differences in the L values of fresh TB (no/with oil) and DS (no/with oil) cheeses. The L^3 and L^4 values of TB (with oil) and TB (no oil) cheeses were not significantly different. Upon cooling the L^5 and L^6 values of TB (with oil) cheeses were higher than those for TB (no oil) cheeses. The L^3 and L^5 values for DS (with oil) and DS (no oil) cheeses were not significantly different while the L^4 and L^6 values for DS (with oil) cheeses were significantly higher than the L^4 and L^6 values for DS (no oil) cheeses. The Hunter a values for TB (no/with oil) and DS (no/with oil) cheeses were similar at the different temperature regimes of pizza-making. The Hunter b^2 , b^4 , b^5 and b^6 values for TB (no/with oil) cheeses were not significantly different but b^3 for TB (with oil) cheeses were significantly higher than the b^3 values for TB (no oil) cheeses. The Hunter b^2 , b^3 , b^4 and b^6 values for DS

(no/with oil) cheeses were not significantly different but b^5 values for DS (with oil) cheeses were significantly greater than those for DS (no oil) cheeses.

4.4.3.4.4 *Effects of baking on Hunter L, a and b values*

The TB (no/with oil) and DS (no/with oil) cheeses showed significantly higher L^3 values compared to L^2 values but the L^4 values were significantly lower than the L^3 values for the same cheeses. The a^3 values were not significantly increased due to baking compared to a^2 values for TB (no/with oil) and DS (no/with oil) cheeses. The a^4 values measured for the brown regions of pizzas showed significantly high values compared to both a^2 and a^3 values for TB (no/with oil) and DS (no/with oil) cheeses. The b^3 values were significantly reduced for baked TB (no/with oil) and DS (no/with oil) cheeses compared to b^2 values and the b^4 values were also significantly reduced compared to b^3 values and b^2 values.

4.4.3.4.5 *Effects of cooling of pizzas on Hunter L, a and b values*

The L^5 values of cooled TB (with oil) and DS (no/with oil) cheeses were not significantly different to their L^3 values, but were significantly different to their L^2 , L^4 and L^6 values. The TB (no oil) cheeses showed L^5 values similar to L^4 values but significantly higher than L^2 and L^6 values. The a^5 values were significantly higher than the a^2 values, significantly lower than a^4 and a^6 values and were similar to a^3 values for TB (no oil) and DS (no oil) cheeses. In case of TB (with oil) and DS (with oil) cheeses, the a^5 values were similar to a^2 and a^3 values but significantly lower than the a^4 and a^6 values. The a^6 values for TB (no/with oil) and DS (no/with oil) cheeses were similar to a^4 values and significantly higher than a^2 , a^3 and a^5 values. Thus application of oil caused a decrease in a^5 values compared to a^3 values for TB (with oil) and DS (with oil) cheeses, which was not observed in the case of both TB (no oil) and DS (no oil) cheeses. The Hunter b^5 values were significantly greater than the b^4 and b^6 values, significantly lower than b^2 values for TB (no/with oil) and DS (no/with oil) cheeses and were similar to b^3 values for only TB (with oil) and DS (with oil) cheeses but significantly lower

than b^3 values of TB (no oil) and DS (no oil) cheeses. The b^6 values were similar to b^4 values and significantly lower than b^2 , b^3 and b^5 values for TB (no/with oil) and DS (no/with oil) cheeses.

4.4.3.4.6 Overall conclusions from pizza bake test after storing TB and DS cheeses for 75 d

Baking of TB (no/with oil) and DS (no/with oil) cheeses at 272°C increased the L and a values but decreased b values compared to fresh cheeses. The brown regions on the pizzas showed reduced L values, increased a values and decreased b values for TB and DS cheeses compared to L, a and b values of white regions. Cooled white regions showed similar L, a and b values as for baked hot white regions and cooled brown regions showed similar L, a and b values as for baked hot brown regions on pizza for all the cheeses. The cooled cheeses showed higher L and a values but lower b values compared to those for the fresh TB and DS cheeses. Application of oil increased the whiteness of cooled pizzas but otherwise there was no significant effect observed in L, a and b values due to application of hydrophobic material. Salting had very little effect on the Hunter L, a and b values of the cheeses although the DS cheeses appeared to have better melt, shred fusion and less browning. The cheeses at 75 d showed increased 'L' values upon baking, which decreased upon cooling. The L values after baking and after cooling pizzas, were higher at 75 d compared to those at 30 d and 44 d for TB and DS cheeses. TB and DS cheeses had similar L values at similar stages of baking and cooling when cheeses were tested at 30 d, 44 d and 75 d. Zerfiridis *et al.* (1989) suggested that the functionality of the cheeses could be greatly improved by ripening at higher temperatures initially (at ~15°C) before storing at <4°C. Although this was out of the scope for this experiment, it would be useful for commercial applications to conduct trials.

4.4.4 Microbiological results

The starter bacteria consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were inoculated each into cheese-milk at approximately 10^6 cfu/mL out of which $\sim 10^4$ cfu/mL of each type of bacteria were lost into the whey after 2 h of inoculation (Table 4.4.9) and the curd contained $\sim 10^6$ cfu/mL of each organism. Further, counts from 0 d cheese showed that the DS cheeses had 1 log higher count of cocci and rods compared to TB cheeses. In both the cheeses, the total bacterial population increased until 16 d of storage after which they decreased in numbers similar to those reported by Girgis *et al.* (1981). TB and DS cheeses showed similar cocci counts at 34 d of storage but the latter had 1 log cycle higher counts of rods. The total manufacturing time for TB and DS cheeses was similar (~ 5.5 h), but rods were in greater numbers in the latter at 0 d and during further storage. This could be due to higher salt concentration (DS cheese), which could have inhibited the non-starter bacteria, thereby allowing better growth of rods. The numbers of cocci and rods increased during manufacture, reduced during stretching, but increased again until 16 d of storage (Table 4.4.9) in both varieties of cheeses. The starter bacteria showed better viability in DS cheeses at 34 d of storage.

4.4.5 Proteolysis

The SDS-PAGE results for TB and DS cheeses are shown in Figure 4.4.5. Lane 1 indicates bands of proteins separated from broad range marker comprising of myosin, β -galactosidase, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme and aprotinin, respectively which are shown as B1, B2..B8 respectively in Figure 4.4.5. Table 4.4.10 shows the molecular weights of the proteins comprised in the broad range marker. Lane 2 indicates protein bands separated from a mixture of milk proteins namely, β -casein, α -casein, κ -casein, β -lactoglobulin A and α -lactalbumin from top to bottom respectively as shown in Figure 4.4.5. Table 4.4.11 shows the concentrations and molecular weights of the milk proteins

that were run in lane 2. The lanes 3 to 8 in Figure 4.4.5 indicates the protein samples that were isolated from TB and DS cheeses after storing them for 1, 8, 15, 22, 29 and 36 d, respectively.

SDS-PAGE for TB cheeses showed that with storage of the cheeses there was increased degradation of β -casein into smaller fractions, and α -casein also was degraded into several fractions. In their study (Feeney *et al.* (2002) found that α_{s1} -casein was degraded into two fractions namely α_{s1} -casein (f24- 199) and α_{s1} -casein (f102- 199). Also κ -casein was found to reduce with increase in storage. The κ -casein band was evident at 1, 8 and 15 d and its concentration appeared to decrease during storage. The β -lactoglobulin A and α -lactalbumin appeared to be stable in TB cheeses throughout storage. The DS cheeses showed breakdown of β -casein, α -casein and κ -casein into smaller proteins (Figure 4.4.5) while β -lactoglobulin A and α -lactalbumin seemed to remain stable. When TB and DS cheeses were compared no major differences in proteolysis could be found which indicates that the method of salting may not play an important role in the proteolytic pattern of unripened cheeses, rather the enzymes released by the starter bacteria could be more important. Similar proteolysis results for different treatments based on salting methods for feta cheeses was found by Pappas *et al.* (1996).

4.5 Conclusions

The cheeses made using the DS method showed higher moisture retention and an increased yield. DS cheeses showed similar hardness values, but poor meltability compared to TB cheeses. Although no significant differences could be seen between TB and DS cheeses by the pizza bake test, results showed that such low fat cheeses require a longer storage period (about 75 d) to improve their functionality. DS cheeses also had increased starter culture populations

Table 4.4.1 Mean ± Standard error of yield and composition of TB and DS cheeses (n = 9).

Characteristic	TB cheese	DS cheese	p-value ¹
Yield ² kg/ 100 kg	8.172 ± 0.011 ^b	8.337 ± 0.007 ^a	0.0011 [*]
Moisture (%)	53.34 ± 0.29 ^a	53.84 ± 0.43 ^a	0.3471
Protein (%)	36.45 ± 0.12 ^a	35.61 ± 0.32 ^b	0.0249 [*]
FDM ³ (%)	12.39 ± 0.60 ^a	12.53 ± 0.36 ^a	0.8404
M:P ⁴	1.46 ± 0.01 ^a	1.51 ± 0.02 ^a	0.0641
Salt (%)	0.49 ± 0.01 ^b	1.08 ± 0.01 ^a	0.0000 [*]
S/M ⁵ (%)	0.92 ± 0.01 ^b	2.01 ± 0.01 ^a	0.0000 [*]

¹ANOVA of means; ²n= 3 ; ³FDM= Fat in dry matter; ⁴M:P= Moisture: Protein; ⁵S/M= salt in moisture content; ^{a,b} Means within same row not sharing common superscripts differ (p<0.05);

^{*}Significant (p<0.05).

despite their higher salt content. Both TB and DS cheeses showed a similar extent of proteolysis during storage.

Table 4.4.1 Mean ± Standard error of yield and composition of TB and DS cheeses (n = 9).

Characteristic	TB cheese	DS cheese	p-value ¹
Yield ² kg/ 100 kg	8.172 ± 0.011 ^b	8.337 ± 0.007 ^a	0.0011 [*]
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S/M ⁵ (%)	0.92 ± 0.01 ^b	2.01 ± 0.01 ^a	0.0000 [*]

¹ANOVA of means; ²n= 3 ; ³FDM= Fat in dry matter; ⁴M:P= Moisture: Protein; ⁵S/M= salt in moisture content; ^{a,b} Means within same row not sharing common superscripts differ (p<0.05);

^{*}Significant (p<0.05).

Table 4.4.2 Hardness values (Mean \pm SE¹) for TB and DS cheeses measured (n = 9) throughout refrigerated storage.

Storage (d)	TB cheese	DS cheese	p-value ²
2	164.60 \pm 5.28 ^{a, A}	172.37 \pm 11.21 ^{a, A}	0.5397
9	133.07 \pm 6.03 ^{b, B}	165.00 \pm 10.41 ^{a, A}	0.0173 [*]
16	94.19 \pm 6.68 ^{def, A}	94.88 \pm 6.55 ^{cdef, A}	0.9422
23	77.60 \pm 4.32 ^{efg, A}	84.10 \pm 11.62 ^{ef, A}	0.6072
34	100.91 \pm 9.49 ^{cdef, A}	106.31 \pm 7.57 ^{bcdef, A}	0.6625
37	59.72 \pm 3.59 ^{g, B}	77.90 \pm 2.65 ^{f, A}	0.0009 [*]
44	76.49 \pm 3.39 ^{fg, A}	86.87 \pm 6.28 ^{def, A}	0.1599
p-value ³	0.0000 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c, d, e, f, g} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05).

Table 4.4.3 Cohesiveness values (Mean \pm SE¹) for TB and DS cheeses measured (n = 9) throughout refrigerated storage.

Storage (d)	TB cheese	DS cheese	p-value ²
2	0.727 \pm 0.008 ^{a, A}	0.718 \pm 0.010 ^{c, A}	0.5037
9	0.746 \pm 0.002 ^{a, A}	0.719 \pm 0.006 ^{bc, B}	0.0009 [*]
16	0.760 \pm 0.004 ^{a, A}	0.753 \pm 0.004 ^{a, A}	0.2453
23	0.774 \pm 0.003 ^{a, A}	0.759 \pm 0.006 ^{a, B}	0.0406 [*]
34	0.711 \pm 0.034 ^{a, A}	0.730 \pm 0.003 ^{abc, A}	0.5894
37	0.773 \pm 0.003 ^{a, A}	0.753 \pm 0.006 ^{a, B}	0.0102 [*]
44	0.757 \pm 0.006 ^{a, A}	0.753 \pm 0.003 ^{a, A}	0.5911
p-value ³	0.0098 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 4.4.4 Springiness values (Mean \pm SE¹) for TB and DS cheeses measured (n = 9)

throughout refrigerated storage.

Storage (d)	TB cheese	DS cheese	p-value ²
2	1.389 \pm 0.065 ^{ab, A}	1.544 \pm 0.073 ^{ab, A}	0.1319
9	1.411 \pm 0.059 ^{ab, A}	1.344 \pm 0.053 ^{b, A}	0.4121
16	1.333 \pm 0.060 ^{b, A}	1.400 \pm 0.047 ^{ab, A}	0.3956
23	1.456 \pm 0.053 ^{ab, A}	1.422 \pm 0.062 ^{ab, A}	0.6878
34	1.400 \pm 0.083 ^{ab, A}	1.467 \pm 0.076 ^{ab, A}	0.5636
37	1.578 \pm 0.049 ^{ab, A}	1.644 \pm 0.087 ^{ab, A}	0.5138
44	1.650 \pm 0.045 ^{a, A}	1.667 \pm 0.045 ^{a, A}	0.7962
p-value ³	0.0020 [*]	0.0021 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^A Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 4.4.5 Meltability (Mean \pm SE¹) for TB and DS cheeses measured (n = 9) in millimeters throughout refrigerated storage.

Storage (d)	TB cheese	DS cheese	p-value ²
9	39.800 \pm 1.619 ^{ab, A}	36.867 \pm 2.426 ^{a, A}	0.3383
16	38.937 \pm 1.304 ^{b, A}	35.983 \pm 1.471 ^{a, A}	0.1638
23	40.263 \pm 0.518 ^{ab, A}	35.913 \pm 0.783 ^{a, B}	0.0009*
34	44.630 \pm 0.846 ^{ab, A}	38.847 \pm 1.428 ^{a, B}	0.0059*
44	48.783 \pm 4.040 ^{a, A}	40.820 \pm 3.050 ^{a, A}	0.1468
p-value ³	0.0123*	0.3705	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05); *Significant (p<0.05).

Table 4.4.6 Mean \pm SE¹ (n = 9) Hunter L a b-values of TB and DS mozzarella cheeses applied with or without canola oil and stored for 30 d at refrigerated temperature, measured fresh² (before baking), after baking (white³ and brown⁴) and after cooling (white⁵ and brown⁶) to room temperature.

Hunter L a b	TB cheese		DS cheese		p-value ⁷
	No oil	With Oil	No oil	With Oil	
L ²	56.55 \pm 1.66 ^{a, A}	55.21 \pm 0.95 ^{a, A}	57.96 \pm 0.68 ^{a, A}	56.85 \pm 0.72 ^{a, A}	0.3658
L ³	49.85 \pm 1.48 ^{bc, A}	49.60 \pm 2.27 ^{ab, A}	44.42 \pm 1.26 ^{bc, A}	50.51 \pm 2.05 ^{bc, A}	0.0858
L ⁴	35.92 \pm 1.03 ^{de, AB}	37.56 \pm 0.70 ^{cd, A}	33.65 \pm 0.96 ^{de, B}	36.10 \pm 0.50 ^{de, AB}	0.0190*
L ⁵	45.22 \pm 1.27 ^{c, A}	48.17 \pm 1.64 ^{b, A}	44.21 \pm 1.54 ^{c, A}	46.88 \pm 1.51 ^{c, A}	0.2682
L ⁶	33.13 \pm 0.96 ^{e, AB}	35.56 \pm 0.41 ^{d, A}	32.79 \pm 0.56 ^{e, B}	34.72 \pm 0.39 ^{e, AB}	0.0106*
a ²	-1.46 \pm 0.28 ^{b, A}	-1.46 \pm 0.21 ^{c, A}	-1.30 \pm 0.12 ^{d, A}	-1.04 \pm 0.15 ^{b, A}	0.4140
a ³	12.90 \pm 0.80 ^{a, A}	14.05 \pm 1.23 ^{a, A}	13.32 \pm 0.69 ^{a, A}	12.64 \pm 0.99 ^{a, A}	0.7375
a ⁴	11.81 \pm 0.59 ^{a, A}	12.38 \pm 0.39 ^{ab, A}	10.62 \pm 0.50 ^{b, A}	11.16 \pm 0.45 ^{a, A}	0.0814
a ⁵	11.94 \pm 0.70 ^{a, A}	13.82 \pm 0.67 ^{ab, A}	11.15 \pm 0.68 ^{ab, A}	11.93 \pm 0.60 ^{a, A}	0.4760
a ⁶	10.27 \pm 0.49 ^{a, A}	10.86 \pm 0.24 ^{b, A}	10.48 \pm 0.40 ^{c, A}	11.04 \pm 0.35 ^{a, A}	0.4882
b ²	15.08 \pm 0.62 ^{b, A}	14.38 \pm 0.49 ^{bcd, A}	15.62 \pm 0.37 ^{b, A}	15.09 \pm 0.51 ^{b, A}	0.3935
b ³	23.50 \pm 1.21 ^{a, A}	22.65 \pm 2.48 ^{a, A}	21.04 \pm 0.96 ^{a, A}	25.06 \pm 1.73 ^{a, A}	0.4169
b ⁴	10.11 \pm 1.21 ^{d, A}	9.24 \pm 0.50 ^{d, A}	10.82 \pm 0.72 ^{cd, A}	9.88 \pm 0.53 ^{cd, A}	0.5695
b ⁵	22.05 \pm 0.94 ^{a, A}	24.86 \pm 1.50 ^{a, A}	19.81 \pm 1.33 ^{a, A}	23.85 \pm 1.52 ^{a, A}	0.0599
b ⁶	10.38 \pm 0.83 ^{cd, A}	10.26 \pm 0.49 ^{cd, A}	9.44 \pm 0.62 ^{d, A}	9.24 \pm 0.58 ^{d, A}	0.5026
L a b ^{2,3,4,5,6} (p-value ⁸)	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked White regions; ⁴Baked brown regions; ⁵Cooled white regions; ⁶Cooled brown regions; ⁷ANOVA of

means arranged within the same row; ⁸ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{^, B} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

Table 4.4.7 Mean \pm SE¹ (n = 9) Hunter L a b-values of TB and DS mozzarella cheeses applied with or without canola oil and stored for 44 d at refrigerated temperature, measured fresh² (before baking), after baking (white³ and brown⁴) and after cooling (white⁵ and brown⁶) to room temperature.

Hunter L a b	TB cheese		DS cheese		p-value ⁷
	No oil		No oil	With Oil	
L ²	56.15 \pm 1.19 ^{a, A}	54.72 \pm 0.83 ^{a, A}	54.82 \pm 0.73 ^{a, A}	53.65 \pm 0.56 ^{a, A}	0.2231
L ³	39.53 \pm 0.59 ^{bc, B}	45.09 \pm 0.98 ^{b, A}	44.95 \pm 1.01 ^{bc, A}	44.82 \pm 1.20 ^{b, A}	0.0022
L ⁴	34.30 \pm 0.52 ^{e, BC}	36.61 \pm 0.45 ^{d, A}	34.24 \pm 0.55 ^{de, C}	36.59 \pm 0.58 ^{cd, A}	0.0014
L ⁵	36.03 \pm 1.00 ^{cd, B}	NA [*]	42.33 \pm 1.76 ^{c, A}	NA [*]	0.0053
L ⁶	34.69 \pm 0.85 ^{de, AB}	37.06 \pm 0.37 ^{cd, A}	33.47 \pm 0.58 ^{e, B}	36.44 \pm 0.54 ^{d, A}	0.0002
a ²	-1.28 \pm 0.21 ^{e, AB}	-1.36 \pm 0.23 ^{d, B}	-0.73 \pm 0.16 ^{b, AB}	-0.55 \pm 0.12 ^{d, A}	0.0034
a ³	11.74 \pm 0.35 ^{a, AB}	14.03 \pm 0.74 ^{a, A}	9.71 \pm 0.40 ^{a, B}	13.16 \pm 0.60 ^{a, A}	0.0000
a ⁴	10.21 \pm 0.26 ^{bcd, AB}	10.91 \pm 0.25 ^{bc, AB}	9.91 \pm 0.34 ^{a, B}	11.26 \pm 0.33 ^{bc, A}	0.0112
a ⁵	9.91 \pm 0.29 ^{cd, A}	NA [*]	9.31 \pm 0.67 ^{a, A}	NA [*]	0.4526
a ⁶	9.35 \pm 0.41 ^{d, A}	10.07 \pm 0.15 ^{c, A}	9.20 \pm 0.30 ^{a, A}	10.20 \pm 0.22 ^{c, A}	0.1960
b ²	14.54 \pm 0.36 ^{a, A}	14.51 \pm 0.49 ^{b, A}	14.80 \pm 0.33 ^{a, A}	14.46 \pm 0.25 ^{b, A}	0.8664
b ³	13.99 \pm 0.63 ^{a, B}	20.25 \pm 1.09 ^{a, A}	16.83 \pm 0.79 ^{a, AB}	18.56 \pm 0.90 ^{a, A}	0.0003
b ⁴	8.03 \pm 0.49 ^{bcd, A}	8.21 \pm 0.44 ^{cd, A}	6.89 \pm 0.63 ^{bc, A}	8.40 \pm 0.51 ^{cd, A}	0.1989
b ⁵	7.51 \pm 0.73 ^{cd, B}	NA [*]	14.31 \pm 1.40 ^{a, A}	NA [*]	0.0018
b ⁶	6.13 \pm 0.57 ^{d, AB}	6.60 \pm 0.33 ^{d, AB}	5.95 \pm 0.63 ^{c, B}	8.05 \pm 0.53 ^{d, A}	0.0278
L a b ²³⁴⁵⁶ (p-value ⁸)	0.0000 [*]	0.0000 [*]	0.0000 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked White regions; ⁴Baked brown regions; ⁵Cooled white regions; ⁶Cooled brown regions; ⁷ANOVA of

means arranged within the same row; ⁸ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); NA= Not available for measurement;

^{*}Significant (p<0.05).

Table 4.4.8 Mean \pm SE¹ (n = 9) Hunter L a b-values of TB and DS mozzarella cheeses applied with or without canola oil and stored for 75 d at refrigerated temperature, measured fresh² (before baking), after baking (white³ and brown⁴) and after cooling (white⁵ and brown⁶) to room temperature.

Hunter L a b	TB cheese		DS cheese		p-value ⁷
	No oil	With Oil	No oil	With Oil	
L ²	57.08 \pm 1.02 ^{d, A}	57.80 \pm 0.95 ^{d, A}	58.85 \pm 0.72 ^{d, A}	56.70 \pm 1.18 ^{d, A}	0.4370
L ³	83.54 \pm 1.82 ^{a, A}	90.53 \pm 3.76 ^{a, A}	86.62 \pm 2.47 ^{a, A}	87.09 \pm 4.50 ^{a, A}	0.4283
L ⁴	69.63 \pm 0.53 ^{bc, A}	72.60 \pm 0.79 ^{bc, A}	65.33 \pm 1.15 ^{bcd, B}	72.60 \pm 0.80 ^{bc, A}	0.0000*
L ⁵	77.11 \pm 2.78 ^{ab, B}	93.37 \pm 5.61 ^{a, A}	82.60 \pm 1.71 ^{a, AB}	90.18 \pm 2.92 ^{a, AB}	0.0079*
L ⁶	65.87 \pm 0.89 ^{c, BC}	70.08 \pm 1.05 ^{c, A}	62.95 \pm 1.02 ^{cd, C}	66.87 \pm 0.65 ^{c, AB}	0.0001*
a ²	-1.74 \pm 0.11 ^{d, A}	-1.94 \pm 0.17 ^{cd, A}	-1.82 \pm 0.07 ^{d, A}	-1.51 \pm 0.14 ^{cd, A}	0.1336
a ³	1.30 \pm 1.13 ^{cd, A}	1.10 \pm 2.48 ^{bcd, A}	-0.74 \pm 0.95 ^{cd, A}	2.51 \pm 3.23 ^{bcd, A}	0.6515
a ⁴	7.55 \pm 0.28 ^{a, A}	7.49 \pm 0.44 ^{a, A}	8.56 \pm 0.31 ^{a, A}	8.83 \pm 0.24 ^{a, A}	0.9500
a ⁵	2.12 \pm 1.15 ^{bc, A}	-2.74 \pm 4.16 ^{d, A}	0.88 \pm 0.55 ^{bc, A}	-4.70 \pm 1.15 ^{d, A}	0.2290
a ⁶	7.23 \pm 0.52 ^{a, A}	7.02 \pm 0.62 ^{a, A}	7.86 \pm 0.29 ^{a, A}	7.14 \pm 0.43 ^{ab, A}	0.6141
b ²	17.61 \pm 0.77 ^{a, A}	17.92 \pm 0.58 ^{a, A}	18.48 \pm 0.34 ^{a, A}	17.68 \pm 0.58 ^{a, A}	0.7142
b ³	-3.10 \pm 0.46 ^{b, B}	3.29 \pm 1.28 ^{bc, A}	-4.50 \pm 1.20 ^{b, B}	-3.39 \pm 2.15 ^{c, B}	0.0012*
b ⁴	-11.55 \pm 0.82 ^{d, A}	-10.86 \pm 0.97 ^{de, A}	-17.67 \pm 0.62 ^{e, B}	-15.79 \pm 0.74 ^{c, B}	0.0000*
b ⁵	-8.87 \pm 1.43 ^{cd, C}	-3.18 \pm 2.05 ^{c, ABC}	-8.80 \pm 1.01 ^{c, BC}	1.48 \pm 0.94 ^{bc, A}	0.0008*
b ⁶	-16.49 \pm 1.13 ^{e, A}	-14.20 \pm 1.61 ^{e, A}	-16.90 \pm 0.89 ^{de, A}	-12.82 \pm 1.51 ^{de, A}	0.1132
L a b ²³⁴⁵⁶ (p-value ⁸)	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked White regions; ⁴Baked brown regions; ⁵Cooled white regions; ⁶Cooled brown regions; ⁷ANOVA of means arranged within the same row; ⁸ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

Table 4.4.9 Starter bacterial population enumerated (n = 6) during manufacture and refrigerated storage of TB and DS mozzarella cheeses.

Sample details	TB cheese (Mean)		DS cheese (Mean)	
	ST	LB	ST	LB
Milk + starter	78 x 10 ⁵	35 x 10 ⁵	65 x 10 ⁵	64 x 10 ⁴
Whey at drain	12 x 10 ⁴	35 x 10 ⁴	< 1 x 10 ⁴	140 x 10 ³
Curd at drain	74 x 10 ⁵	23 x 10 ⁶	19 x 10 ⁶	43 x 10 ⁵
0 d cheese	30 x 10 ⁵	20 x 10 ⁶	53 x 10 ⁶	38 x 10 ⁶
5 d cheese	84 x 10 ⁶	87 x 10 ⁶	193 x 10 ⁶	93 x 10 ⁶
16 d cheese	21 x 10 ⁶	40 x 10 ⁷	49 x 10 ⁷	98 x 10 ⁶
23 d cheese	42 x 10 ⁶	33 x 10 ⁵	175 x 10 ⁶	22 x 10 ⁶
34 d cheese	12 x 10 ⁵	48 x 10 ⁴	65 x 10 ⁵	36 x 10 ⁵

ST = *Streptococcus thermophilus*; LB = *Lactobacillus delbrueckii* ssp. *bulgaricus*

Table 4.4.10 The molecular weights of proteins comprised in the broad range marker separated on an SDS-PAGE gel.

Protein	Molecular Weight (Dalton)
Myosin	205000
β-galactosidase	120000
Bovine Serum Albumin	84000
Ovalbumin	52200
Carbonic Anhydrase	36300
Soybean Trypsin Inhibitor	30200
Lysozyme	21900
Aprotinin	7400

Source: Biorad Laboratories Ltd catalogue.

Table 4.4.11 The molecular weights and concentrations of milk proteins loaded and separated on the SDS-PAGE gel.

Type of milk protein	Concentration loaded on Gel	Molecular Weight (Dalton)
β -casein	2.0 μ L	24000
α -casein	2.0 μ L	23500
κ -casein	2.5 μ L	19000
β -lactoglobulin A	2.0 μ L	18300
α -lactalbumin	2.0 μ L	14146

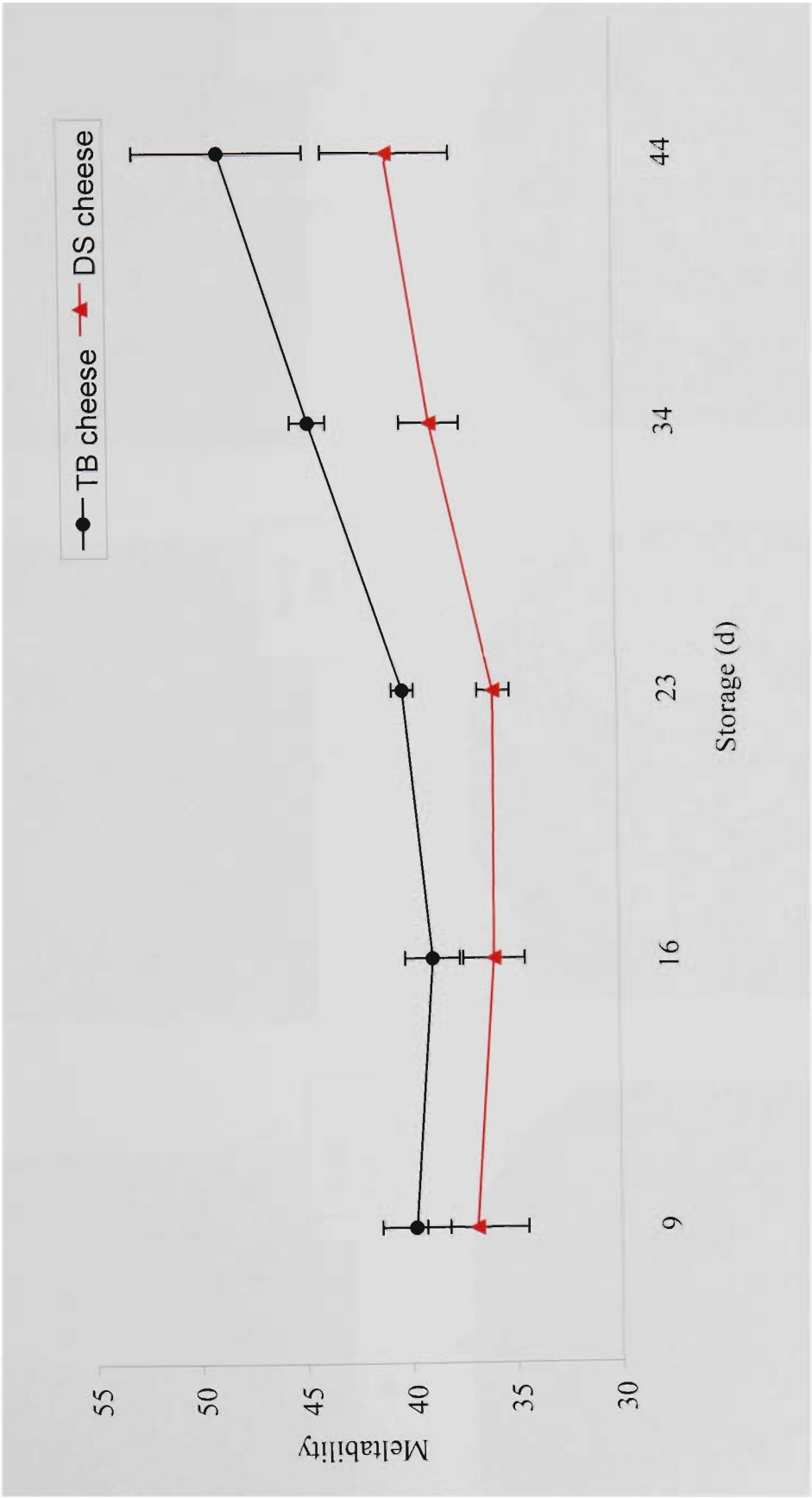


Figure 4.4.1 Meltability (Mean \pm SE¹) measured in mm for TB and DS cheeses during storage at refrigeration temperature.

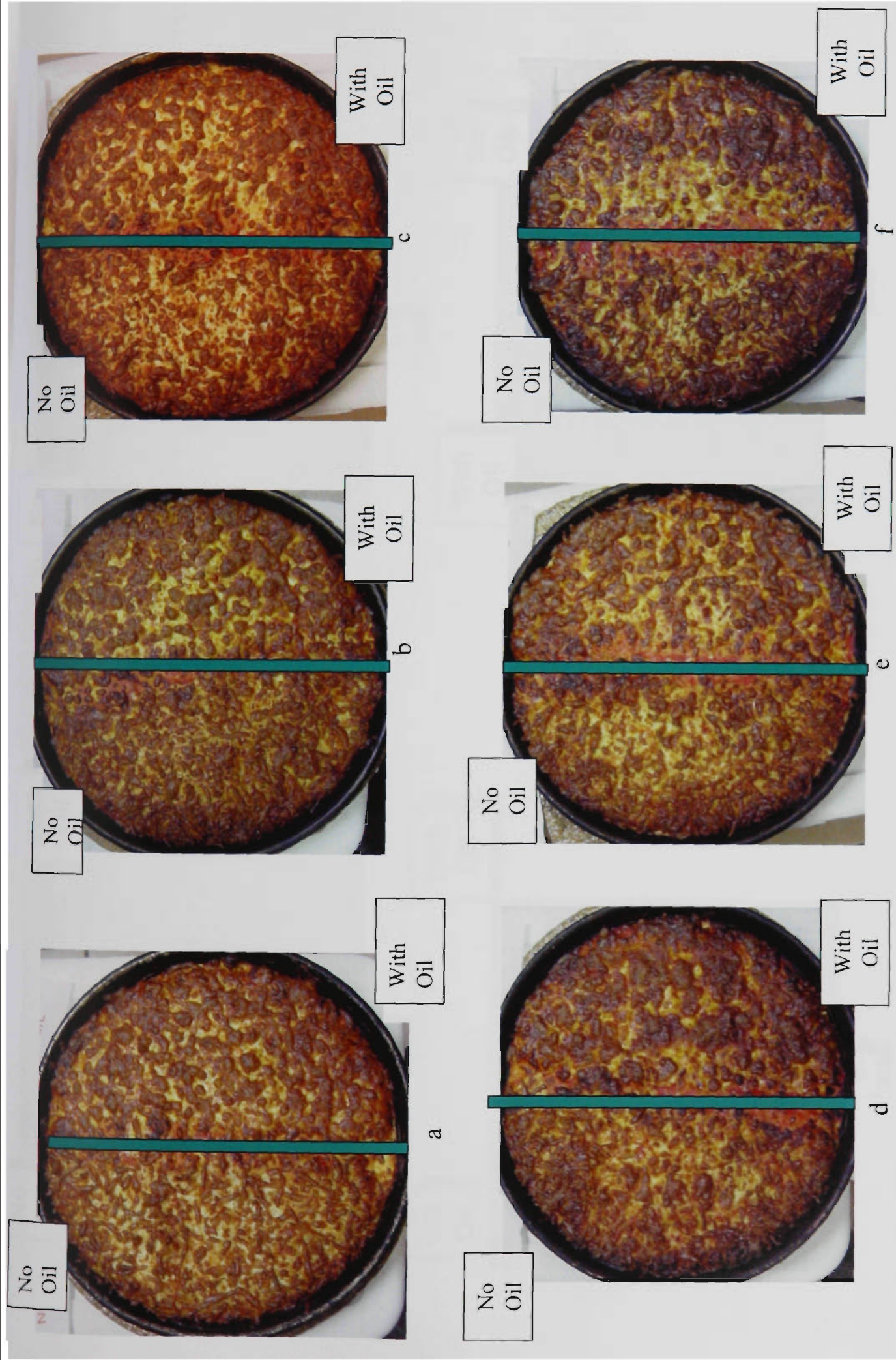


Figure 4.4.2 Pizzas a, b, c were made using DS cheeses and d, e, f with TB cheeses stored for 30 d. The pizzas were divided into two halves and one half was covered with cheese shreds that were coated with oil and the other half was covered with cheese shreds that were not applied with oil.

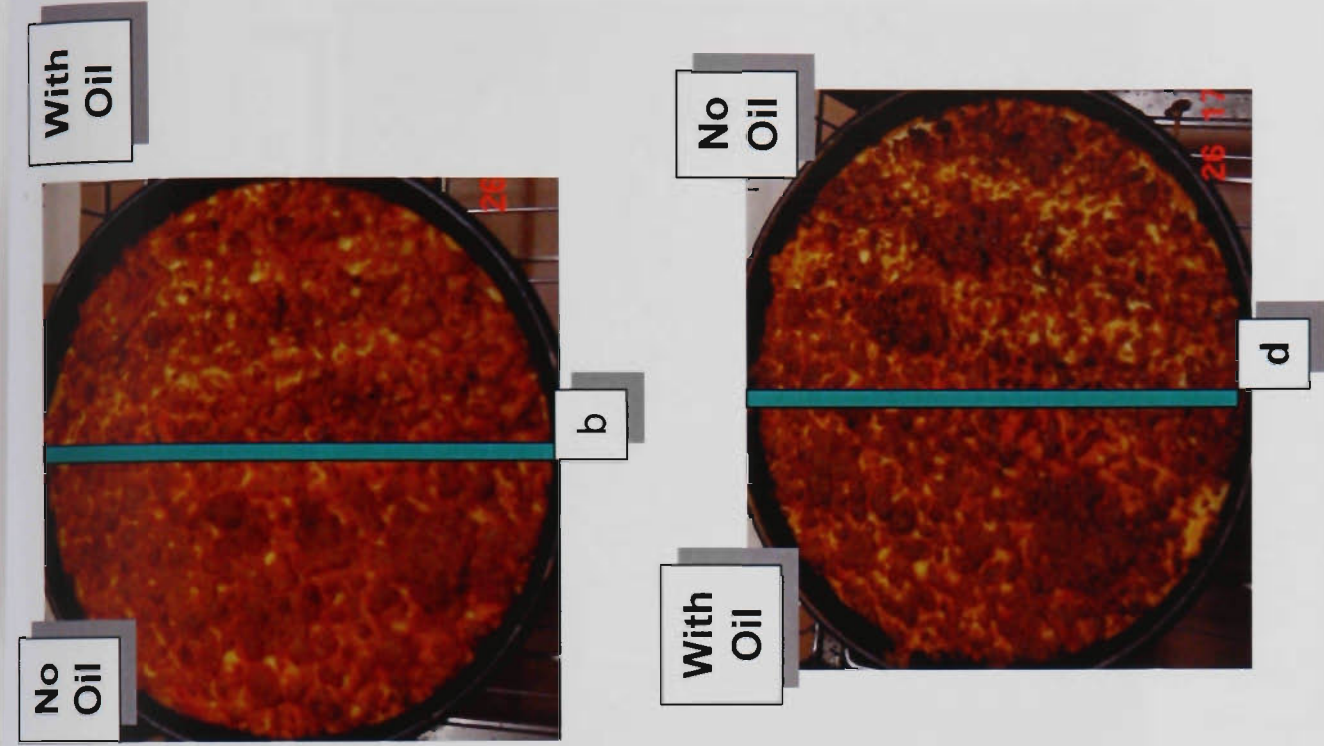
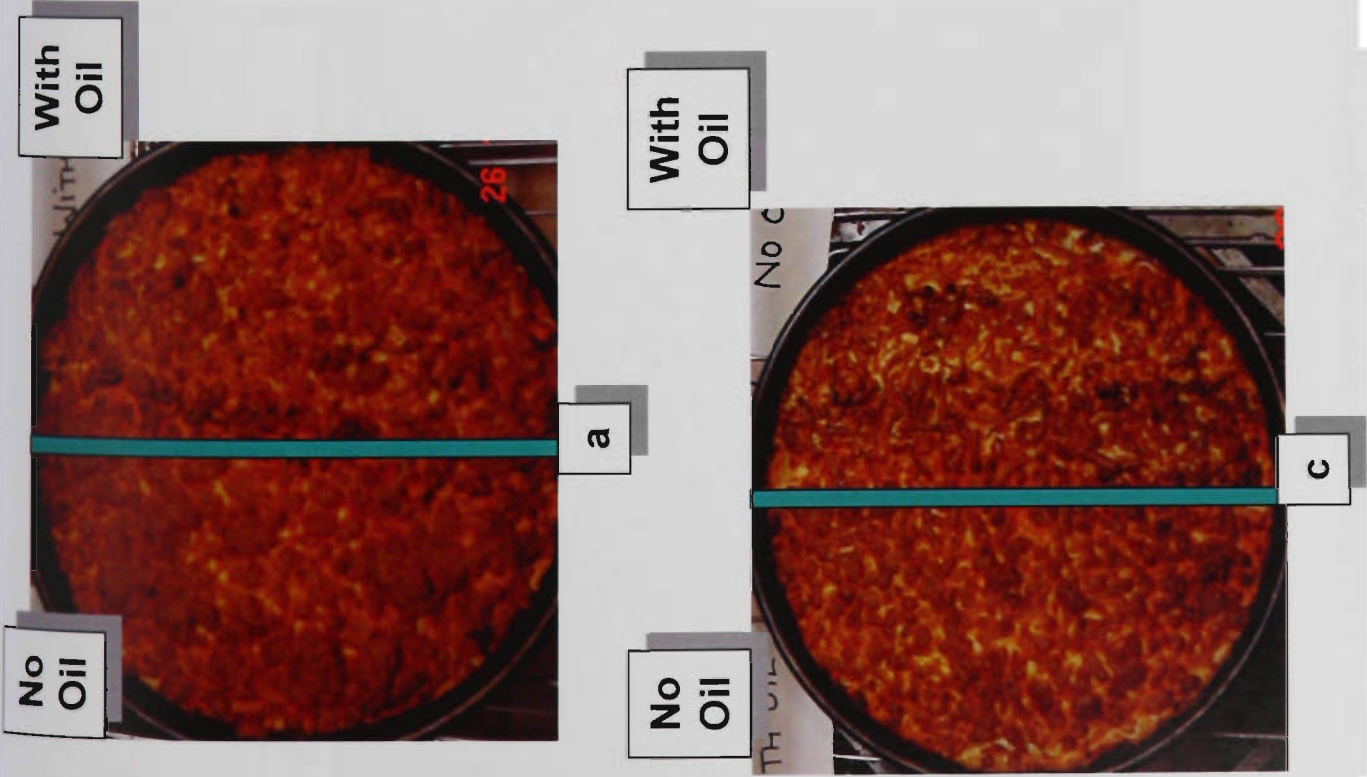


Figure 4.4.3 Pizzas a and b were made using DS cheeses and c and d with TB cheeses stored for 44 d. The pizzas were divided into two halves and one half was covered with cheese shreds that were coated with oil and the other half was covered with cheese shreds that were not applied with oil.



Figure 4.4.4 Pizzas TB cheese and DS cheese were made using TB and DS cheeses, respectively which were stored for 75 d. The pizzas were divided into two halves and one half was covered with cheese shreds coated with oil and the other half was covered with cheese shreds that were not applied with any oil.

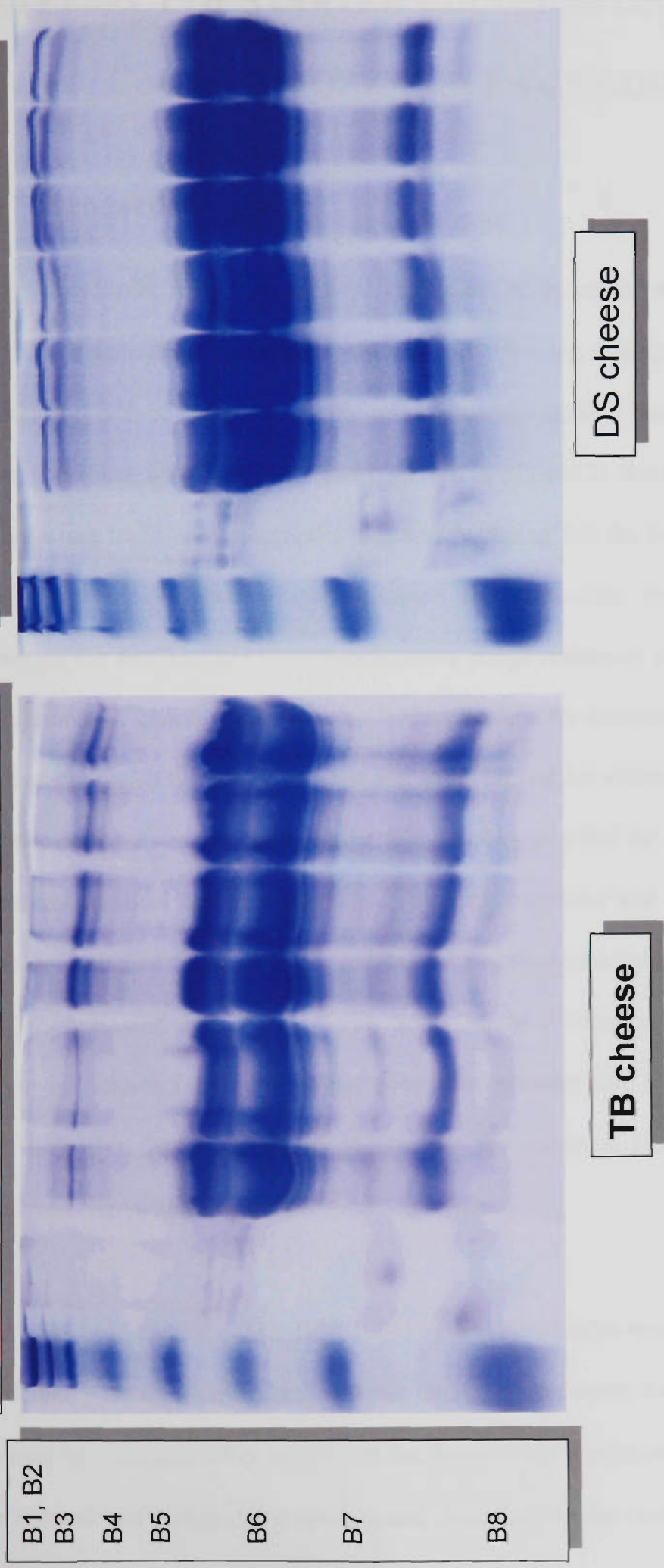


Figure 4.4.5 SDS-PAGE gel electrophoresis results showing lane 1 consisting of broad range standard marker, lane 2 consisting of a mixture of milk proteins namely, β -casein, α -casein, Kappa-casein, β -lactoglobulin A and α -lactalbumin and lanes 3-8 indicating protein samples isolated from TB or DS cheeses at 1, 8, 15, 22, 29 and 36 d of storage.

5.0 EFFECT OF STARTER CULTURES ON ULTRA LOW FAT MOZZARELLA CHEESES

5.1 Introduction

Early cheese milk fermentations were carried out using natural starter organisms found in raw milk. But due to defects in cheeses such as early blowing, late blowing, production of biogenic amines and lack of control in cheese production and quality, starter cultures were isolated and have since been used for cheese making (Hull *et al.*, 1992). Starter cultures have been used to manufacture traditional mozzarella cheeses containing full fat. Such starter bacteria have been studied for mainly their phage resistance and proteolytic abilities. Coffey *et al.* (2002) reviewed the developments made to improve phage resistance in starter bacteria. The current requirement of cheese manufacturers to develop low fat mozzarella cheeses has made several researchers revisit the starter bacteria and study them more extensively. The basic tenet for low fat mozzarella cheese to have similar functionality as a full fat mozzarella requires increased moisture retention. Starter bacteria such as *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* have been studied for their EPS production by several researchers (Low *et al.*, 1998; Perry *et al.*, 1998; Hassan and Frank, 1997). Some of the *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* strains are able to utilise lactose and produce EPS (Cerning *et al.*, 1986 and 1988) and such EPS has been found to increase the moisture retention in mozzarella cheeses (Bhaskaracharya and Shah, 2001a).

Work has been carried out (Reddy, 1984) to develop suitable media for growing starter cultures particularly for use in mozzarella cheese manufacture. Starter cultures are important in low fat cheeses for their capability to enhance the flavour characteristics (Baxter *et al.*, 1997). improve the textural and functional properties and demineralise the curd (Kindstedt and Kiely, 1990)

through increased proteolysis and glycolysis. Lactic acid production by the starter cultures causes demineralisation of the curd and in low fat mozzarella cheeses makes the curd softer and easily workable. The rate of acid production affects the rate of syneresis taking place from the curd and most of the calcium associated with the casein is leached out along with the whey. Thus starter activity indirectly controls the demineralisation of the curd, moisture retention and the functional properties of the mozzarella cheese. A too slow acid production during cheddaring leads to longer holding time of the curd and reduced moisture content in the final cheese (McMahon *et al.*, 1993). Coliform contamination and growth in cheeses could also be indirectly caused by a very slow reduction in pH due to slow starter cultures as observed by Winterer (1986). Also, inconsistent starter cultures could lead to erratic cheese production schedules and such cheeses would vary in their compositional characteristics. Use of excess starter cultures could reduce the fat, total solids (TS) and pH of cheeses as observed by Dulay *et al.* (1986).

Proteolytic changes brought about by starter culture activity have been associated with changes in functional characteristics of cheeses. Recent work using attenuated starter cultures (Johnson *et al.*, 1995; Muir *et al.*, 1992) in making low fat cheeses has shown tremendous improvements in texture, functionality and sensory characteristics of the cheeses. Studies of the proteolytic pattern and extent of casein breakdown in cheeses (El-Soda *et al.*, 2000) that were mixed with attenuated *L. helveticus* bacteria in the form of adjunct addition showed freeze shocked cells having increased levels of peptidase activity and increased rate of proteolysis compared to heat shocked cells.

Use of mixed/combination starter culture is preferred due to their phage resistance and a choice for the manufacturers to quickly modify the ratio of the starter culture mix in order to reduce any defects that may arise over prolonged use of starter cultures. The lactobacilli (*L.*

delbrueckii ssp. *bulgaricus*) exist in a symbiotic relationship with *S. thermophilus*. Growth of both *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were faster when mixed and inoculated rather than when inoculated alone (Patel *et al.*, 1983). They observed faster rate of growth and faster acid production in the first 6 h for *S. thermophilus* and after 6 h for *L. delbrueckii* ssp. *bulgaricus* when they were inoculated individually. Their mixed cultures showed faster utilisation of lactose in 4 h.

The ratio of rods to cocci used for the manufacture of mozzarella cheese influences its characteristics. An increase in the rods to cocci ratio increases the proteolytic effect on the cheese especially during storage (Yun *et al.*, 1992). Also, the cocci being fewer could lead to slower acid production in the curd and longer ripening times. *L. delbrueckii* ssp. *bulgaricus* provides more proteolytic enzymes than to *S. thermophilus* (Oberg and Broadbent, 1993). The latter organisms stimulate growth of *L. delbrueckii* ssp. *bulgaricus* through production of carbon dioxide, formic acid and decrease of the oxidation-reduction potential. *L. helveticus* is a highly proteolytic organism similar to *L. delbrueckii* ssp. *bulgaricus* and produces peptides and free amino acids from caseins. The proteolysis brought about by the starter cultures in mozzarella cheeses helps to modify the functional characteristics of the cheeses (Oberg *et al.*, 1991 and 2002). The proteolytic activity varies among the different strains and species of *L. delbrueckii* ssp. *bulgaricus* and *L. helveticus* (Farkyne *et al.*, 1995).

The type of milk protein, whether from cow milk or buffalo milk, also affects the proteolytic pattern of the same bacterial strains. Variation in the genetic casein variant affects the proteolysis rate of starter cultures as observed by Subramanian *et al.* (1992 a, b). Excessive proteolysis, such as by the use of *L. casei* has been associated with a soft body defect (McMahon *et al.*, 1993; Hull *et al.*, 1983). Some of the extracellular enzymes produced by lactobacilli may be denatured during stretching of mozzarella curd as suggested by Lawrence

et al. (1983), but the starter bacteria survive the stretching temperature and produce enzymes during cheese storage.

Presently, work is being carried out on identification and genetic manipulation of starter bacteria to gain an insight into their endopeptidase activities. Chen and Steele (1998) have studied the nucleotide sequence of some of the *L. helveticus*, which produced endopeptidases. They have been able to create a mutant with the same growth rate and acid production as the non-mutant, by removing the endopeptidase related gene, similar to the work carried out by Dudley *et al.* (1996). Yuksel and Steele (1996) by cloning a gene isolated from *L. helveticus* CNRZ32 and reintroducing the gene into a CNRZ32-negative derivative and obtaining production of an amino peptidase, showed that it was possible to create tailor made starter bacteria having the desired proteolytic or other fermentation capabilities with no effect on their growth rates.

The carbohydrate metabolism is a second aspect that is affected by the change in rate or type of starter bacteria used in cheese production. It is well known that browning in mozzarella cheeses is caused by Maillard reaction between reducing sugars such as galactose with amino groups of protein fractions (Johnson and Olson, 1985). Utilisation of galactose would decrease such reactions but if an excessively proteolytic starter is used, it could also cause an increase in the free amino groups due to increased casein breakdown (Kindstedt, 1993). Normally, *S. thermophilus* bacteria are able to utilise lactose and glucose while the galactose sugars are accumulated in the cheese. *L. delbrueckii* ssp. *bulgaricus* cultures previously used in the mozzarella cheese industry were also galactose negative strains. Owing to the importance of galactose fermentation in order to reduce the non-enzymatic browning that occurs after storage during baking of cheese, strains of *L. delbrueckii* ssp. *bulgaricus* with galactose fermenting ability are being used in mozzarella cheese manufacture. In contrast, *L. helveticus* organisms

are able to utilise glucose, galactose and lactose, which increases their suitability in mozzarella cheese manufacture (Rowney *et al.*, 1999). A balance is essential in the amount of proteolysis desired and the ability of the organisms to utilise galactose in mozzarella cheese manufacture. Significant reduction in browning of low fat mozzarella cheeses during baking was observed (Mukherjee and Hutkins, 1994) by using galactose fermenting thermophilic starter cultures. Galactose positive streptococci used alone in manufacture of mozzarella cheeses caused accumulation of galactose in the cheeses (Tunick *et al.*, 1993), whereas when inoculated with *L. delbrueckii* ssp. *bulgaricus* (galactose negative strains) complete utilisation of galactose from the cheeses was observed suggesting that *L. delbrueckii* ssp. *bulgaricus* had a stimulating effect on galactose metabolism by *S. thermophilus*.

Studies have also been carried out to increase the rate of acid production by starter cultures by homogenising the bulk starter. Hicks and Ibrahim (1992) showed that homogenisation at low pressures could help break the clumps and chains of agglutinating cultures and thereby increase their rate of acid production. At the same time, they also found that homogenisation of non-agglutinating bulk starter cultures could lead to cell damage and decrease their rate of acid production.

Milk preservation techniques such as the lacto-peroxidase (LP) system on starter bacterial growth and performance have been shown (Shive-Kumar and Mathur, 1989) to increase the cheddaring time and cause decreased moisture retention in mozzarella cheeses made from such LP treated milk. *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* grown in milk with reduced a_w showed slower acid production but reduction in a_w did not influence the optimum growth temperature of these bacteria. A decrease in dissolved oxygen from 5.5 to 2.9 ppm in milk was observed (Shekar and Bhat, 1983) to increase the rate of acid production by *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* while an increase to 9.0 ppm of dissolved oxygen caused a

reduction in the rate of acid production. These effects were also correlated with heat treatment of milk, which decreased the dissolved oxygen and increased the starter culture activity.

5.2 Aims

The aims of this study were to manufacture low fat mozzarella cheeses using either *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus helveticus*, along with *Streptococcus thermophilus* and to analyse the cheeses for their yield, composition, textural and functional characteristics.

5.3 Materials and methods

5.3.1 Cheese making

Low-moisture, part skim (LMPS) mozzarella cheeses containing ~6.0% fat were manufactured using starter cultures namely *Streptococcus thermophilus* (ST) and either *Lactobacillus helveticus* (LB1) or with *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lb2515). Three batches of cheeses were made using ST2000 + LB1 (Lh based cheese) or ST2000 + Lb2515 (Lb based cheese) starter cultures as described in sections 3.2.6.1 and 3.2.6.2. The cheese were dry salted, stretched in hot brine, vacuum packed and stored at 4°C.

5.3.2 Analysis of cheeses

The yield of cheeses was measured and the cheese samples were prepared for estimation of composition and meltability as described in section 3.2.9, for measurement of texture characteristics (section 3.2.8) and for pizza bake characteristics (section 3.2.7). The composition including moisture/total solids was estimated using the method described in section 3.2.20, fat content (section 3.2.22) and protein content (section 3.2.25) were

determined. Texture characteristics including hardness, cohesiveness and springiness were measured for cheeses stored for 2, 9, 16, 23 and 34 d by compressing cylindrical cheese samples to 50% height as described in section 3.2.23 using an Instron. according to the method of Bhaskaracharya (2000). Meltability test was carried out on 9, 16, 23 and 34 d of storage as described in section 3.2.15 using 10 g of grated cheese sample heated in glass tube at 110°C for 100 min. Pizza-bake tests were carried out (as described in section 3.2.24) after 20 d of storage and the colour, size of blisters and their distribution upon baking were examined. The Hunter L, a and b values for fresh, baked and cooled pizzas (cheeses) were also measured at 20 d.

5.3.3 Statistical analysis

Statistical analysis of the results was carried out using StatPro[®] software on Microsoft[®] excel. The sample sizes, sample means, sample standard deviations and standard error were calculated. The means from at least two populations were compared. The analysis of variance table was prepared and two sources of variation: the variation *within* each population and variation *among* sample means from the different populations was compared. If the latter variation was large relative to the former, as was measured using an F test, this was deemed to be evidence of differences between the population means. The p-value obtained from this table and the confidence intervals (at 95% confidence level) for all the differences between pairs of means were considered for describing the statistical differences and their significance in the results tables. A small p-value was a result of large differences in population means and the confidence intervals that did not include zero were taken to indicate that the means that were compared were not equal.

5.4 Results and discussion

5.4.1 Yield and composition

Table 5.4.1 shows the yield and compositional results for the two varieties of cheeses made using different starter culture systems. The yield of the cheeses although varying were not significantly different. The Lh based cheeses showed significantly higher moisture and higher FDM content but lower protein content compared to the Lb based cheeses. The fat contents on wet basis were similar for both the cheeses. The variation in moisture content of the Lh based cheeses could be due to higher proteolysis by *Lactobacillus helveticus* than *Lactobacillus delbrueckii* ssp. *bulgaricus*.

5.4.2 Texture characteristics

Table 5.4.2 shows the mean and standard error for hardness values measured at 2, 9, 16, 23 and 34 d of storage for the two varieties of dry salted cheeses. The Lh based cheeses were significantly softer compared to the Lb based cheeses throughout storage, which could be due to their higher moisture content (Olson and Johnson, 1990; Tunick *et al.*, 1991). Our previous studies (Bhaskaracharya and Shah, 1999) on commercial mozzarella cheeses showed similar reduction in hardness with increase in moisture content. Also both varieties of cheeses showed reduction in hardness values with storage up to 16 d after which the hardness values did not vary significantly.

Table 5.4.3 shows the mean and standard error for cohesiveness values measured at 2, 9, 16, 23 and 34 d of refrigerated storage for Lh based and Lb based cheeses. The two varieties of cheeses showed similar cohesiveness values at 2d and 9d, however at 16, 23 and 34 d the Lh based cheeses had lower cohesiveness values compared to Lb based cheeses, which could be due to physico-chemical changes during storage. Higher cohesiveness values in Lb based

cheeses could be related to the higher amount of protein (thus increased protein network) that was available for compression. Such cheeses with more intact casein and reduced proteolysis would show higher cohesiveness. Similar correlations have been made for the protein content of the cheeses and increased cohesiveness, in earlier studies on commercial mozzarella cheeses by Bhaskaracharya and Shah (1999). Although the mean cohesiveness values decreased for Lh based cheeses with storage they were not significantly different. The Lb based cheeses showed lower cohesiveness values at 2 d compared to 16 d, 23 d and 34 d of storage. After 9 d the cohesiveness values remained similar for the Lb based cheeses.

Table 5.4.4 shows the springiness values for Lh based and Lb based cheeses measured at 2, 9, 16, 23 and 34 d of refrigerated storage. The Lh based cheeses showed significantly higher springiness values throughout storage compared to Lb based cheeses. The higher springiness values could be due to their higher FDM values, which gave resilience to the Lh based cheeses, similar to the observations made by Bhaskaracharya and Shah (1999). The springiness values for the Lh based cheeses increased up to 16 d and then remained stable at 23 and 34 d. The Lb based cheeses showed similar springiness through out storage.

5.4.3 Functional characteristics

5.4.3.1 Meltability

Table 5.4.5 shows the meltability measured at 9, 16, 23 and 34 d of storage for Lh based and Lb based cheeses. The meltability was significantly higher for the Lh based cheeses compared to Lb based cheeses throughout the storage. This could be due to increased casein degradation by the Lh bacteria during manufacture and storage. During storage to 34 d both Lh based and Lb based cheeses showed an increase in meltability although not statistically significant. Studies by Yun *et al.* (1995) showed that increased rod: coccus ratio did not impact on meltability characteristic of the cheeses and they concluded that the total amount of starter

culture inoculated may have more impact than the rod: coccus ratio. Thus during storage, small increases in the extent of proteolysis and the pattern of proteolytic activity by either Lh or Lb bacteria may not be sufficient to cause significant changes in meltability of ULFM cheeses. But between use of Lh or Lb starter bacteria for cheese-making, the Lh based cheeses showed higher meltability than Lb based cheeses.

5.4.3.2 Pizza bake results at 20 d of storage

5.4.3.2.1 Effect on physical properties as observed with the naked eye on low fat Lh based and Lb based mozzarella cheeses (with/no oil) due to baking in oven.

The ULFM cheeses showed marked differences upon baking. The Lb based cheeses when baked had excessive browning compared with the Lh based cheeses as shown in Figure 5.4.1. The Lb based (no oil) cheeses had large blisters, improper fusion of cheese shreds, burning and browning on pizza, and were unappealing compared to Lb based (with oil) and Lh based (with/no oil) cheeses. Similarly, the Lb based (with oil) cheeses had very large blisters compared to even Lb based (no oil) cheeses, and although most of the cheese shreds were completely melted, due to excessive browning the pizza made with such cheese was unappealing. The Lh based (no oil) cheeses showed good melt, a few shreds were not completely fused and were burnt, large burnt surface areas on the melted cheese and lack of flow of the cheese on pizza. These characteristics made the Lh based (no oil) cheeses undesirable. Only the Lh based (with oil) cheeses showed a glistening surface and resembled a full fat mozzarella in aesthetic appeal, but the melted cheese even though was completely fused did not flow as desired on the pizza base. Some areas on the pizza also showed blister formation, which turned brown during baking.

Table 5.4.6 shows the mean and standard error of Hunter L, a and b values for fresh, baked and cooled Lh based and Lb based (with/no oil) cheeses during pizza baking after 20 d of storage. The various effects of starter bacteria, baking and cooling and application of oil on the Hunter

L, a and b values are discussed below. Table 5.4.6 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

5.4.3.2.2 Effects on Hunter L, a and b values due to coating with a hydrophobic material

The Lh based cheeses showed no significant differences in the Hunter L, a and b values due to application of oil on the cheese shreds. The Lb based cheeses also showed no significant differences in the Hunter L, a and b values due to application of oil except the L^3 values of Lb based (with oil) cheeses were significantly higher than the L^3 values of Lb based (no oil) cheeses after baking due to application of oil and that the hydrophobic material could improve the functionality of ULFM cheeses.

5.4.3.2.3 Effects on Hunter L, a and b values due to starter cultures

The fresh L^2 values of Lh based (no oil) and Lh based (with oil) cheeses were similar and were significantly higher than those for Lb based (no oil) and Lb based (with oil) cheeses. The baked or cooled Lh based (no oil) cheeses showed higher L^3 and L^4 values compared to those of Lb based (no oil) cheeses. Similarly the baked or cooled Lh based (with oil) cheeses showed higher L^3 and L^4 values compared to those of Lb based (with oil) cheeses. The increase in whiteness of Lh based cheeses compared to Lb based cheeses could be due to higher proteolytic activity of *L. helveticus*, higher moisture content, greater galactose utilisation or higher FDM content. Thus *L. helveticus* was able to improve not only the compositional parameters but also the functionality of the cheeses. The fresh a^2 values for Lh based (with/no oil) cheeses were significantly higher than those for Lb based (with/no oil) cheeses. But these values were close to zero and so may not indicate redness in colour as perceived by the chromameter. The a^3 and a^4 values of Lb based (with/no oil) cheeses were significantly higher compared to those of Lh based (with/no oil) cheeses indicating increased burning due to baking. The Hunter b values (b^2 , b^3 and b^4) for Lh based (no oil) cheeses were higher than both the Lb based (with/no oil) cheeses whereas the b^2 , b^3 and b^4 values for Lh based (with oil)

cheeses were similar to Lb based (with oil) cheeses. In general, the Lh based (with/no oil) cheeses had more yellowness (b values) when fresh and after baking compared to Lb based (with/no oil) cheeses.

5.4.3.2.4 *Effects of baking and cooling on Hunter L, a and b values*

The L^3 and L^4 values of Lh based (with oil) cheeses were similar (not significantly different) and were markedly ($p < 0.05$) higher than their L^2 values. The L^3 values of Lh based (no oil) cheeses were significantly higher than their L^2 and L^4 values and the L^4 values of these cheeses were also higher than their L^2 values. Thus baking improved the whiteness of Lh based (with/no oil) cheeses. The L^2 values for Lb based (with/no oil) cheeses were significantly higher than their L^3 and L^4 values and their L^3 and L^4 values were similar for each treatment (applying with/no oil). Thus fresh Lb based cheeses were whiter than baked cheeses. These cheeses became dark or had increased redness due to burning, which is reflected in their L^3 and L^4 values that were lower than their L^2 values. The Lh based (with oil) cheeses showed significantly increased a values upon baking and after cooling while the Lh based (no oil) cheeses and Lb based (with oil) cheeses showed significantly higher a values upon baking but the latter values did not change upon cooling. The increased a values indicate increase in burning of the cheeses upon baking and after cooling due to loss in glossiness these values could be further increased. The a values for Lb based (no oil) cheeses increased with baking and then decreased with cooling but both a^3 and a^4 values were significantly higher than their a^2 values, which shows that baking increased burning or browning of the cheeses. The b^4 values for the cooled Lh based (with/no oil) and Lb based (with/no oil) cheeses were not significantly different to their respective b^3 values except for the Lh based (with oil) cheeses. This shows that the yellowness of the cheeses does not change upon cooling the cheeses after baking. The b^4 values for Lh based (with/no oil) and Lb based (with/no oil) cheeses were

significantly higher than their b^2 values indicating increased yellowness of baked and cooled cheeses compared to fresh cheeses.

5.5 Conclusions

Cheeses made using *L. helveticus* showed increased moisture, FDM and M:P contents compared to those made using *L. delbrueckii* ssp. *bulgaricus*. Also the latter cheeses had higher protein content compared to the former cheeses. Due to the compositional changes the Lh based cheeses had reduced hardness (increased softness), lower cohesiveness and increased springiness compared to Lb based cheeses. Meltability of the Lh based cheeses was significantly higher throughout storage and these cheeses also had better performance when baked on pizzas with an increased whiteness (L values), reduced redness (a values) and increased yellowness (b values) compared to their fresh state. The Lb based cheeses had poor melt characteristics and did not fuse completely when baked, had excessive blistering and browning. All the ULFM cheeses showed improvement in pizza bake characteristics due to application of hydrophobic material.

Future experiments would use Lh starter bacteria and make further modifications in cheese-making methods to improve functionality of ULFM cheeses. Further, application of oil shows some promise in improving pizza bake performance and hence will be used as an additional step during storage of cheese for pizza bake test.

Table 5.4.1 Mean \pm SE¹ of yield and composition (n = 9) of dry salted cheeses made using starter cultures consisting of ST and LH (Lh based cheese) or ST and LB (Lb based cheese).

Characteristic	Lh based cheese	Lb based cheese	p-value ²
Yield ³ (g/ 50 kg)	4275.0 \pm 175.0 ^a	4188.5 \pm 4.5 ^a	0.6702
Moisture (%)	57.05 \pm 0.48 ^b	53.85 \pm 0.43 ^a	0.0003 [*]
Protein (%)	31.11 \pm 0.51 ^b	35.61 \pm 0.32 ^a	0.0000 [*]
Fat (%)	6.00 \pm 0.00 ^a	5.78 \pm 0.15 ^a	0.2445
FDM ⁴ (%)	13.98 \pm 0.16 ^a	12.53 \pm 0.36 ^b	0.0087 [*]
M:P ⁵	1.85 \pm 0.04 ^a	1.51 \pm 0.02 ^b	0.0000 [*]

¹Mean \pm Standard error; ²ANOVA of means; ³n= 3; ⁴FDM = Fat in dry matter; ⁵M:P = Moisture: Protein; ST = *S. thermophilus*; LH = *L. helveticus*; LB = *L. delbrueckii* ssp. *bulgaricus*; ^{a,b} Means within same row not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05).

Table 5.4.2 Hardness values (Mean \pm SE¹) measured (n = 9) at 2, 9, 16, 23 and 34 d of refrigerated storage for dry salted cheeses made using starter cultures consisting of ST and LH (Lh based cheese) or ST and LB (Lb based cheese).

Storage (d)	Lh based cheese	Lb based cheese	p-value ²
2	63.18 \pm 2.64 ^{a, B}	172.37 \pm 11.21 ^{a, A}	0.0000 [*]
9	27.32 \pm 3.34 ^{cde, B}	165.00 \pm 10.41 ^{a, A}	0.0000 [*]
16	22.23 \pm 4.00 ^{de, B}	94.88 \pm 6.55 ^{cd, A}	0.0000 [*]
23	32.90 \pm 3.90 ^{bcde, B}	84.10 \pm 11.62 ^{d, A}	0.0041 [*]
34	20.88 \pm 2.16 ^{e, B}	106.31 \pm 7.57 ^{bcd, A}	0.0000 [*]
p-value ³	0.0000 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ST = *S. thermophilus*; LH = *L. helveticus*; LB = *L. delbrueckii* ssp. *bulgaricus*; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p < 0.05); ^{*}Significant (p<0.05).

Table 5.4.3 Cohesiveness values (Mean \pm SE¹) measured (n = 9) at 2, 9, 16, 23 and 34 d of refrigerated storage for dry salted cheeses made using starter cultures consisting of ST and LH (Lh based cheese) or ST and LB (Lb based cheese).

Storage (d)	Lh based cheese	Lb based cheese	p-value ²
2	0.687 \pm 0.020 ^{a, A}	0.718 \pm 0.010 ^{c, A}	0.1131
9	0.708 \pm 0.010 ^{a, A}	0.719 \pm 0.006 ^{bc, A}	0.3245
16	0.690 \pm 0.004 ^{a, B}	0.753 \pm 0.004 ^{a, A}	0.0000*
23	0.668 \pm 0.010 ^{a, B}	0.759 \pm 0.006 ^{a, A}	0.0000*
34	0.678 \pm 0.007 ^{a, B}	0.730 \pm 0.003 ^{abc, A}	0.0000*
p-value ³	0.0996	0.0000*	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ST = *S. thermophilus*; LH = *L. helveticus*; LB = *L. delbrueckii* ssp. *bulgaricus*; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05); *Significant (p<0.05).

Table 5.4.4 Springiness values (Mean \pm SE¹) measured (n = 9) at 2, 9, 16, 23 and 34 d of refrigerated storage for dry salted cheeses made using starter cultures consisting of ST and LH (Lh based cheese) or ST and LB (Lb based cheese).

Storage (d)	Lh based cheese	Lb based cheese	p-value ²
2	2.017 \pm 0.07 ^{b, A}	1.544 \pm 0.07 ^{a, B}	0.0006*
9	2.317 \pm 0.07 ^{ab, A}	1.344 \pm 0.05 ^{a, B}	0.0000*
16	2.517 \pm 0.04 ^{a, A}	1.400 \pm 0.05 ^{a, B}	0.0000*
23	2.500 \pm 0.07 ^{a, A}	1.422 \pm 0.06 ^{a, B}	0.0000*
34	2.467 \pm 0.08 ^{a, A}	1.467 \pm 0.08 ^{a, B}	0.0000*
p-value ³	0.0001*	0.2480	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ST = *S. thermophilus*; LH = *L. helveticus*; LB = *L. delbrueckii* ssp. *bulgaricus*; ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05); *Significant (p<0.05).

Table 5.4.5 Meltability (Mean ± SE¹) measured (n = 9) at 9, 16, 23 and 34 d of refrigerated storage for dry salted cheeses made using starter cultures consisting of ST and LH (Lh based cheese) or ST and LB (Lb based cheese).

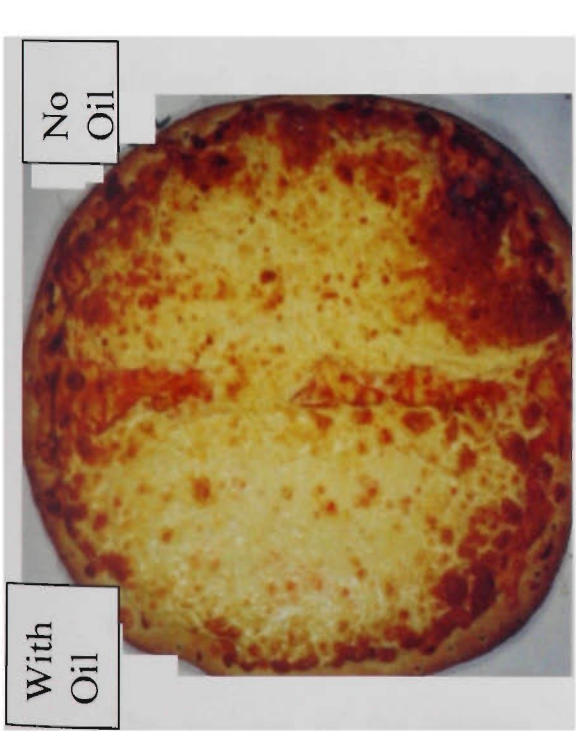
Storage (d)	Lh based cheese	Lb based cheese	p-value ²
9	51.83 ± 1.12 ^{a, A}	36.867±2.43 ^{a, B}	0.0015 [*]
16	51.88 ± 0.97 ^{a, A}	35.983±1.47 ^{a, B}	0.0000 [*]
23	52.19 ± 2.59 ^{a, A}	35.913±0.78 ^{a, B}	0.0001 [*]
34	53.48 ± 3.09 ^{a, A}	38.847±1.43 ^{a, B}	0.0016 [*]
p-value ³	0.9515	0.5640	

¹Mean ± Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ST = *S. thermophilus*; LH = *L. helveticus*; LB = *L. delbrueckii* ssp. *bulgaricus*; ^a Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05).

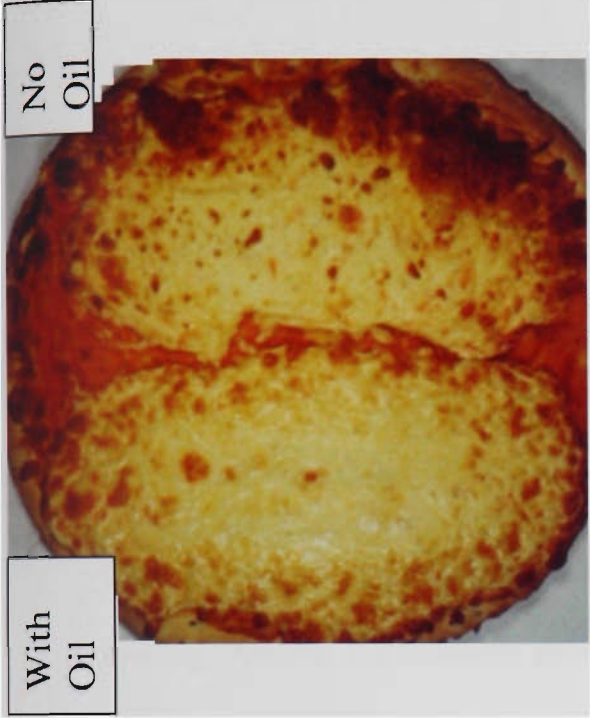
Table 5.4.6 Mean \pm SE¹ (n = 9) Hunter L a b-values for fresh² (before baking), after baking³ and after cooling⁴ of dry salted mozzarella cheeses made using starter cultures consisting of ST and LH (Lh based cheese) or ST and LB (Lb based cheese) applied with or without canola oil and stored for 20 d at refrigerated temperature.

Hunter L a b	Lb based cheese		Lb based cheese		p-value ⁵
	With Oil	No oil	With Oil	No oil	
L ²	59.54 \pm 1.74 ^{b, ABC}	62.49 \pm 0.50 ^{c, A}	56.85 \pm 0.72 ^{a, C}	57.96 \pm 0.68 ^{a, BC}	0.0022 [*]
L ³	73.68 \pm 0.36 ^{a, A}	72.49 \pm 0.26 ^{a, A}	50.51 \pm 2.05 ^{bc, B}	44.42 \pm 1.26 ^{bc, C}	0.0000 [*]
L ⁴	71.08 \pm 0.51 ^{a, A}	69.98 \pm 0.67 ^{b, A}	46.88 \pm 1.51 ^{c, BC}	44.21 \pm 1.54 ^{c, C}	0.0000 [*]
p-value ⁶	0.0000 [*]	0.0000 [*]	0.0004 [*]	0.0000 [*]	
a ²	0.06 \pm 0.18 ^{bc, A}	-0.40 \pm 0.15 ^{b, A}	-1.04 \pm 0.15 ^{b, BC}	-1.30 \pm 0.12 ^{c, C}	0.0000 [*]
a ³	-0.02 \pm 0.12 ^{c, C}	3.00 \pm 0.45 ^{a, BC}	12.64 \pm 0.99 ^{a, A}	13.32 \pm 0.69 ^{a, A}	0.0000 [*]
a ⁴	1.36 \pm 0.35 ^{a, C}	2.69 \pm 0.72 ^{a, BC}	11.93 \pm 0.60 ^{a, A}	11.15 \pm 0.68 ^{b, A}	0.0000 [*]
p-value ⁶	0.0006 [*]	0.0003 [*]	0.0000 [*]	0.0000 [*]	
b ²	17.11 \pm 0.60 ^{c, ABC}	18.42 \pm 0.36 ^{b, A}	15.09 \pm 0.51 ^{b, C}	15.62 \pm 0.37 ^{b, BC}	0.0002 [*]
b ³	25.59 \pm 0.59 ^{b, ABC}	30.87 \pm 1.16 ^{a, A}	25.06 \pm 1.73 ^{a, BC}	21.04 \pm 0.96 ^{a, C}	0.0003 [*]
b ⁴	28.30 \pm 0.67 ^{a, A}	29.25 \pm 1.01 ^{a, A}	23.85 \pm 1.52 ^{a, AB}	19.81 \pm 1.33 ^{a, B}	0.0001 [*]
p-value ⁶	0.0000 [*]	0.0000 [*]	0.0000 [*]	0.0015 [*]	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; ST = *S. thermophilus*; LH = *L. helveticus*; LB = *L. delbrueckii* ssp. *bulgaricus*; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B C} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).



Lh based cheese



Lh based cheese



Lb based cheese



Lb based cheese

Figure 5.4.1 Pizzas labelled Lh based cheese were made using *S. thermophilus* and *L. helveticus* and Lb based cheese were made using *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* which were stored for 20 d. The pizzas were divided into two halves and one half was covered with cheese shreds coated with oil and the other half was covered with cheese shreds that were not applied with any oil.

6.0 QUANTIFICATION OF CALCIUM IN MILK AND CHEESE BY-PRODUCTS USING AAS AND ROLE OF CALCIUM IN MOZZARELLA CHEESE-MAKING

6.1 Quantification of calcium in milk, whey, cheese and stretch water using atomic absorption spectroscopy

6.1.1 Introduction

Calcium like other milk constituents is concentrated approximately ten fold during cheese making. Among the minerals present in milk, calcium affects the functionality of mozzarella cheese. Loss of calcium occurs during mozzarella cheese making, in whey and stretch water and some of the ionic calcium in cheese is replaced by sodium ions at the salting stage. Several methods have been developed for estimating calcium content including atomic absorption spectroscopy.

Although studies have been made to standardise the methods to obtain reproducible and repeatable results for calcium estimation, very little work has been done on the effect of sample temperature on measuring their absorbance values of calcium. Thus this study would look into the aspects of estimation of calcium and repeatability of the results when the samples were tempered at different temperatures.

6.1.1.1 Methods of Estimation

The analysis of the mineral content in foods can be carried out by several traditional instrumental techniques, such as compleximetric titrations (Kindstedt and Kosikowski, 1985a,

b), ion chromatography, potentiometry and spectroscopy. The last method such as atomic absorption spectroscopy and its related techniques are the most widely used (Welz, 1985).

Wenner (1958) reported a method for the rapid determination of cations in milk by flame spectrophotometry where a sample of casein free milk serum was obtained by acid precipitation and treated with ion-exchange resin to remove the interfering anions. Murthy and Rhea (1967) showed that the major cations in milk could be determined by atomic absorption spectroscopy using ashed milk sample. Similar methods have been used for the determination of cations in milk products. The samples used for the determination of metal ions were processed by decomposition of organic material completely to avoid the interference by organic residue (Kolz *et al.*, 1979; Katz and Jenniss, 1981). The separation of the organic fraction from the mineral fraction can be carried out by:

- a) wet digestion of sample using concentrated acid mixtures (Cabanis *et al.*, 1988; Solchaga *et al.*, 1986; Farre *et al.*, 1986; IDF, 1980; Puchyr and Shapiro, 1986; Metzger *et al.*, 2000)
or
- b) dry ashing in a muffle furnace (IDF, 1964; Pollman, 1991; de Ruig, 1986).

Trichloroacetic acid (TCA) was used in several studies (Hankinson, 1975; IDF, 1966; Zucchetti and Contarini, 1993) to remove organic matter from dairy products. The preliminary interference studies by Brooks *et al.* (1970) revealed that calcium absorption was depressed by TCA. Moreover, the TCA precipitation method could be routinely used for laboratory analysis of relatively large numbers of samples while holding the sample volume to a minimum and this method was applicable for determination of the major anions and all the major cations. The use of nitrous oxide-acetylene flame overcame many of the chemical interferences found with conventional flames. Concentration of calcium measured using AAS with nitrous oxide

acetylene flame was not influenced by 100 times its concentration of phosphorous (Willis, 1965).

A back-titration procedure for the determination of calcium and magnesium in biological fluids was originally developed by Kamal (1960), which was refined by Ntailianas and Whitey (1964) for use in milk. Calcium in cottage cheese was determined titrimetrically, using disodium ethylene diamine tetra acetate as a calcium binder and calcein as an indicator (Demott, 1988). The average values of several samples analysed using this method were not statistically different from those obtained from the samples analysed by atomic absorption spectroscope. Similarly, several of the sampling improvements in atomic absorption spectroscopy have been well demonstrated by Kahn and Kerber (1971).

Further, a collaborative study was conducted (Pollman, 1991) to determine the calcium, phosphorous and magnesium contents in 5 varieties of cheeses using a single sample preparation procedure and estimated with a graphite furnace method of AAS. The range of values of calcium measured were from 625 to 680 mg/100 g for low moisture part skim mozzarella cheese. The method used in the collaborative study was adopted as official first action by AOAC (Pollman, 1991).

Kindstedt and Kosikowski (1985 a and b) used a single sample preparation procedure for both Ca and P based on complexiometric titrations with ethylene diamine tetra acetic acid (EDTA). But the endpoint determination using this method was found unsatisfactory, especially with grated cheese containing silica-based anti-caking agents (Pollman, 1991). The IDF (1982) method used for determination of P was found unsatisfactory for Ca and Mg determination and sample preparation was found to be labour intensive as well as reagents for P determination

had limited shelf life (Pollman, 1991). The collaborative study by Pollman (1991) identified four conditions to obtain good repeatability and reproducibility:

1. All glassware must be free of contamination. The study indicated that Ca, P and Mg contamination may be a more common problem than most analysts realize (Moffett, 1995).
2. The response of AAS instruments must meet manufacturers expectations and response must be averaged for a reasonable period of time. In addition, all instruments must be zeroed properly (Moffett, 1988; Dahmen *et al.*, 1989).
3. Standard curves must be linear within reasonable criteria, which can be achieved through instrument design or by experimental conditions used (Limbeck and Rowe, 1986; Moffett, 2000).
4. Standards must be prepared from reagents of guaranteed purity. Substitution of reagents where purity may deteriorate must be avoided.

In the presence of phosphates, ions of calcium, strontium and barium are converted to pyrophosphates at temperatures greater than 1000°C . These pyrophosphates of calcium do not decompose completely in the flame of AAS. When lanthanum is added to solutions containing phosphates and calcium, precipitates are formed of lanthanum phosphate and the free ions of calcium are completely decomposed (Elwell and Gidley, 1966) in the AAS flame. The extent of vaporization of metal ions, which determines the absorbance values, is governed by the rate of vaporization and the transit time through the flame. The rate of vaporization is determined by the characteristics of solution namely, boiling point, vapour pressure and size of particles. Suggestions to improve absorbances reported by Elwell and Gidley (1966) included, increased dilution and to reduce in the concentration of metal ions, increase in atomisation thereby achieving smaller droplet size (particles) and adjustments to the flame height. The increase in dilution of aspirated solution and increased atomisation produces smaller particles, which increases the total surface area per unit volume. Also increasing the flame height increases the

transit time for the particles through the flame. Elwell and Gidley (1966) also reported that during calcium estimation keeping the flame low decreases the transit time and increases the interferences but the sensitivity is the greatest, while higher flame height decreases the sensitivity.

6.1.2 Aims

The aim of this part of the study was to examine the effect of sample temperature on the absorbance values of calcium in skim milk, whey, mozzarella cheese and stretch water using atomic absorption spectroscopy.

6.1.3 Materials and methods

6.1.3.1 Cheese making

Mozzarella cheese was made from standardised milk (0.5% fat content) using *Streptococcus thermophilus* (TS2000) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB2515) at 2:1 ratio of starter cultures at the rate of 2.4%. Rennet was added and the milk coagulated in 30 min. When the cheese pH dropped to 5.2, milling was carried out followed by dry salting and stretching in 3% brine solution as described in sections 3.2.6.1, 3.2.6.2.

6.1.3.2 Atomic absorption spectroscopy analysis

Samples of mozzarella cheese, whey, skim milk and stretch water were collected at different stages during mozzarella cheese manufacture. The estimation of calcium was carried out using the method similar to that of Metzger *et al.* (2000) after making several dilutions of the samples of mozzarella cheese, whey, skim milk and stretch water as described in section 3.2.11; subsections 3.2.11.2, 3.2.11.3, 3.2.11.4 and 3.2.11.5. The total calcium content was estimated with AAS flame absorption method using N₂O/acetylene flame (N₂O flow: 11.00

L/min, acetylene flow: 5.96 L/min) at 422.7 nm wavelength, 0.5 nm slit width. 17.2 mm burner height, 285 volts and 7 mA. Eight standards for calcium from 2 mg/L to 16 mg/L with 2 mg increments were prepared as described in section 3.2.11.1. The absorbance values were measured for estimating the total calcium content of three diluted samples of skim milk, three diluted samples of whey, 6 samples of mozzarella cheese diluted and three samples of diluted stretch water at room temperature (22°C) with or without warming for 20 min at 30°C, 40°C and 50°C prior to aspirating the samples.

6.1.4 Results and discussion

The concentration of skim milk, whey, cheese and stretch water samples are shown in Table 6.1.1. These samples were prepared in 12% trichloroacetic acid added with lanthanum oxide (in hydrochloric acid) and aspirated into the AAS at 22°C. The table shows the samples arranged in ascending order. All absorbance values measured had very small standard deviations and the standards for calcium showed an R^2 value of 0.999 (Figure 6.1.1).

Figure 6.1.2 shows the effect of warming skim milk to 30, 40 and 50°C. The samples were cooled to 22°C and immediately measured for absorbance values. Skim milk A showed increased absorbance values at 30, 40 and 50°C compared to 22°C. although at 50°C the values were lower than at 40°C. Skim milk B showed increased absorbance values at 30, 40 and 50°C compared to those at 22°C. The absorbance values in general seemed to increase with increase in temperature, except at lower calcium concentrations (skim milk C) showed similar absorbance values at all temperature treatments.

Figure 6.1.3 shows the effects of warming whey to 30, 40 and 50°C and cooling to 22°C prior to measuring absorbance values. The whey samples namely, Whey C, whey B and whey A having increasing calcium concentrations showed increasing absorbance values. Whey A had

the highest calcium ion concentration and showed similar absorbance values at 22 and 30°C. the values increased at 40°C but decreased at 50°C to values similar to those at 30°C. Whey B showed a similar trend as whey A at the different temperature treatments while whey C showed a slight increase in absorbance values at 30°C compared to 22°C which remained similar at 40 and 50°C.

Figure 6.1.4 shows the effects of warming cheese samples to 22, 30, 40 and 50°C followed by cooling to 22°C on the absorbance values measured. Cheese F which had the highest calcium ion concentration showed the highest absorbance values compared to the other cheese samples, followed by cheese D, cheese A, cheese E, cheese B and cheese C with decreasing calcium concentrations and showing decreasing absorbance values in the same order. Cheese F showed a decrease in absorbance values at 30°C compared to 22°C and further when warmed to 40 and 50°C the absorbance values decreased. The lowest absorbance values for cheese F were recorded when the samples were warmed to 50°C. Cheese D, cheese A, cheese E and cheese B showed an increase when warmed to 30°C but the absorbance values did not significantly change upon increased warming to 40°C. These cheeses warmed to 50°C showed a decrease in absorbance values compared to 30 and 40°C warmed samples. The absorbance values for the samples warmed to 50°C were lower than the values for samples warmed to 22°C. The absorbance values for cheese C remained constant and no change in values were seen with warming to 30, 40 or 50°C. In cheeses containing higher calcium ion concentration, excess heating (such as 50°C) caused a decrease in absorbance values, which could be due to loss in detection of the excited calcium ions that were scattered outside the flame-zone during burning.

Figure 6.1.5 shows the effect of warming stretch water to 22, 30, 40 and 50°C, followed by cooling to 22°C and measuring the absorbance values. The calcium ion concentrations

decreased in the order- stretch water A, stretch water B followed by stretch water C. The stretch water samples showed absorbance values at 22 and 30°C, which correlated with the amount of calcium in the samples. Stretch water A showed lower absorbance values than stretch water B at 40 and 50°C. This could be due to the reason that when the sample concentrations produced more than 1.0 absorbance values the ions are excited beyond the zone of the flame and thus were not combusted thus causing lower absorbance values. Stretch water C had the least calcium ion concentration and also showed the least absorbance values for all the temperature treatments. The absorbance values showed no significant change due to warming at 22, 30, 40 or 50°C. The differences in concentrations of calcium measured at 22, 30, 40 and 50°C could also be due to decrease in viscosity and surface tension of the samples as suggested by Lyster (1965) which probably increased the aspiration rates (Anderson *et al.*, 1973).

6.1.5 Conclusions

Overall very small standard deviations in absorbance values were found for samples at similar temperatures. However, the temperature for treating samples was found to have an effect on the absorbance values recorded by the AAS. The skim milk and whey samples showed an increase in absorbance values with increased temperature of warming at all dilutions. In contrast, the absorbance values increased with increased warming of the cheese samples up to 40°C and then decreased at 50°C. Stretch water having higher calcium ion concentrations showed an indefinite trend to an increase in temperature when the sample concentrations produced more than 1.0 absorbance values while low calcium containing stretch water did not show any significant change in absorbance values due to warming of the samples. The change in measured absorbance values for the same sample due to effect of temperature could be because of change in solution characteristics such as surface tension and viscosity, which could alter the aspiration rates and also could be due to level of excitation energy available to the

calcium ions. Thus it is important to maintain uniform sample temperature and similar dilution levels during their preparation in order to obtain reproducible and comparable results.

Table 6.1.1 Concentration of calcium in skim milk, whey, mozzarella cheese and stretch water measured using atomic absorption spectroscopy.

Sample ID	Sample concentration in aspirated solution (µg/g)
Skim milk C	1251.03
Skim milk B	6326.18
Skim milk A	12482.67
Whey C	1246.74
Whey B	6260.43
Whey A	12521.36
Cheese C	190.42
Cheese B	379.43
Cheese E	748.60
Cheese A	757.26
Cheese D	1516.62
Cheese F	3030.71
Stretch water C	8338.52
Stretch water B	41611.86
Stretch water A	83344.35

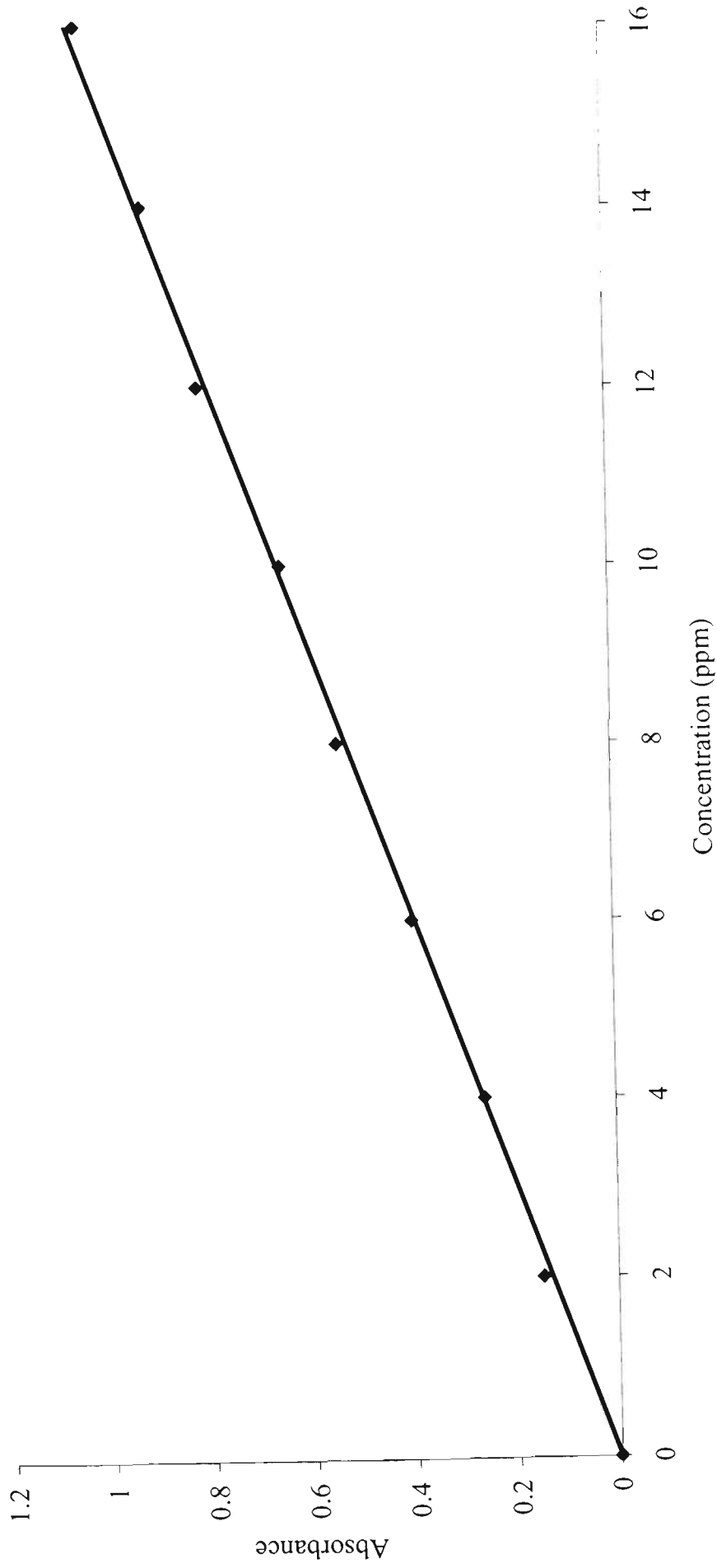


Figure 6.1.1 Absorbance values plotted against concentration (ppm) for calcium standards showing a $R^2 = 0.9986$ and $y = 0.0658x$.

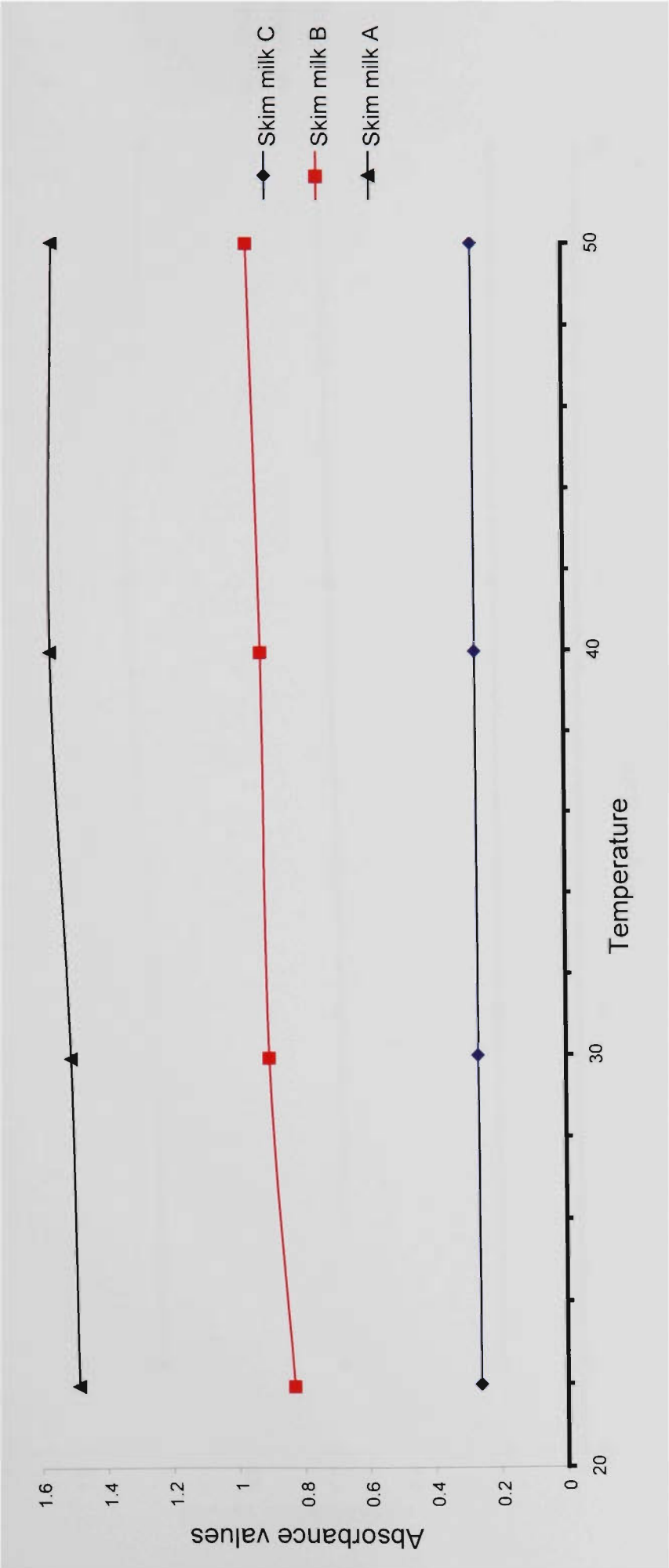


Figure 6.1.2 Effect of warming skim milk samples to 22, 30, 40 and 50°C on the absorbance values of calcium measured at 22°C. The skim milk samples are arranged in ascending order of sample concentration.

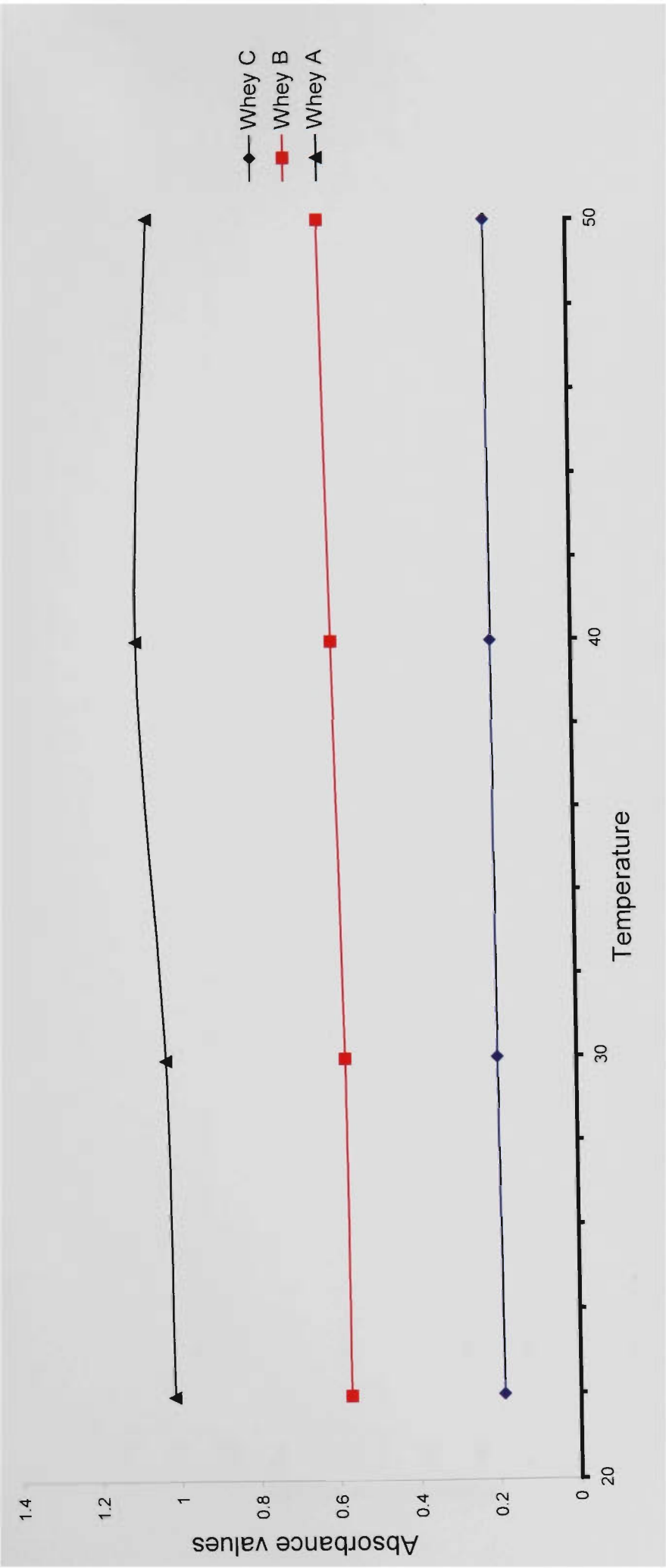


Figure 6.1.3 Effect of warming whey samples to 22, 30, 40 and 50 C on the absorbance values of calcium measured at 22 C. The whey samples are arranged in ascending order of sample concentration.

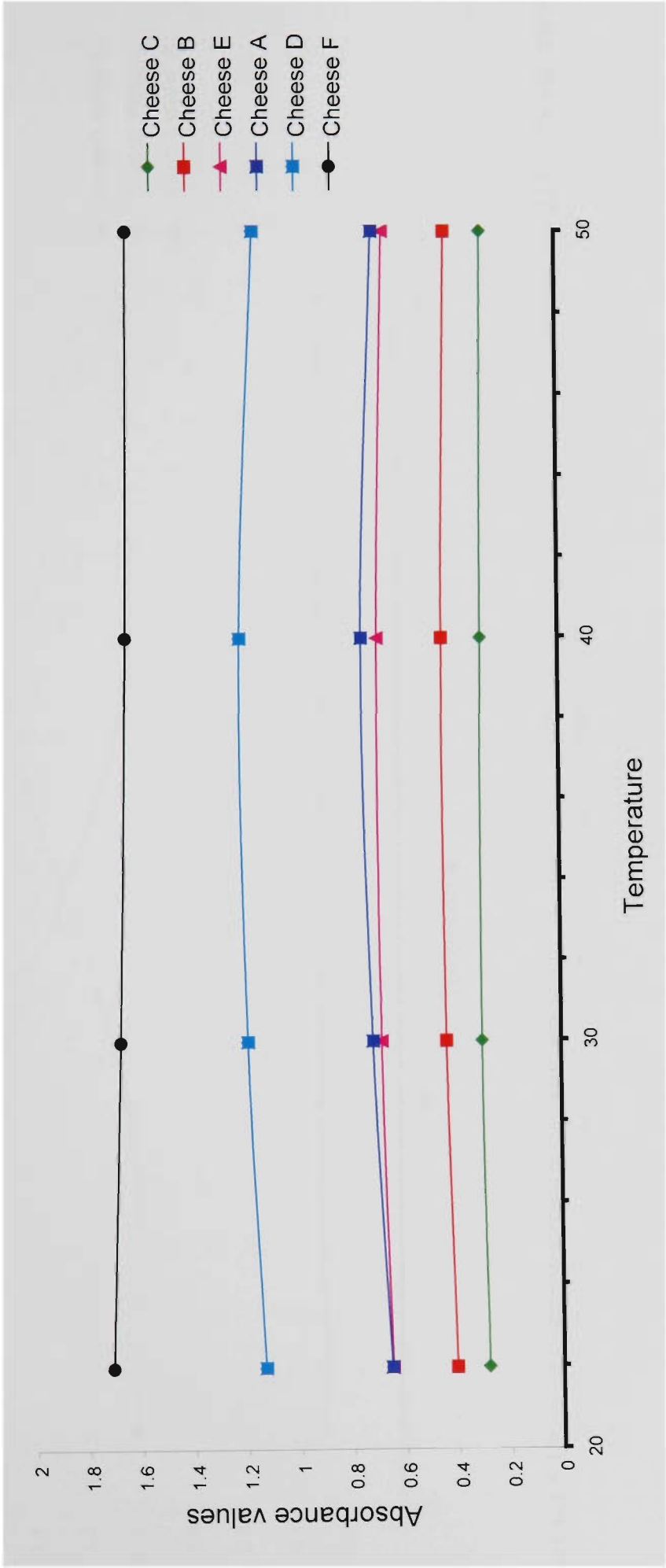


Figure 6.1.4 Effect of warming cheese samples to 22, 30, 40 and 50 °C on the absorbance values of calcium measured at 22 °C. The cheese samples are arranged in ascending order of sample concentration.

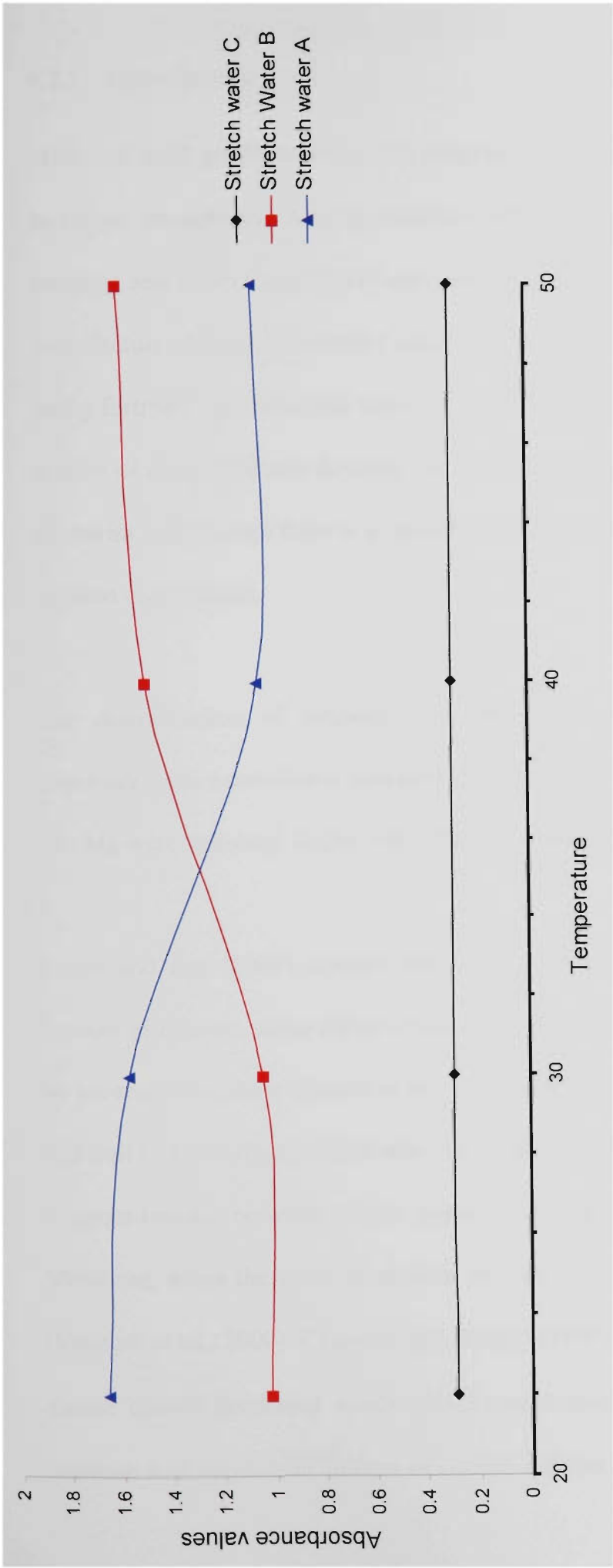


Figure 6.1.5 Effect of warming stretch water samples to 22, 30, 40 and 50 °C on the absorbance values of calcium measured at 22 °C. The stretch water samples are arranged in ascending order of sample concentration.

6.2 Role of calcium in milk and cheese

6.2.1 Introduction

Milk and milk products are major sources for calcium, magnesium, sodium, potassium and inorganic phosphates. Among minerals calcium is important for human nutrition for bone building and tooth formation (Taylor *et al.*, 1956). The milk products are altered during their manufacture (Martin-Hernandez and Juarez, 1989; Wong *et al.*, 1975) and are increasingly being fortified with minerals such as calcium, iron, manganese, potassium and vitamins. The quality of dairy products depends on chemical and microbiological parameters (Zucchetti and Contarini, 1993), and there is a need for qualitative and quantitative evaluation of the mineral fraction constituents.

The determination of calcium (Ca), phosphorus (P) and magnesium (Mg) in cheese is important from a nutritional standpoint. In a study by Pollman (1991), ratios between Ca: P and Ca: Mg were indicated as possible criteria for judging the authenticity of grated hard cheese.

Lucey and Fox (1993) reported the importance of calcium in cheese texture. The effect of amount of calcium cross-linked to proteins on the texture of cheese has been well documented by several researchers (Geurts *et al.*, 1972; Yun *et al.*, 1994; Solarza and Bell, 1995). Solarza and Bell (1995) suggested that when the total calcium content of cheese is reduced, the amount of cross-linking between casein polymers is also reduced and the cheese becomes softer. Moreover, when the calcium content of curd was decreased, the curd stretched more easily (Metzger *et al.*, 2000). Creamer and Waugh (1966) showed that increased calcium binding in cheese caused decreased solubility of α_s -caseinate. The firmness of cheese was shown to decrease with increase in amount of soluble calcium (Fox and Ernstrom, 1969).

The extent of syneresis of curd is influenced by the calcium content of milk (Aiyar and Wallace, 1970). Increased calcium content, decreased pH and increased acidity of the whey resulting from pre-acidification were suggested to have an impact on whey processing and whey product functionality (Metzger *et al.*, 2000). The effects of pre-acidification on protein-protein interactions and gel structure during setting of curd, causes changes in the fat retained and is related to reduction in calcium content (van Hooydonk *et al.*, 1986).

6.2.1.1 Factors affecting calcium content during manufacture

The mineral content of raw milk was found to be affected by the cow's physiological state (Salih *et al.*, 1987), the production season and the feed provided to the cow (Brendehaug and Abrahamsen, 1986; Murthy *et al.*, 1972). Thus variation in the mineral content of raw milk altered the mineral balance in milk products prepared from it.

Studies carried out by Wong *et al.*, (1977) on cottage cheese showed recoveries of 18%, 39%, 4.7% and 21% of Zn, Fe, Cu and Mn in cottage cheese curd. The remaining amounts of these trace minerals were drained off along with the whey and washings given to the curd. Their study also showed that addition of CaCl_2 , cutting large cubes of curd with large cheese knives and reducing the number of curd washings, increased the amount of Fe and Zn that was retained in the cottage cheese curd.

Similarly Rudan *et al.* (1999) showed that reduction of fat in mozzarella cheese from 26.7 to 4.1% caused an increase in the total calcium content from 0.67 to 1.0% as compared to low moisture part skim mozzarella cheese. The calcium content of mozzarella cheese can be manipulated by modifying cheese manufacturing conditions. When the whey is drained off, micellar calcium is retained in the curd, while most of the non-micellar calcium is lost in the whey (Hill *et al.*, 1985; Yun *et al.*, 1995). A decrease in the pH of curd at draining stage has been shown to increase the level of non-micellar calcium and lower the total calcium content of

the cheese (Hill *et al.*, 1985; Yun *et al.*, 1995). Also reports have shown that prior to renneting, a portion of micellar calcium can be destabilized by addition of acid and lowering of pH of milk (Dalglish and Law, 1989; van Hooydonk *et al.*, 1986) and thus the calcium content of the cheese can be lowered. Keller *et al.* (1974) determined that the type of acid used to manufacture direct acid mozzarella cheese had an effect on cheese calcium content while Shehata *et al.*, (1967) established that there existed a direct relationship between calcium, phosphate, viscosity and modulus of elasticity.

The calcium content of the curd decreased while the calcium content of the whey increased with increase in level of pre-acidification (Metzger *et al.*, 2000) for acetic and citric acid based pre-acidification although the latter acid when used caused a greater decrease in cheese calcium and greater increase in whey calcium than the former acid. Care needs to be taken with the choice of acids, as some acids can be strong calcium chelating agents and cause curd demineralisation (Keller *et al.*, 1974). The controlled level of calcium removal from milk would be a deciding factor in obtaining desirable characteristics in the finished mozzarella cheese manufactured from such milk.

Curd drained at pH 5.9 had 17% less calcium than curd drained at pH 6.4, even though both were cheddared to pH 5.2 (Kiely *et al.*, 1992). The cheese milled at a higher pH had higher calcium content and retained more moisture, probably due to shorter cheese manufacturing time (Yun *et al.*, 1993). The calcium content on the surface of freshly brine-salted cheese was found to be lower than in the centre of the cheese block (Kindstedt *et al.*, 1992).

Pre-acidification to pH 5.8 with citric acid was found to chelate more calcium than with acetic acid (Metzger *et al.*, 2000). The addition of acid to milk caused an increase in non-micellar calcium (Dalglish and Law, 1988; van Hooydonk *et al.*, 1986). During cheese manufacture,

the micellar calcium in milk is retained in the cheese, while the non-micellar calcium is lost in the whey. Therefore, as a result of pre-acidification, the high level of non-micellar calcium caused the observed decrease in cheese calcium content and increase in whey calcium content. The binding affinity of acetic acid for calcium was observed to be less than that of phosphate for calcium, while the binding affinity of citric acid for calcium was greater than that of phosphate (Inczey, 1976). Thus during pre-acidification, citric acid removed a larger amount of calcium from the micelles than acetic acid at the same whey pH and the calcium citrate acid was lost into whey (Metzger *et al.*, 2000).

The calcium content of the stretch water was low in the pre-acidification treatments with citric and acetic acids to pH 6.0 and pH 5.8 compared to control treatments. The lower the pH of pre-acidification, the lower was the calcium content of the stretching water. The pre-acidification treatments, which led to the lowest cheese calcium content also led to the lowest stretch water calcium content (Metzger *et al.*, 2000).

6.2.2 Aims

The aims of this part of the study were to estimate the calcium content in milk, whey, curd and stretch water and pre-acidified cheeses during mozzarella cheese-making from milk pre-acidified to pH 6.3, 6.1 and 5.9 using citric acid and to compare them against control cheeses made without pre-acidification.

6.2.3 Materials and methods

6.2.3.1 Cheese making

ULFM cheeses containing ~6.0% fat were manufactured from milk pre-acidified with citric acid and further acidified using starter cultures namely *Streptococcus thermophilus* (TS2000)

and *Lactobacillus helveticus* (LB1). Three batches of cheeses were made using methods as described in sections 3.2.6.1, 3.2.6.2 and 3.2.6.3 and were stored at 4°C.

6.2.3.2 Estimation of calcium content using AAS

Samples of standardized milk; milk mixed with starter cultures; milk mixed with starter cultures and pre-acidified to pH 6.3, 6.1 and 5.9; whey at whey draining stage; curd at whey draining stage; milled curd before salting; stretched curd; stretch water after use; and 2 d old mozzarella cheeses were collected during cheese-making. The estimation of calcium was carried out using the method described by Metzger *et al.* (2000) after making appropriate dilutions of the samples collected during manufacture of mozzarella cheeses as described in section 3.2.11 including subsections 3.2.11.2, 3.2.11.3, 3.2.11.4 and 3.2.11.5. The total calcium content was estimated with AAS flame absorption method using N₂O/acetylene flame (N₂O flow: 11.00 L/min, acetylene flow: 5.96 L/min) at 422.7 nm wavelength, 0.5 nm slit width, 17.2 mm burner height, 285 v and 7 mA. Eight standards for calcium from 2 mg/L to 16 mg/L with 2 mg increments were prepared as described in section 3.2.11.1. The absorbance values were measured for total calcium content for all samples at room temperature (22°C).

6.2.4 Results and discussion

Table 6.2.1 shows the mean and standard error values for calcium contents of in-line samples collected for control cheese, and cheeses made using citric acid pre-acidification to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1 and PAC5.9 cheeses). Standardized milks used for the manufacture of the different cheeses had similar (statistically not different) calcium content. The control cheeses showed variation in calcium content although manufactured under similar conditions, which could possibly be attributed to variation in calcium content contributed from bulk starter culture added to pre-acidified milk.

Samples of pre-acidified milk used for preparation of different cheeses showed no significant differences in calcium content. Whey collected at the whey draining stage for control and PAC6.3 cheeses showed similar calcium contents but the PAC6.1 and PAC5.9 showed lower calcium contents which could be due to samples being drawn immediately from the cheese vats and not allowing sufficient time for the calcium to be chelated from the cheese matrix. The curd samples were taken after allowing 20 min for complete whey draining along with an intermediate turning of the curd, followed by sampling from several areas of the curd slabs. The curd at whey drain for control cheese had the highest calcium content followed by a decreasing order with increase in pre-acidification. The curd samples collected from PAC6.3 had higher calcium content than PAC6.1 and the latter had higher calcium content than PAC5.9 cheeses. These curd samples showed that with increase in pre-acidification of milk there was a decrease in the amount of calcium retained in their curd.

Milling was carried out manually by cutting the slabs of curd into about 4 cm x 2 cm x 2 cm cuboids and salt was externally applied. The samples of milled curd collected at this stage showed variation in estimated calcium contents, which could be due to differences in the moisture contents, serum pockets trapped in the curd and ion exchange between Ca and Na.

The stretched curd from each of the cheeses showed a significant difference in their calcium contents. The control cheeses, prepared with only lactic fermentation by the starter bacteria, showed the highest levels of calcium, while the pre-acidified cheeses showed decreasing amounts of calcium with increased levels of pre-acidification. PAC5.9 cheeses showed the least amount of calcium. Samples of stretch water after stretching the control cheeses had the highest calcium content, followed by the pre-acidified cheeses, which showed decreasing calcium contents with increasing levels of pre-acidification. These results indicate that due to lack of further acidification during stretching under hot conditions (70°C) no further calcium

was lost into stretch water. The losses of calcium into stretch water could be attributed to the calcium, which was exchanged by the sodium ions and all the calcium trapped in serum pockets. Lastly, the 2 d old mozzarella cheeses (Table 6.2.1) indicated that cheeses made using pre-acidified milk contained less calcium compared to control. Also, the lower the pre-acidification pH, the lower was the calcium content in cheeses. Thus, 2 d old control cheese had the highest calcium content, followed by PAC6.3, PAC6.1 and PAC5.9 cheeses.

6.2.5 Conclusions

Calcium contents measured at several stages of mozzarella cheese manufacture are important to ascertain the amount of calcium that would be present in the stretched cheese. This would affect the characteristics of the cheeses prepared. Online measurement of calcium content during cheese preparation would give a real time control of the desired cheese. Pre-acidification reduced the calcium content in the cheeses. Increase in level of pre-acidification decreased the calcium retained in the cheeses. Control cheeses made without pre-acidification had the highest levels of calcium. Calcium is chelated out into the whey at whey draining stage due to mainly acidification and later into stretch water at stretching stage due to replacement of calcium with sodium ions. Also the physical forces of cutting and stretching may affect the calcium retained in the cheeses.

Table 6.2.1 Calcium content (Mean \pm SE¹) measured (n = 6) at several stages of manufacture and for cheese stored for 2 d at refrigeration temperature for control cheese and citric acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1, PAC5.9) cheeses.

Stage of manufacture	Control cheese	PAC6.3 cheese	PAC6.1 cheese	PAC5.9 cheese	p-value ²
	mg/1000g	mg/ 1000g	mg/ 1000g	mg/ 1000g	
Standardized milk	1487.64 \pm 6.73 ^A	1481.41 \pm 58.19 ^A	1204.91 \pm 92.57 ^A	1445.27 \pm 3.20 ^A	0.0576
Milk mixed with Starter culture	564.14 \pm 2.44 ^B	893.16 \pm 102.01 ^A	615.75 \pm 1.80 ^A	668.95 \pm 1.18 ^A	0.0359*
Whey at draining	12682.21 \pm 213.65 ^A	11969.744 \pm 207.92 ^A	9519.27 \pm 18.46 ^{BC}	8448.12 \pm 16.25 ^C	0.0001*
Curd at draining	12557.71 \pm 75.08 ^A	11631.25 \pm 34.98 ^B	10107.97 \pm 112.73 ^C	9177.84 \pm 22.77 ^D	0.0000*
Milled curd before salting	174.94 \pm 15.17 ^C	268.670 \pm 6.78 ^B	288.87 \pm 8.43 ^{AB}	335.59 \pm 4.75 ^A	0.0013*
Stretched curd	12612.91 \pm 125.71 ^A	11640.91 \pm 72.96 ^B	10306.05 \pm 2.96 ^C	9372.07 \pm 27.56 ^D	0.0000*
Stretch water after use	12813.37 \pm 6.26 ^A	11553.97 \pm 90.26 ^B	10099.67 \pm 167.57 ^C	9471.82 \pm 68.87 ^D	0.0000*
Cheese 2 d	12657.44 \pm 3.62 ^A	11653.38 \pm 18.17 ^B	10161.44 \pm 2.49 ^C	9248.99 \pm 46.94 ^D	0.0000*

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

7.0 EFFECT OF PRE-ACIDIFICATION OF MILK USING LACTIC, CITRIC OR ACETIC ACID TO PH 6.3 ON ULFM CHEESE CHARACTERISTICS

7.1 Introduction

A low fat mozzarella cheese does not have the functional characteristics of a full fat mozzarella. Improvement to the functional characteristics of low fat mozzarella cheeses can be brought about by a preliminary step of pre-acidification of cheese-milk using lactic, acetic, citric, phosphoric or glucono- δ -lactone, added either singly or in combination. Merrill *et al.* (1994) successfully developed reduced fat mozzarella cheese with greater meltability, using lactic acid as acidulant to lower the pH of milk to 6.0 prior to starter culture addition as compared to reduced fat cheese without pre-acidification. One of the easiest ways to manufacture mozzarella cheese is by direct acidification and such cheeses perform with uniform functionality batch after batch. The pH of fresh milk (about 6.65) can be slowly reduced to the desired pH using starter cultures or one or more of the food grade acidifying agents.

When mozzarella cheeses are made using starter cultures, the curd that is formed is stretched at about pH 5.2. Alternatively, acidifying agents can be added to cheese milk to lower pH to a suitable level to allow the curd to stretch. It is important to note that when the mozzarella cheese is made using direct acidification the pH for stretching the curd slightly shifts towards neutral probably due to shift in calcium ion concentration and over-acidification could lead to crumbly curd. Skim milk when acidified at 30°C to pH 4.6 showed complete solubilisation of colloidal calcium but at the same time caused casein precipitation, while at 2°C it showed partial solubilisation of colloidal calcium and no precipitation of casein (Fox and Ernstrom,

1969). Fox and Ernstrom (1969) suggested that a minimum level of calcium was required to solvate the casein and although at higher temperature of acidification of milk more calcium could be removed from the bound state with casein into the serum. It is important to have a balance with casein solvation and calcium remaining in the curd. The ability of calcium to stabilize casein in milk influences the loss of protein into whey due to acidification of cheese-milk during mozzarella cheese manufacture. The ratio of calcium: phosphate could translate into variations in the mineral balance in the finished cheese and needs to be considered while classifying grated cheeses as imitation cheeses (Pollman, 1991).

An acceptable low fat mozzarella cheese was made by Kim *et al.*, (1995) from a mixture of 15% soymilk and 85% cows milk using lactic acid for direct acidification. Ather and Anwar (1992) manufactured a buffalo milk mozzarella cheese using direct acidification technique, which had better sensory qualities compared to the cheese made using starter cultures consisting of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. The study showed that although mozzarella cheeses made using the starter culture technique had significantly higher protein and minerals and reduced lactose content, their acceptability (sensory score) was lower than that for mozzarella cheeses made by direct acidification using lactic acid. Similarly Patel *et al.* (1986) were able to manufacture acceptable buffalo milk mozzarella cheese by direct acidification with lactic acid.

Although it is feasible to manufacture mozzarella cheese using direct acidification, the typical flavour of a fresh cheese made using starter cultures is absent. The flavour characteristic in low fat mozzarella cheese may cause a lower sensory score for such direct acidified cheeses (Keller, 1978). Moreover, direct acidification in commercial conditions to reduced pH levels is difficult to manage due to faster rate of curd setting, increase in curd fines that are lost into whey. Thus a low level of pre-acidification of milk followed by addition of starter cultures may

provide the finished cheese with an improved flavour due to starter bacteria and better textural and functional properties due to acidification with acidulating agents.

7.2 Aims

The aims of this study were to manufacture ULFM cheeses from cheese milk pre-acidified with either lactic, citric or acetic acids to a pH of 6.3 and further using *Lactobacillus helveticus* and *Streptococcus thermophilus* to ripen the milk for mozzarella cheese making, and to analyse the cheeses for their yield, composition, textural and functional characteristics.

7.3 Materials and methods

7.3.1 Cheese making

Cheese milk was standardised to contain 0.5% fat and 3.0% casein as described in section 3.2.6.1. The milk was mixed with *Streptococcus thermophilus* (TS2000) and *Lactobacillus helveticus* (LB1) starter cultures and further acidified using lactic (PAL6.3), citric (PAC6.3) or acetic (PAA6.3) acid to pH 6.3 (section 3.2.6.3). Three batches of control and pre-acidified ULFM cheeses were made using dry salting method, containing ~6.0% fat (section 3.2.6.2) and were stored at 4 °C.

7.3.2 Analysis of cheeses

The yield of cheeses was determined on wet basis and the cheese samples were prepared (section 3.2.9) for estimation of composition, melt, texture characteristics (section 3.2.8) and for pizza bake characteristics (section 3.2.7 and 3.2.24). The composition including moisture/total solids was estimated using the method described in section 3.2.20, fat content as in section 3.2.22 and protein content as in section 3.2.25 were determined. Textural

characteristics including hardness, cohesiveness and springiness were measured by compressing cylindrical cheese samples to 50% height as described in section 3.2.23 using an Instron according to the method of Bhaskaracharya (2000) after storing the cheeses for 2, 8, 15, 22 and 29 d. Meltability test was carried out as described in section 3.2.15 at weekly intervals during storage using 10 g of grated cheese sample heated in glass tube at 110°C for 100 min similar to the method used by Poduval and Mistry (1999). Pizza-bake test was carried out (section 3.2.24) at 20 and 45 d of storage and the colour, size of blisters and their distribution upon baking were assessed.

7.3.3 Statistical analysis

Statistical analysis of the results was carried out using StatPro[®] software on Microsoft[®] excel. The sample sizes, sample means, sample standard deviations and standard error were calculated. The means from at least two populations were compared. The analysis of variance table was prepared and two sources of variation: the variation *within* each population and variation *among* sample means from the different populations was compared. If the latter variation was large relative to the former, as was measured using an F test, this was deemed to be evidence of differences between the population means. The p-value obtained from this table and the confidence intervals (at 95% confidence level) for all the differences between pairs of means were considered for describing the statistical differences and their significance in the results tables. A small p-value was a result of large differences in population means and the confidence intervals that did not include zero were taken to indicate that the means that were compared were not equal.

7.4 Results and discussion

7.4.1 Yield and composition

The yield (g/50 Kg) and composition of control cheese and citric/acetic/lactic-pre-acidified cheeses are shown in Table 7.4.1. PAL6.3 cheeses showed the least yield while control cheeses had the highest yield followed by PAA6.3, PAC6.3 cheeses. The moisture contents (in descending order) were 57, 57, 53 and 52%; protein contents (in ascending order) were 31, 31, 35 and 36% for PAA6.3, control, PAC6.3 followed by PAL6.3 cheeses respectively. The control and PAA6.3 cheeses had a high yield, moisture, FDM and M:P content along with a low protein content compared to those of PAC6.3 and PAL6.3 cheeses. The PAL6.3 cheeses had the least yield, moisture, FDM and M:P contents along with a high protein content which are undesirable characteristics for commercial manufacture of ULFM cheeses. The PAC6.3 cheeses were also unsuitable for the same reasons as for PAL6.3 cheeses although the former had better yield and compositional characteristics than the latter cheeses. Thus significant differences in yield and composition (as shown in Table 7.4.1) were observed for pre-acidified (PAC6.3, PAA6.3, PAL6.3) cheeses as compared to the control cheeses.

7.4.2 Texture characteristics

Hardness values for control, PAC6.3, PAA6.3 and PAL6.3 cheeses, measured on 2, 8, 15, 22 and 29 d of storage are shown in Table 7.4.2. The hardness values for control and PAA6.3 cheeses were lower compared to PAC6.3 and PAL6.3 cheeses throughout storage. The PAL6.3 cheeses were significantly harder compared to control, PAA6.3 and PAC6.3 cheeses except that on 8 d the hardness values for PAL6.3 and PAC6.3 cheeses were similar. Analysis of PAC6.3 cheeses could not be carried out on 22 and 29 d of storage due to sample shortage. All the cheeses showed a significant decrease ($p<0.05$) in hardness values during storage. The hardness decreased for the control cheeses from 8 d, PAC6.3 and PAA6.3 cheeses from 15 d, and PAL6.3 cheeses from 22 d. Thus PAL6.3 cheeses, which had the highest protein content

were significantly harder than the other cheeses (Bhaskaracharya and Shah 1999 and 2001c). The delay in the decrease in hardness values for the pre-acidified cheeses could be due to less ripening time that was available for the ripening of milk by the starter cultures leading to decreased proteolysis during manufacture.

Cohesiveness values for control, PAC6.3, PAA6.3 and PAL6.3 cheeses measured on 2, 8, 15, 22 and 29 d of storage are shown in Table 7.4.3. All the cheeses showed similar cohesiveness on 2 and 8 d of storage except PAA6.3 cheeses, which had lower cohesiveness values on 8 d compared to the other cheeses. PAA6.3 cheeses had the highest moisture and showed the least cohesiveness values (Bhaskaracharya and Shah, 1999). PAL6.3 cheeses had the highest cohesiveness values followed by PAC6.3 cheeses and then control and PAA6.3 cheeses at 15 d. At 22 and 29 d, again PAL6.3 cheeses had significantly higher cohesiveness compared to control and PAA6.3 cheeses. From 15 d onwards both control and PAA6.3 cheeses showed similar (non-significant differences) values. Due to lack of sample PAC6.3 cheeses could not be analysed on 22 and 29 d of storage. All the cheeses had similar cohesiveness, which did not significantly change due to storage from 2 d to 29 d except for PAL6.3 cheeses, which exhibited significantly ($p < 0.05$) low values on 2 d followed by similar values from 8 d to 29 d of storage.

The mean \pm standard error of springiness values for control, PAC6.3, PAA6.3 and PAL6.3 cheeses measured on 2, 8, 15, 22 and 29 d of storage are shown in Table 7.4.4. The PAA6.3 cheeses showed higher springiness on 2 d compared to other cheeses while PAL6.3 cheeses had the least springiness. PAL6.3 cheeses had the least M:P content and also showed the least springiness values. The effect of M:P contents upon the cheeses is similar to the observations made by Bhaskaracharya and Shah (1999). Both control and PAC6.3 cheeses showed similar springiness on 2 d of storage. At 8 d of storage, control and PAA6.3 cheeses had higher

springiness while PAC6.3 and PAL6.3 cheeses had lower springiness. After 15 d of storage (i.e. on 15, 22 and 29 d) control, PAC6.3 and PAA6.3 cheeses had similar and higher springiness compared to PAL6.3 cheeses. Thus during storage PAA6.3 cheeses showed high springiness, control and PAC6.3 cheeses had moderate springiness, while PAL6.3 cheeses had the least springiness. Control, PAC6.3, PAA6.3 and PAL6.3 cheeses showed lowest springiness at 2 d followed by 8 d, and then springiness values increased after 15 d to remain similar till 29 d of storage.

7.4.3 Functional characteristics

7.4.3.1 Meltability results

Meltability results measured at 11, 18 and 25 d for control, PAC6.3, PAA6.3 and PAL6.3 cheeses are shown in Table 7.4.5. The meltability values for control and PAC6.3 cheeses were similar throughout storage and their melt distance was significantly greater than PAA6.3 and PAL6.3 cheeses on 11, 18 and 25 d. The PAL6.3 cheeses showed the least meltability throughout storage. The meltability values for PAA6.3 and PAL6.3 cheeses were similar. During storage no significant changes in meltability were observed for control, PAC6.3 and PAL6.3 cheeses but PAA6.3 cheeses seemed to have decreased meltability after 18 and 25 d of storage ($p < 0.05$). Thus PAL6.3 cheeses, which had lower moisture content and higher protein content melted the least. The PAA6.3 cheeses had the highest moisture content but contrary to our expectations showed less meltability. Correlation could not be established between their textural characteristics and functional characteristics of melt and flow. Thus pre-acidification did not markedly improve meltability characteristic of cheeses.

7.4.3.2 Pizza bake results for 20 d old cheeses

7.4.3.2.1 *Physical properties of blister formation and burning as observed with the naked eye on 20 d old PAC6.3, PAA6.3, PAL6.3 and control ULFM cheeses.*

The control (no oil) cheeses showed (Figure 7.4.1) most of the cheese shreds as intact and had not melted during baking. These cheese shreds were brown, had burnt appearance and the cheese did not melt and flow on the pizza base. Very few small blisters were formed. The control (with oil) cheeses showed almost complete shred fusion and some melting of the cheese. There were a few large blisters (burnt) and many small blisters (also burnt) on this sample. Overall, the control (with oil) cheeses showed some improvement in shred fusion due to application of oil, but both control (with oil) and control (no oil) cheeses did not show any whiteness, rather they were burnt and showed poor melt and flow on the pizza base during baking.

The PAC6.3 (with/ no oil) cheeses showed increased shred fusion, increased whiteness, better melt and flow characteristics compared to control, PAA6.3 and PAL6.3 cheeses as shown in Figure 7.4.1. PAC6.3 (no oil) cheeses showed complete shred fusion but some regions on the surface of cheeses were excessively burnt. A few large blisters and many small blisters were formed that showed scorching. The PAC6.3 (with oil) cheeses showed decreased burning but such cheeses lacked the shine/gloss of a typical full fat mozzarella cheese.

Figure 7.4.1 shows that PAA6.3 (with/no oil) cheeses were whiter than control (with/no oil) and PAL6.3 (with/no oil) cheeses and these cheeses showed increased shred fusion, better melt and flow properties. PAA6.3 (no oil) cheeses showed a few intact cheese shreds, which appeared to be submerged under the melted cheese. This could suggest that the temperature and time of baking was insufficient to cause complete melting of all ULFM cheese shreds, but higher temperatures would increase scorching. Although all the cheeses namely, control, PAC6.3, PAA6.3 and PAL6.3 cheeses were baked under the same temperature-time regimes,

PAA6.3 (no oil) cheeses were not completely melted and fused. These cheeses showed small blisters that were brown (although not completely burnt). The PAA6.3 (with oil) cheeses showed complete shred fusion with no intact cheese shreds. Many large blisters were formed on the cheeses surface, which were not completely burnt and showed browning. Figure 7.4.1 shows that there were an equal number of small sized blisters with similar extent of browning as the large blisters could also be seen on such cheeses. The cheeses showed some flow although more could be desired.

The PAL6.3 (no oil) cheeses shown in Figure 7.4.1 had better melt, improved shred fusion and slightly less burning compared to control (with/no oil) cheeses. But some of the cheeses shreds of PAL6.3 (no oil) cheeses were intact and the cheeses were brown upon baking. The PAL6.3 (with oil) cheeses had improved shred fusion, better melt and more blister formation than the PAL6.3 (no oil) cheeses. PAL6.3 (with oil) cheeses showed a few large sized brown blisters and many small blisters, which were also brown due to scorching. These cheeses showed slightly reduced browning compared to control (with/ no oil) cheeses, but were still not of acceptable characteristics.

Overall the PAC6.3 (with oil) cheeses had the best pizza baked characteristics of reduced browning and burning, complete shred fusion, better melt and flow properties, but lacked whiteness and gloss on surface. Storage of such cheeses or increased level of pre-acidification and further modifications in manufacturing methodology could possibly improve the glossiness in order to obtain the desired ULFM cheeses.

Table 7.4.6 shows the mean and standard error of Hunter L, a and b values for fresh, baked and cooled ULFM (with/no oil) cheeses during pizza baking after 20 d of storage. The various effects of pre-acidification of cheese milk using citric; acetic or lactic acid, baking and cooling

and application of oil on the Hunter L, a and b values are discussed below. Table 7.4.6 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

7.4.3.2.2 Effect of pre-acidification with citric/acetic/lactic acids to pH 6.3 on the Hunter L, a and b values of cheeses

The Hunter L values for fresh samples of control, PAC6.3, PAA6.3 and PAL6.3 cheeses were similar before baking. Cheeses made using citric acid (PAC6.3) showed higher L^3 and L^4 values compared to control, PAA6.3 and PAL6.3 cheeses. Control (with oil) and PAA6.3 (with oil) cheeses showed similar L^3 and L^4 values to control (no oil) cheeses whereas PAA6.3 (no oil) cheeses had lower L^3 and L^4 values. The PAL6.3 cheeses showed very low L^3 and L^4 values due to excessive browning. Thus PAC6.3 cheeses were whiter compared to control, PAA6.3 and PAL6.3 cheeses.

The measured Hunter a values were significantly different for fresh control and for pre-acidified cheeses. The a^2 values for control (with oil) cheeses were higher than those for pre-acidified (with oil) cheeses. Similarly, the a^2 values for control (no oil) cheeses were higher than those for pre-acidified (no oil) cheeses. The PAA6.3 (with/no oil) cheeses showed the least a^2 values among fresh cheeses. Upon baking and further upon cooling PAC6.3 and PAL6.3 (with/no oil) showed the highest a^3 and a^4 values while PAA6.3 (with/no oil) cheeses showed the least a^3 and a^4 values. Thus PAA6.3 cheeses showed reduced browning upon baking. This could be the effect of acetic acid pre-acidification or because these PAA6.3 cheeses had low a^2 values and so after baking, their a^3 and a^4 values continued to be lower than that for the other cheeses. The Hunter b values for fresh control and pre-acidified cheeses were significantly different ($p < 0.05$) with the PAA6.3 (with/no oil) and control (with/no oil) cheeses having higher b^2 values compared to PAC6.3 (with/no oil) and PAL6.3 (with/no oil) cheeses. Similar trends in b^3 and b^4 values were seen for the pre-acidified and control cheeses. Thus the

PAA6.3 and control cheeses showed increased yellowness compared to PAC6.3 and PAL6.3 cheeses. This indicates a negative impact upon yellowness for PAC6.3 and PAL6.3 cheeses. Baking or cooling did not alter this trend for the pre-acidified cheeses.

7.4.3.2.3 Effect of oil application on Hunter L, a, and b values.

The Hunter L, a and b values for control, PAC6.3, PAA6.3 and PAL6.3 cheeses measured after 20 d of storage are shown in Table 7.4.6. The L, a, and b values for control (with oil) cheeses were similar (not statistically different) to control (no oil) cheeses. Similarly the L, a, and b values for pre-acidified (with oil) cheeses were similar to the pre-acidified (no oil) cheeses. Thus there was no significant influence on any of the cheeses when fresh, after baking or after cooling due to application of hydrophobic material on the cheese shreds.

7.4.3.2.4 Effects of baking and cooling of cheeses on Hunter L, a and b values

The data shown column-wise in Table 7.4.6 indicates the statistical differences or similarities for the same cheese in their Hunter L, a, and b values due to the effects of baking or cooling. The control (with/no oil) cheeses showed increased ($p<0.05$) L^3 and L^4 values compared to L^2 (fresh cheeses) values. The L^4 values were also significantly lower than the L^3 values for control cheeses. Similarly, the L^3 and L^4 values for PAC6.3 (with/no oil) and PAA6.3 (with/no oil) cheeses were significantly higher than their L^2 values although L^3 and L^4 values were similar for each cheese. The PAL6.3 cheeses showed a significant decrease ($p<0.05$) in L^3 values due to baking and the latter values further decreased significantly ($p<0.05$) due to cooling. Thus except for PAL6.3 cheeses, the PAA6.3, PAC6.3 and control cheeses showed increase in whiteness when baked.

The a^3 and a^4 values increased for the control, PAC6.3, PAA6.3 (with/no oil) cheeses compared to their a^2 values except for a decrease in a^3 values for control (with oil) cheeses. The a^4 values for control, PAC6.3 and PAA6.3 (with/no oil) cheeses were higher than their a^3

values, but PAL6.3 (with/no oil) cheeses showed lower a^4 values than their a^3 values. Thus the PAL6.3 cheeses showed an opposite trend to the other cheeses in relation to their a^3 and a^4 values. Overall, due to increased browning in all the cheeses except for control (with oil) cheeses, their a^3 and a^4 values seemed to increase. The b values increased with baking (b^3 values) and further increased upon cooling (b^4 values) for the control, PAC6.3, PAA6.3 and PAL6.3 (with/no oil) cheeses. The b^3 and b^4 values were higher than the b^2 values for control (no oil) and PAA6.3 (with/no oil) cheeses whereas for PAC6.3 (with/no oil) and PAL6.3 (with/no oil) cheeses these values were similar to their b^2 values.

7.4.3.3 Pizza bake analysis for PAL6.3 cheeses compared after storing for 20 and 45 d.

7.4.3.3.1 Physical properties of blister formation and burning as observed with the naked eye on 20 d and 45 d old ULFM cheeses made from milk pre-acidified using lactic acid to pH of 6.3.

Figure 7.4.2 shows pizzas made from PAL6.3 (with/no oil) cheeses that were stored for 20 d and 45 d. Due to the use of flash while taking pictures the 20 d old PAL6.3 cheeses appear red in colour compared to the 45 d old PAL6.3 (with/no oil) cheeses. Increase in storage period to 45 d slightly improved the melt and shred fusion of the cheeses. Larger blisters were formed, which were more numerous than for the 20 d old cheeses. There was no increase in whiteness of the cheeses due to prolonged storage. Thus prolonged storage did not improve the overall acceptability of the PAL6.3 (with/no oil) cheeses.

Table 7.4.7 shows the mean and standard error of Hunter L, a and b values for fresh, baked and cooled PAL6.3 (with/no oil) cheeses during pizza baking after 45 d of storage. The various effects of pre-acidification of cheese milk using lactic acid, baking and cooling and application of oil on the Hunter L, a and b values are discussed below. Table 7.4.7 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

7.4.3.3.2 *Effects of storage on Hunter L, a, and b values for PAL6.3 cheeses.*

The Hunter L values for fresh PAL6.3 cheeses did not significantly change with storage although PAL6.3 (with oil) cheeses stored for 20 d showed lower L^2 values compared to PAL (no oil) cheeses, which were stored for 45 d. Also the L^3 and L^4 values for PAL6.3 (with/no oil) cheeses stored for 45 d were higher than the L^3 and L^4 values for cheeses stored for 20 d. Thus longer period of storage helped to increase whiteness in baked PAL6.3 cheeses. The a^2 values of 20 d old PAL6.3 (with oil) cheeses were significantly higher than the a^2 values of 45 d old PAL6.3 (with oil) cheeses. There were no significant differences between the 20 d and 45 d stored PAL6.3 (no oil) cheeses. Again, the a^3 values of 20 d stored PAL6.3 (with/no oil) cheeses were significantly higher ($p<0.05$) compared to the a^3 values of 45 d stored PAL6.3 (with/no oil) cheeses. The a^4 values for the 20 d old PAL6.3 (with oil) cheeses were similar to a^4 values of 45 d old PAL6.3 (with oil) cheeses but PAL6.3 (no oil) cheeses showed significantly higher a^4 values after 20 d of storage than those after 45 d of storage. The a^4 values of 20 d old PAL6.3 (no oil) cheeses were similar to those for the 45 d stored PAL6.3 (no oil) cheeses. The b^2 values of 45 d stored PAL6.3 (with/no oil) cheeses were higher ($p<0.05$) than those values for the 20 d stored PAL6.3 (with/no oil) cheeses. But, the b^3 and b^4 values of 20 d old PAL6.3 (with/no oil) cheeses were significantly higher than those for 45 d stored PAL6.3 (with/no oil) cheeses. There was no significant improvement observed in the pizza bake characteristics due to prolonged storage of PAL6.3 cheeses.

7.4.3.3.3 *Effects of oil application on the Hunter L, a and b values*

The Hunter L, a, and b values indicated row-wise in Table 7.4.7. The PAL6.3 cheeses after 20 d or after 45 d storage did not show a significant effect due to application of oil on the shredded cheeses on the Hunter L, a and b values measured for the fresh, baked and cooled cheeses.

7.4.3.3.4 *Effects of baking and cooling on the Hunter L, a and b values*

The data shown column-wise in Table 7.4.7 indicates the statistical differences and similarities for the PAL6.3 cheeses in their Hunter L, a and b values due to the effects of baking and cooling. At 20 d the PAL6.3 (with/no oil) cheeses showed a significant decrease in L values due to baking (L^3 values), which further decreased upon cooling (L^4 values). Unlike the trend of L^2 , L^3 and L^4 values shown by 20 d old PAL6.3 (with/no oil) cheeses, these 45 d old cheeses after the prolonged storage and then baking showed increased L^3 and L^4 values compared to their L^2 values. Thus storage improved the whiteness of baked PAL6.3 cheeses. The a^3 values at 20 d were significantly decreased compared to those for 45 d old PAL6.3 (with/no oil) cheeses. The a^4 values for 20 d old and for 45 d old cheeses were similar. Thus storage decreased the a^3 values of PAL6.3 cheeses upon baking. The b^2 values improved for PAL6.3 (with/no oil) cheeses that were stored for 45 d compared to 20 d old cheeses. The b^3 and b^4 values decreased significantly upon storage for 45 d compared to those stored for 20 d.

7.5 Conclusions

The PAA6.3 cheeses showed increased yield, similar to control cheeses and had higher moisture, FDM and M:P contents and lower protein content, while PAC6.3 and PAL6.3 cheeses had poor yield and higher protein content. The PAA6.3 cheeses were softer, had lower cohesiveness values and higher spring. The pre-acidified cheeses required longer storage to soften compared to control cheeses probably due to shorter starter culture activity time during the manufacture. The PAC6.3 and PAL6.3 cheeses did not have the desired textural characteristics, but PAC6.3 cheeses showed good meltability compared to PAA6.3 and PAL6.3 cheeses. Also the PAC6.3 and PAA6.3 cheeses showed increased whiteness upon baking while PAL6.3 cheeses were only better compared to control cheeses. Storage of PAL6.3 cheeses for 45 d increased the size of blisters and improved shred fusion characteristics which were indicated as higher L values, lower a values, which are desirable, but their b values were not

affected. The PAL6.3 cheeses were not of acceptable pizza bake characteristics even after the prolonged storage due to excessive burning and scorching. Application of oil seemed to improve the cheese shred fusion and melt of all cheeses during baking.

Overall, acetic and citric acid when used as pre-acidification agents seemed to improve some of the functional characteristics such as meltability, stretchability and/ or pizza bake characteristics. A lower pH of acidification of milk may improve the characteristics of the ULFM cheeses.

Table 7.4.1 Yield and composition of control cheese and citric, acetic or lactic acid pre-acidified to pH 6.3 (PAC6.3, PAA6.3, PAL6.3) cheeses, (n = 9).

	Control cheese	PAC6.3 cheese	PAA6.3 cheese	PAL6.3 cheese	p-value ²
	Mean ± SE ¹	Mean ± SE ¹	Mean ± SE ¹	Mean ± SE ¹	
Yield ³ (g/ 50 Kg)	4275.0 ± 175.0 ^a	4000.0 ± 11.5 ^{ab}	4012.5 ± 12.5 ^{ab}	3852.3 ± 30.0 ^b	0.0272 [*]
Moisture (%)	57.050 ± 0.48 ^a	53.174 ± 0.13 ^{bc}	57.590 ± 0.14 ^a	52.422 ± 0.11 ^c	0.0000 [*]
Protein (%)	31.107 ± 0.51 ^d	35.093 ± 0.07 ^b	31.430 ± 0.11 ^{cd}	36.868 ± 0.29 ^a	0.0000 [*]
FDM ⁴ (%)	13.978 ± 0.15 ^a	12.814 ± 0.04 ^{bc}	14.148 ± 0.05 ^a	12.611 ± 0.03 ^c	0.0000 [*]
M:P ⁵	1.851 ± 0.04 ^a	1.515 ± 0.00 ^b	1.832 ± 0.01 ^a	1.422 ± 0.01 ^c	0.0000 [*]

¹Mean ± Standard error; ²ANOVA of means; ³n= 3; ⁴FDM = Fat in dry matter; ⁵M:P = Moisture: Protein; ^{a, b, c, d} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 7.4.2 Hardness values (Mean \pm SE¹) for control cheese and citric, acetic or lactic acid pre-acidified to pH 6.3 (PAC6.3, PAA6.3, PAL6.3) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAC6.3 cheese	PAA6.3 cheese	PAL6.3 cheese	p-value ²
2	63.18 \pm 2.64 ^{a, CD}	117.29 \pm 5.36 ^{a, B}	51.04 \pm 2.87 ^{a, D}	180.07 \pm 13.02 ^{a, A}	0.0000 [*]
8	27.32 \pm 3.34 ^{cde, C}	124.80 \pm 5.01 ^{a, A}	48.00 \pm 4.08 ^{a, BC}	132.92 \pm 7.37 ^{cd, A}	0.0000 [*]
15	22.23 \pm 4.00 ^{de, D}	62.46 \pm 2.20 ^{b, B}	31.32 \pm 4.73 ^{bcd, CD}	139.57 \pm 7.54 ^{bc, A}	0.0000 [*]
22	32.90 \pm 3.90 ^{bcd, BC}	NA	18.17 \pm 1.41 ^{d, C}	154.10 \pm 4.83 ^{abc, A}	0.0000 [*]
29	20.88 \pm 2.16 ^{e, BC}	NA	19.77 \pm 3.24 ^{cd, C}	100.97 \pm 6.35 ^{d, A}	0.0000 [*]
p-value ³	0.0000 [*]	0.0000 [*]	0.0000 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05); NA= Data not available; ^{*} Significant (p<0.05).

Table 7.4.3 Cohesiveness values (Mean \pm SE¹) for control cheese and citric, acetic and lactic acid pre-acidified to pH 6.3 (PAC6.3, PAA6.3, PAL6.3) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAC6.3 cheese	PAA6.3 cheese	PAL6.3 cheese	p-value ²
2	0.687 \pm 0.02 ^{a, A}	0.696 \pm 0.01 ^{a, A}	0.670 \pm 0.01 ^{a, A}	0.648 \pm 0.03 ^{b, A}	0.3231
8	0.708 \pm 0.01 ^{a, A}	0.717 \pm 0.00 ^{a, A}	0.660 \pm 0.01 ^{a, B}	0.732 \pm 0.00 ^{a, A}	0.0000 [*]
15	0.690 \pm 0.00 ^{a, CD}	0.717 \pm 0.01 ^{a, B}	0.683 \pm 0.01 ^{a, D}	0.744 \pm 0.00 ^{a, A}	0.0000 [*]
22	0.668 \pm 0.01 ^{a, C}	NA	0.682 \pm 0.01 ^{a, BC}	0.727 \pm 0.00 ^{a, A}	0.0000 [*]
29	0.678 \pm 0.01 ^{a, BC}	NA	0.672 \pm 0.01 ^{a, C}	0.758 \pm 0.00 ^{a, A}	0.0000 [*]
p-value ³	0.0996	0.1934	0.5292	0.0000 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column. ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05); NA= Data not available; ^{*} Significant (p<0.05).

Table 7.4.4 Springiness values (Mean \pm SE¹) for control cheese and citric, acetic and lactic acid pre-acidified to pH 6.3 (PAC6.3, PAA6.3, PAL6.3) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAC6.3 cheese	PAA6.3 cheese	PAL6.3 cheese	p-value ²
2	2.017 \pm 0.07 ^{b, C}	2.089 \pm 0.06 ^{b, BC}	2.620 \pm 0.14 ^{a, A}	1.478 \pm 0.06 ^{d, D}	0.0000*
8	2.317 \pm 0.07 ^{ab, A}	1.578 \pm 0.06 ^{c, BC}	2.233 \pm 0.07 ^{a, A}	1.500 \pm 0.04 ^{cd, C}	0.0000*
15	2.517 \pm 0.04 ^{a, A}	2.456 \pm 0.08 ^{a, A}	2.533 \pm 0.13 ^{a, A}	1.933 \pm 0.04 ^{a, B}	0.0000*
22	2.500 \pm 0.07 ^{a, A}	NA	2.433 \pm 0.06 ^{a, A}	1.578 \pm 0.03 ^{bcd, B}	0.0000*
29	2.467 \pm 0.08 ^{a, A}	NA	2.600 \pm 0.23 ^{a, A}	1.867 \pm 0.05 ^{a, B}	0.0009*
p-value ³	0.0001*	0.0000*	0.3109	0.0000*	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column. ^{a, b, c, d} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05); NA= Data not available; * Significant (p<0.05).

Table 7.4.5 Mean \pm SE¹ of melt distance measured (mm) for control cheese and citric, acetic or lactic acid pre-acidified to pH 6.3 (PAC6.3, PAA6.3, PAL6.3) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAC6.3 cheese	PAA6.3 cheese	PAL6.3 cheese	p-value ²
11	51.885 \pm 0.97 ^{a, AB}	53.370 \pm 0.59 ^{a, A}	43.310 \pm 2.69 ^{a, B}	31.307 \pm 2.21 ^{a, C}	0.0000 [*]
18	52.195 \pm 2.59 ^{a, A}	54.263 \pm 0.54 ^{a, A}	34.070 \pm 0.94 ^{b, C}	36.050 \pm 3.31 ^{a, BC}	0.0000 [*]
25	53.483 \pm 3.09 ^{a, A}	NA	37.000 \pm 1.14 ^{ab, BC}	35.720 \pm 3.45 ^{a, C}	0.0016 [*]
p-value ³	0.9515	0.2899	0.0141 [*]	0.4854	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column. ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); NA= Data not available; ^{*} Significant (p<0.05).

Table 7.4.6 Mean \pm SE¹ (n = 9) Hunter L a b-values of 20 d old control cheese and citric, acetic and lactic acid pre-acidified to pH 6.3 (PAC6.3, PAA6.3, PAL6.3) cheeses, applied with or without canola oil, measured fresh² (before baking), after baking³ and after cooling⁴.

Control cheese		PAC6.3 cheese			PAA6.3 cheese			PAL6.3 cheese			p-value ⁵
Hunter L a b	With oil	No Oil	With oil	No Oil	With oil	No Oil	With oil	No Oil			
L ²	59.53±1.74 ^{b, A}	62.48±0.50 ^{c, A}	60.71±0.64 ^{b, A}	61.65±0.65 ^{b, A}	60.53±1.05 ^{b, A}	63.08±1.26 ^{b, A}	59.27±0.68 ^{a, A}	61.00±0.87 ^{a, A}	0.0759		
L ³	73.68±0.36 ^{a, CDE}	72.49±0.26 ^{a, DE}	97.44±2.15 ^{a, A}	91.92 ± 3.10 ^{a, A}	74.85±0.33 ^{a, BCDE}	72.05±1.64 ^{a, E}	37.62±1.17 ^{b, FG}	36.98±0.77 ^{b, G}	0.0000 [*]		
L ⁴	71.08±0.51 ^{a, CDE}	69.98±0.67 ^{b, DE}	94.00±2.20 ^{a, A}	87.71±2.23 ^{a, A}	73.68±0.30 ^{a, BCDE}	69.89±1.60 ^{a, E}	32.15±1.01 ^{c, FG}	31.60±1.21 ^{c, G}	0.0000 [*]		
p-value ⁶	0.0000 [*]	0.0000 [*]	0.0000 [*]	0.0000 [*]	0.0000 [*]	0.0020 [*]	0.0000 [*]	0.0000 [*]			
a ²	0.06±0.18 ^{bc, A}	-0.40±0.15 ^{b, ABC}	-1.00±0.12 ^{b, BCDE}	-1.30±0.09 ^{b, EF}	-1.82±0.09 ^{c, F}	-2.15±0.10 ^{b, G}	-1.06±0.12 ^{c, CDE}	-1.22±0.11 ^{c, DEF}	0.0000 [*]		
a ³	-0.02±0.12 ^{c, EF}	3.00±0.45 ^{a, CDE}	14.68±0.82 ^{a, A}	13.64±0.45 ^{a, AB}	-1.37±0.18 ^{bc, F}	2.39±1.36 ^{a, DEF}	12.35±0.25 ^{a, AB}	11.40±0.31 ^{a, B}	0.0000 [*]		
a ⁴	1.36±0.35 ^{a, FG}	2.69±0.72 ^{a, DEF}	14.58±0.29 ^{a, A}	12.47±0.44 ^{a, AB}	-0.71±0.18 ^{a, G}	1.78±0.94 ^{a, EFG}	10.61±0.47 ^{b, BC}	9.05±0.43 ^{b, C}	0.0000 [*]		
p-value ⁶	0.0006 [*]	0.0003 [*]	0.0000 [*]	0.0000 [*]	0.0006 [*]	0.0085 [*]	0.0000 [*]	0.0000 [*]			
b ²	17.11±0.60 ^{c, ABCDE}	18.41±0.36 ^{b, ABC}	15.14±0.27 ^{a, E}	16.69±0.54 ^{a, BCDE}	18.94±0.38 ^{b, AB}	19.35±0.37 ^{b, A}	15.98±0.47 ^{a, CDE}	15.49±0.36 ^{a, DE}	0.0000 [*]		
b ³	25.59±0.59 ^{b, A}	30.87±1.16 ^{a, A}	16.69±1.87 ^{a, BCDE}	14.03 ± 0.74 ^{a, CDE}	27.36±0.38 ^{a, A}	30.59±1.13 ^{a, A}	13.87±1.28 ^{a, DE}	13.63±0.76 ^{a, E}	0.0000 [*]		
b ⁴	28.30±0.67 ^{a, A}	29.25±1.01 ^{a, A}	15.33±0.51 ^{a, CDE}	15.73±0.90 ^{a, BCDE}	28.69±0.38 ^{a, A}	30.02±0.72 ^{a, A}	12.75±1.15 ^{a, E}	13.06±1.09 ^{a, DE}	0.0000 [*]		
p-value ⁶	0.0000 [*]	0.0000 [*]	0.5779	0.0768	0.0000 [*]	0.0000 [*]	0.0962	0.0986			

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D, E, F, G} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 7.4.7 Mean \pm SE¹ (n = 9) Hunter L a b-values of lactic acid pre-acidified to pH 6.3 (PAL6.3) cheeses, applied with or without canola oil and stored for 20 and 45 d at refrigerated temperature, measured fresh², after baking³ and after cooling⁴.

Hunter L a b	After 20 d storage		After 45 d storage		p-value ⁵
	With oil	No Oil	With oil	No Oil	
L ²	59.27±0.68 ^{a, C}	61.00±0.87 ^{a, BC}	62.40±0.64 ^{b, ABC}	65.86±1.07 ^{b, A}	0.0000 [*]
L ³	37.62±1.17 ^{b, BC}	36.98±0.77 ^{b, C}	85.26±3.05 ^{a, A}	78.72±2.46 ^{a, A}	0.0000 [*]
L ⁴	32.15±1.01 ^{c, BC}	31.60±1.21 ^{c, C}	76.11±5.03 ^{a, A}	73.64±1.80 ^{a, A}	0.0000 [*]
p-value ⁶	0.0000 [*]	0.0000 [*]	0.0003 [*]	0.0002 [*]	
a ²	-1.06±0.12 ^{c, A}	-1.22±0.11 ^{c, AB}	-1.52±0.08 ^{c, B}	-1.35±0.12 ^{c, AB}	0.0364 [*]
a ³	12.35±0.25 ^{a, A}	11.40±0.31 ^{a, A}	1.60±1.56 ^{bc, C}	3.83±1.83 ^{b, BC}	0.0000 [*]
a ⁴	10.61±0.47 ^{b, A}	9.05±0.43 ^{b, AB}	9.35±0.45 ^{a, AB}	8.43±0.47 ^{a, B}	0.0161 [*]
p-value ⁶	0.0000 [*]	0.0000 [*]	0.0000 [*]	0.0000 [*]	
b ²	15.98±0.47 ^{a, BC}	15.49±0.36 ^{a, C}	18.50±0.40 ^{a, A}	18.84±0.49 ^{a, A}	0.0000 [*]
b ³	13.87±1.28 ^{a, A}	13.63±0.76 ^{a, A}	-4.30±1.27 ^{b, BC}	-7.10 1.65 ^{b, C}	0.0000 [*]
b ⁴	12.75±1.15 ^{a, A}	13.06±1.09 ^{a, A}	-20.15±1.19 ^{c, C}	-19.27±0.84 ^{c, BC}	0.0000 [*]
p-value ⁶	0.0962	0.0986	0.0000 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05).

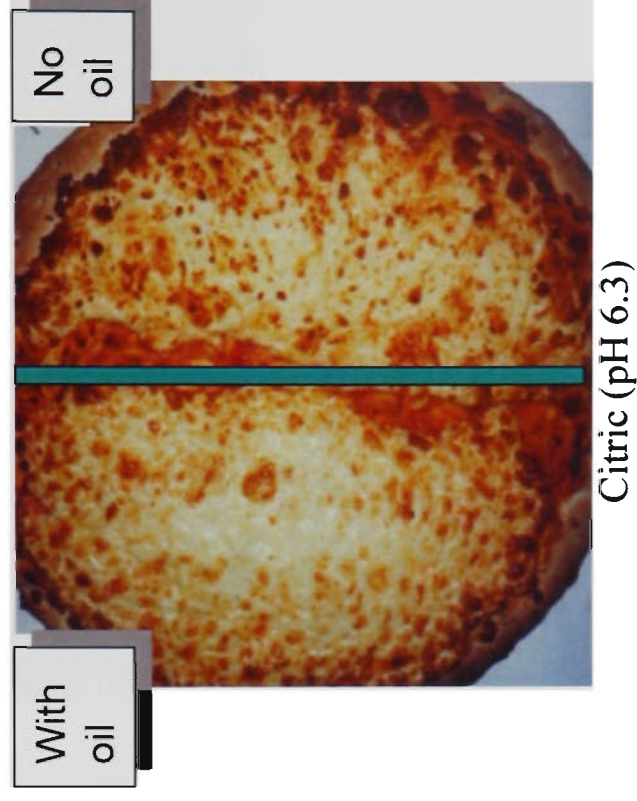
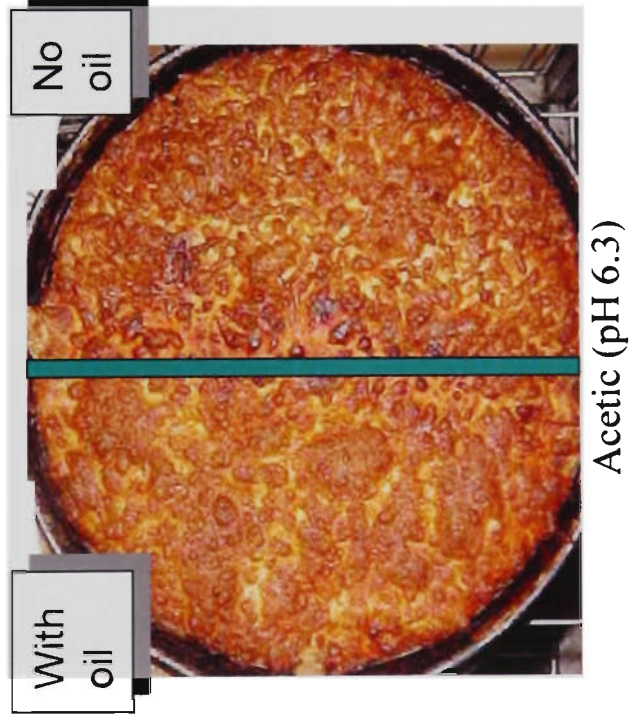
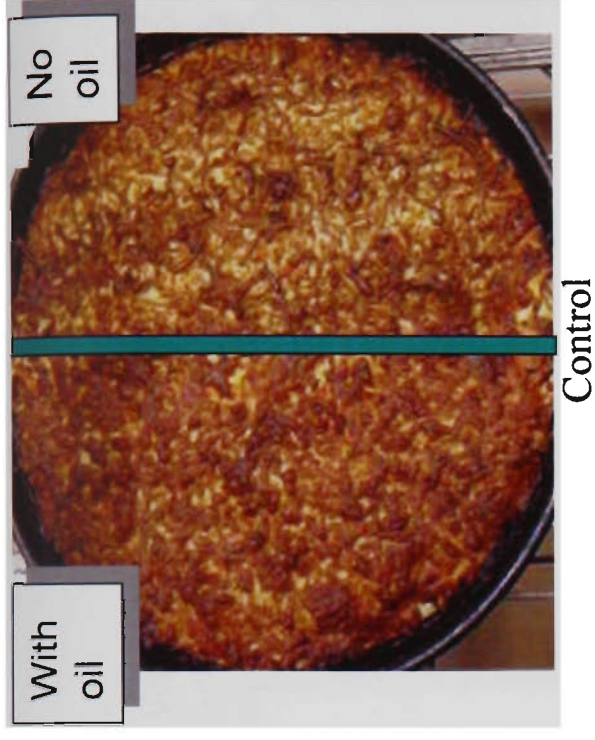


Figure 7.4.1 Pizza bake analysis of PAL6.3 (with/no oil), control (with/no oil), PAA6.3 (with/no oil) and PAC6.3 (with/no oil) cheeses carried out after 20 d of storage.

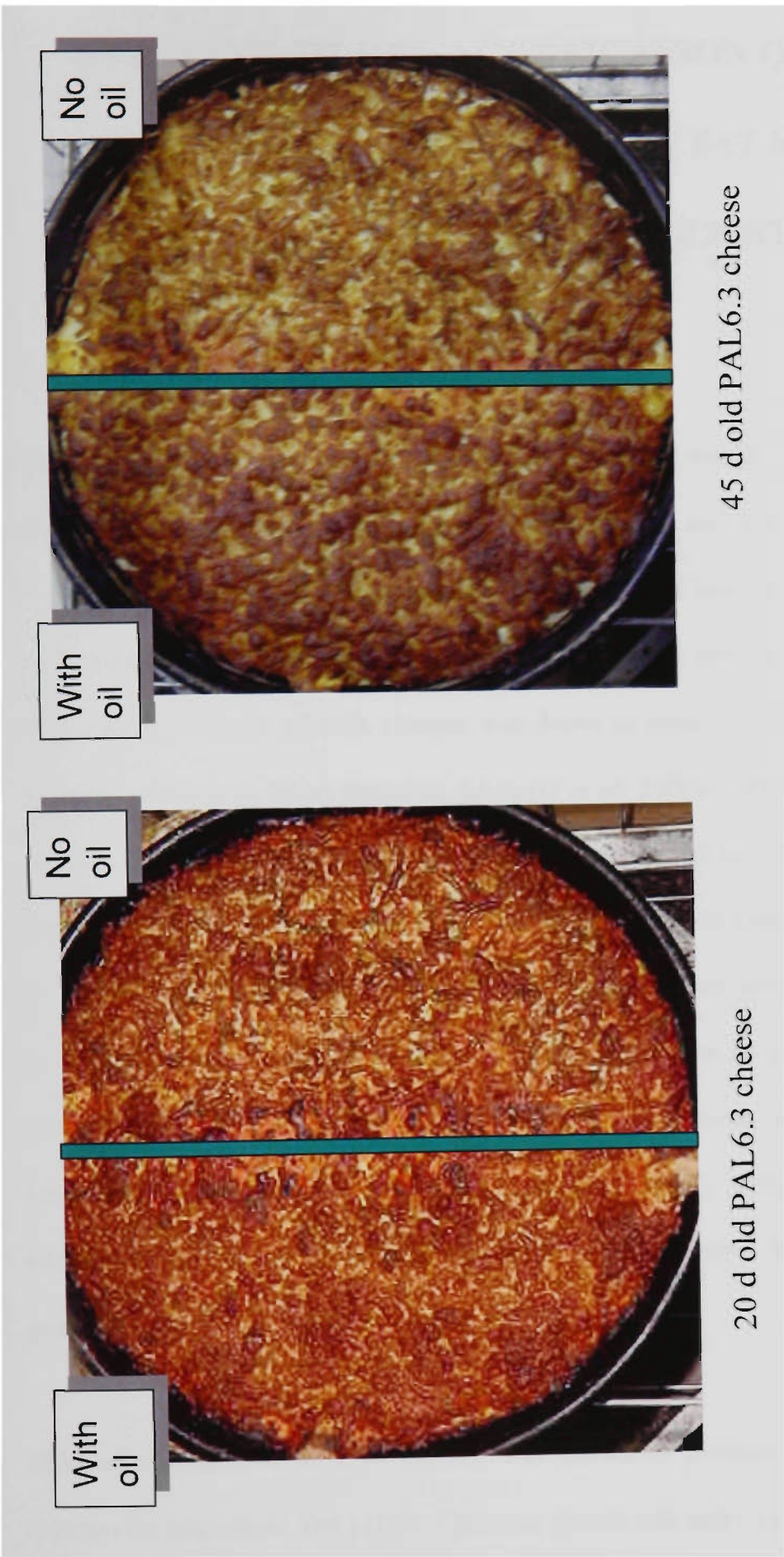


Figure 7.4.2 Pizza bake analysis of PAL6.3 (with/no oil) cheeses carried out after 20 d and 45 d of storage.

8.0 EFFECT OF PRE-ACIDIFICATION OF MILK USING CITRIC ACID ON ULTRA LOW FAT MOZZARELLA CHEESE CHARACTERISTICS

8.1 Introduction

The calcium content of low fat mozzarella cheeses is usually higher than that for full fat mozzarella cheeses due to increase in protein content and the associated colloidal calcium bound to the protein. The tough and rubbery texture of low fat mozzarella cheeses does not allow proper melt, flow and stretch characteristics when the cheeses are heated. Reduction in total calcium content of such cheeses was found to make the cheeses softer, melt better and form smooth strings when stretched (Metzger *et al.* 2000). Direct acidification of cheese milk using citric acid has been tried by several researchers such as Verruma-Bernardi *et al.* (2000), Gasperi *et al.* (2001), Junghee *et al.* (1998), do Valle and de Leita0 (1995 a and b), Coppola *et al.* (1990) and Prato (1993) to manufacture full fat mozzarella cheeses without the use of starter cultures. However, the problems associated with low fat mozzarella cheese manufacture were not studied. Also the small amount of free amino acids produced due to proteolysis by starter bacteria was further reduced (la Notte *et al.*, 1980) along with the amount of calcium retained in curd when mozzarella cheeses were made using direct acidification using citric acid.

Mild acidification of milk to pH 6.3 was shown to produce a rubbery and less cohesive mozzarella curd while low pH curd became plastic and tacky (Keller *et al.*, 1974). Among the cheeses made, the citric and malic acid cheeses had higher moisture but lower fat content compared to those made using acetic, hydrochloric or phosphoric acids (Keller *et al.*, 1974). The lower the pH of acidification, the higher were the calcium losses, and the amount of

calcium retained in the cheeses was affected by the type of acid used for acidification (Keller *et al.* 1974, Metzger *et al.*, 2000). Increased acidification was also found to increase meltability of the cheeses and viscous and elastic moduli were correlated with the calcium and phosphate content (Metzger *et al.*, 2000). The cheeses showed a decrease in phosphate content with the type of acid used for acidification namely, phosphoric> acetic> malic> hydrochloric> citric acid. At very low pH of direct acidification there was an increase in bitterness perceived upon sensory evaluation of the cheeses and preference was indicated to the cheese made by acidification to pH 5.4 using phosphoric acid (Keller *et al.*, 1974). Similarly, Metzger *et al.* (2000) reported that citric acid was able to remove more calcium from the curd than acetic acid.

The main problem with pre-acidification was the loss of some protein into whey, which decreased the yield of the cheeses. Yield efficiency was found to be significantly reduced (~ 5.5%) for cheeses made using citric acid while ~3.4% reduction in yield efficiency was found for cheeses made using acetic acid (Metzger *et al.*, 2000). The decrease in the colloidal calcium associated with the casein has been reported to increase the hydration of paracasein. This was suggested to be the reason for greater losses of casein due to pre-acidification. A gradual acidification of milk to pH 5.0 was reported to progressively increase the solubilization of calcium from its colloidal state (Law, 1996). Simultaneously an increase in casein concentrations in the serum was observed with decrease in pH up to 5.5 and below pH 5.5 the casein concentrations in the serum decreased. The solvation of casein was found to be higher for heat treated milk compared to raw milk (Law, 1996) at a higher pH. The dissociation of calcium was affected by the heat treatment but increased at lower pH of acidification.

Some of the changes such as dissociation of β -casein from the casein micelles and increase in its concentration in the serum are reported to be partly reversible (Davies and Law, 1983;

Downey and Murphy, 1970). Reducing the pH of milk at higher temperatures ($\sim 30^{\circ}\text{C}$) was found to remove most of the colloidal calcium without dissociation of caseins. At lower temperatures ($<20^{\circ}\text{C}$) of pre-acidification, greater loss in caseins into serum was reported (Dalglish and Law, 1988 and 1989). Thus, temperature and pH of milk were found to affect the amount of calcium removed from the curd and into the whey.

Law (1996) observed that mainly κ -casein was dissociated and lost in whey during pre-acidification of milk and that the amount of κ -casein dissociated increased when milk had previously been subjected to heat treatment. Such dissociated caseins (including κ -casein and some minor caseins) were readily centrifuged out from the skim milk after heat treatment and acidification. Similarly, β -casein was also readily dissociated along with κ -casein. The dissociation of β -casein at $<4^{\circ}\text{C}$ was reported to be higher than at $>4^{\circ}\text{C}$. When milk was pre-acidified to pH 5.4 (raw milk) or pH 5.7 (heat treated milk $\sim 85^{\circ}\text{C}$ for 10 min), the maximum casein concentration was observed in the serum.

During manufacture of direct acidified mozzarella cheese the stretching is usually carried out at pH 5.5- 5.4 and the dissociation of casein would be expected to cause losses in yield as was observed by Metzger *et al.* (2000). Heating milk to 90°C for 10 min has been shown to cause complexing of β -lactoglobulin with κ -casein (Singh and Fox, 1987) and α -lactalbumin with κ -casein (Noh and Richardson, 1989), which would provide a stabilizing effect to the κ -casein in the micelles ($<\text{pH } 6.5$). However, the milk used for manufacture of ULFM cheeses is not treated to 90°C for 10 min, thus such stabilizing effect does not take place.

The results from previous experiments showed that lowering the acidification pH of milk reduced hardness and in some instances improved the pizza bake characteristics. In this study

cheeses were made from milk pre-acidified using citric acid to three levels namely- 6.3, 6.1 and 5.9 and comparisons made between their functionality.

8.2 Aims

The aims of this study were to manufacture ULFM cheeses from cheese milk pre-acidified with citric acid to pH of 6.3, 6.1 or 5.9 using *Lactobacillus helveticus* and *Streptococcus thermophilus* and to determine the most suitable pH of pre-acidification using citric acid that achieves the best textural and functional characteristics.

8.3 Materials and methods

8.3.1 Cheese making

Cheese milk was standardised to 0.5% fat and 3.0% casein as described in section 3.2.6.1. The milk was inoculated with *Streptococcus thermophilus* (TS2000) and *Lactobacillus helveticus* (LB1) starter cultures and further pre-acidified using citric acid to pH 6.3, 6.1 or 5.9 as described in section 3.2.6.3. ULFM cheeses containing ~6.0% fat were manufactured following the steps described in section 3.2.6.2. Three batches were made of control (without any pre-acidification) and pre-acidified cheeses using dry salting method and the cheeses were stored at 4°C.

8.3.2 Analysis of cheeses

The yield of cheeses was measured and the cheese samples were prepared as described in section 3.2.9 for estimation of composition, melt, texture characteristics (section 3.2.8) and for pizza bake characteristics (section 3.2.7 and 3.2.24). The composition including moisture/total solids was estimated using the method described in section 3.2.20, fat content (section 3.2.22)

and protein content (section 3.2.25) were determined. Meltability test was carried out at 11 d as described in section 3.2.15 using 10.0 g of grated cheese sample heated in glass tube at 110°C for 100 min similar to the method used by Poduval and Mistry (1999). Textural characteristics including hardness, cohesiveness and springiness were measured by compressing cylindrical cheese samples to 50% height as described in section 3.2.23 using an Instron according to the method of Bhaskaracharya (2000) after storing the cheeses for 2, 8 and 15 d. Pizza-bake test was carried out (section 3.2.24) at 20 d of storage and the colour, size of blisters and their distribution upon baking were assessed.

8.3.3 Statistical analysis

Statistical analysis of the results was carried out using StatPro[®] software on Microsoft[®] excel. The sample sizes, sample means, sample standard deviations and standard error were calculated. The means from at least two populations were compared. The analysis of variance table was prepared and two sources of variation: the variation *within* each population and variation *among* sample means from the different populations was compared. If the latter variation was large relative to the former, as was measured using an F test, this was deemed to be evidence of differences between the population means. The p-value obtained from this table and the confidence intervals (at 95% confidence level) for all the differences between pairs of means were considered for describing the statistical differences and their significance in the results tables. A small p-value was a result of large differences in population means and the confidence intervals that did not include zero were taken to indicate that the means that were compared were not equal.

8.4 Results and discussion

8.4.1 Yield and composition

Table 8.4.1 shows the yield, composition and meltability at 11 d for control cheese and citric acid pre-acidified to pH 6.3, 6.1 or 5.9 (PAC6.3, PAC6.1 and PAC5.9) cheeses. Cheeses were made in triplicate and the average yield of control cheeses was similar (not statistically different) to PAC6.3, PAC6.1 and PAC5.9 cheeses. Thus pre-acidification with citric acid to any of the pH's had no significant effect (statistically) on the yield of the cheeses. The moisture, FDM and M:P contents of control cheeses and PAC6.1 and PAC5.9 cheeses were similar and significantly ($p<0.05$) higher than for PAC6.3 cheeses. The protein content of the PAC6.3 cheeses was significantly higher than the control, PAC6.1 or PAC5.9 cheeses, which had similar protein contents. The melt characteristic of the cheeses was significantly different. PAC6.1 cheeses showed the highest melt distance of 95.4 mm, control and PAC6.3 cheeses showed similar melt while PAC5.9 cheeses showed poor melt of 40.9 mm. The observations are correlating to the results reported by Metzger *et al.* (2000) although PAC5.9 does not follow similar trend.

8.4.2 Texture characteristics

The hardness values measured on 2, 8 and 15 d of storage for control cheeses and citric acid pre-acidified to pH 6.3, 6.1 or 5.9 (PAC6.3, PAC6.1 and PAC5.9) cheeses is represented in Table 8.4.2. The hardness values were highest for PAC6.3 cheeses while those for control cheeses, which had the most moisture, were the least on 2, 8 and 15 d of storage. PAC6.1 and PAC5.9 cheeses showed similar hardness values on 2 d of storage, but on 8 and 15 d the PAC6.1 cheeses were significantly harder than the PAC5.9 cheeses. Decrease in pH of pre-acidification to 5.9 could have caused increased interaction of the negatively charged casein micelles with the serum phase, while opposing interactions between the casein micelles, thus

making the cheese softer (Fox and Ernstrom, 1969). The PAC6.3 and PAC6.1 cheeses showed similar hardness at 15 d while control and PAC5.9 cheeses had similar hardness values. During storage the hardness values for control cheeses were highest at 2 d, which decreased significantly at 8 d and then did not change at 15 d. The PAC6.3 cheeses showed high hardness at 2 d and the values showed no change up to 8 d followed by a decrease at 15 d. This could be due to the higher protein content and lower moisture content of PAC6.3 cheeses. The PAC6.1 cheeses had a high hardness at 2 d, which decreased at 8 d and remained similar (statistically not different) at 15 d similar to the trend shown by control cheeses. The PAC5.9 cheeses also had high hardness values similar to the control, PAC6.3 and PAC6.1 cheeses and the values decreased significantly at 8 d and further decreased at 15 d. Thus pre-acidified cheeses required longer storage period to show decreased hardness possibly due to starter culture activity during storage.

Table 8.4.3 shows the cohesiveness values for control, PAC6.3, PAC6.1 and PAC5.9 cheeses measured at 2, 8 and 15 d of refrigerated storage. The control and PAC6.3 cheeses were similar (statistically) to both PAC6.1 and PAC5.9 cheeses at 2 d but PAC5.9 cheeses had significantly higher cohesiveness values compared to PAC6.1 cheeses. At 8 d of storage, control, PAC6.3, PAC6.1 and PAC5.9 cheeses showed similar cohesiveness. The control cheeses showed the least cohesiveness at 15 d while PAC6.3 and PAC5.9 cheeses showed the highest values. An increase in hydration of caseins due to pre-acidification to pH 5.9 could have caused increased interactions within the protein matrix leading to higher cohesiveness values. The PAC6.1 cheeses had similar cohesiveness as control, PAC6.3 and PAC5.9 cheeses at 15 d of storage. During storage the control cheeses showed similar cohesiveness at 2, 8 and 15 d while PAC6.3 and PAC5.9 cheeses showed no effect of storage. PAC6.1 cheeses had the least cohesiveness values on 2 d and the values that increased at 8 and 15 d of storage.

The mean and standard error for springiness values measured at 2, 8 and 15 d of storage for control, PAC6.3, PAC6.1 and PAC5.9 cheeses are shown in Table 8.4.4. PAC6.3 cheeses showed significantly higher springiness than PAC6.1 cheeses at 2 d. The latter cheeses had the least springiness and control and PAC5.9 cheese had similar springiness as those for PAC6.3 and PAC6.1 cheeses at 2 d. At 8 d, control and PAC5.9 cheeses had similar springiness values, which were significantly higher than those for PAC6.3 and PAC6.1 cheeses. Also PAC6.3 and PAC6.1 cheeses had similar springiness values at 8 d. The control, PAC6.3 and PAC5.9 cheeses showed similar springiness at 15 d and these values were significantly higher than the values for PAC6.1 cheeses. During storage control cheeses showed an increase in springiness from 2 d to 8 d but remained similar (statistically not different) at 8 d and 15 d. The PAC6.3 cheeses showed a significant decrease in springiness from 2 d to 8 d but the values significantly increased at 15 d. PAC6.1 cheeses showed no change in springiness due to storage while PAC5.9 cheeses showed a significantly increased springiness at 15 d compared to 2 d. The springiness values seemed to increase with storage of the cheeses irrespective of pre-acidification but there was no definite relation between the level of acidification and springiness values.

8.4.3 Functional characteristics

8.4.3.1 Pizza bake characteristics

8.4.3.1.1 Physical properties of blister formation and burning as observed with the naked eye on 20 d old ULFM cheeses made from milk pre-acidified using citric acid to pH of 6.3, 6.1 and 5.9 and control cheeses.

The control (no oil) cheeses (Figure 8.4.1) showed most of the shreds as intact and did not melt during baking. These cheese shreds were brown, had burnt appearance and the cheese did not melt and flow on the pizza base. Very few blisters were formed and these were small in size. The control (with oil) cheeses showed almost complete shred fusion and some melting of the cheese. There were a few large blisters (burnt) and many small blisters (also burnt) of the

control (with oil) cheeses. Overall, the control (with oil) cheeses showed some improvement in shred fusion due to application of oil, but both control (with oil) and control (no oil) cheeses did not show any whiteness, rather they were burnt and had not melted and flowed on the pizza base during baking.

PAC6.3 (no oil) cheeses when baked showed that the cheese shreds had melted but some structural intactness of the shreds was visible. Such shreds were swollen, partially blistered and were deformed during baking. The melted cheese formed large blisters, which were brown and these covered most of the cheese surface. The cheese seemed to have melted but did not show the flow characteristics. PAC6.3 (with oil) cheeses showed the shreds to have completely fused and the cheese surface was covered with very large size blisters. Most of these blisters were brown in colour and the cheese lacked whiteness and gloss. Thus PAC6.3 (with/no oil) cheeses were not of acceptable quality.

PAC6.1 (no oil) cheeses showed a good melt and most of the shreds had disintegrated uniformly although some parts of cheese shreds had not fused completely and showed burning during baking. The PAC6.1 (no oil) cheeses showed formation of a few blisters of small size, which were brown. The cheese seemed to have melted and started flowing although not to a great extent (not beyond the pizza base). In comparison, PAC6.1 (with oil) cheese showed very good melt and fusion of cheese shreds, a few large blisters were formed but mostly the melted cheese was white. The patches of brown colour on the cheese surface would prove to be detrimental to its acceptability. The cheese had thoroughly melted and flowed all over the pizza base and had started to overflow. Thus PAC6.1 (with oil) cheeses had improved melt, flow and shred fusion characteristics compared to PAC6.3 (with/no oil) cheeses. There was an improvement in whiteness of PAC6.1 cheeses compared to PAC6.3 cheeses.

The PAC5.9 (no oil) cheeses showed that the cheese shreds had completely fused after melting. The cheeses also showed flow characteristics but did not overflow from the pizza base. Large regions on the cheese surface showed browning although the cheese was not burnt. The shreds of PAC5.9 (with oil) cheeses showed very good melt and fusion and the entire cheese had good flow characteristics. Large blisters were observed which had turned light brown during baking but most of the melted cheese surface was white and had a good gloss, which are desirable characteristics of a pizza cheese. The edges of the cheese showed some browning probably due to harsh conditions at the periphery of the pizza base.

Table 8.4.5 shows the mean and standard error for Hunter L, a and b values for control cheeses and cheeses made from milk pre-acidified using citric acid to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1 and PAC5.9 cheeses). The pizzas were made from cheeses applied with or without oil and stored for 20 d. Table 8.4.5 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

8.4.3.1.2 *Effect of pre-acidification on L, a and b values.*

The Hunter L^2 values for control, PAC6.3, PAC6.1 and PAC5.9 cheeses were similar. This indicates that L-values did not change for the fresh cheeses made with or without pre-acidification. Upon baking PAC6.3 (with/no oil) cheeses had significantly higher L^3 values compared to control (with/no oil), PAC6.1 (with/no oil) or PAC5.9 (with/no oil) cheeses. PAC6.1 (no oil) cheeses had the least L^3 values compared to control (with/no oil), PAC6.3 (with/no oil), PAC5.9 (with/no oil) and PAC6.1 (with oil) cheeses. PAC5.9 (with/no oil) cheeses had similar L^3 values as control (with/no oil) cheeses. When the pizzas were cooled PAC6.3 (with/no oil) cheeses had significantly higher L^4 values compared to control (with/no oil), PAC6.1 (with/no oil) or PAC5.9 (with/no oil) cheeses. The PAC6.1 (no oil) cheeses had the least L^4 values compared to control (with/no oil), PAC6.3 (with/no oil) or PAC5.9 (with/no

oil) cheeses and PAC6.1 (with oil) cheeses. PAC5.9 (with/no oil) cheeses had similar L^* values as control (with/no oil) cheeses. Thus measurements using Minolta chroma meter showed that PAC6.3 cheeses had better whiteness compared to PAC6.1, PAC5.9 and control cheeses after baking and also after cooling the pizzas. Differences between the whiteness measured using a chroma meter to that observed by naked eye could be due to selection of white areas on pizzas after the cheeses were baked to measure the L , a , and b values.

The a^2 values for control (with oil) cheeses were significantly higher than those for PAC6.3 (with oil) cheeses. The a^2 values for PAC6.1 and PAC5.9 (with oil) cheeses were similar to each other and were not significantly different to control and PAC6.3 (with oil) cheeses. The PAC6.1 (no oil), control (no oil) and PAC5.9 (no oil) cheeses had the highest a^2 values, while PAC6.3 (no oil) cheeses had the least a^2 values. Among the cheeses that were applied with oil and baked, the a^3 values of PAC6.3 cheeses were significantly higher than the control, PAC6.1 and PAC5.9 cheeses. The a^3 values for PAC6.1 (with oil) cheeses were similar to those for PAC5.9 (with oil) cheeses but significantly higher than the a^3 values for control (with oil) cheeses. Among the cheeses that were not applied with oil, the a^3 values for PAC6.3 and PAC6.1 cheeses were similar to a^3 values for PAC5.9 cheeses and higher than those for control cheeses. Upon cooling PAC6.3 (with oil) cheeses had the highest a^4 values followed by PAC6.1 (with oil) cheeses and PAC5.9 (with oil) cheeses and the least values were for control (with oil) cheeses. The a^4 values for PAC6.3 (with oil) cheeses were significantly higher than the PAC6.1 (with oil), PAC5.9 (with oil) and control (with oil) cheeses. The a^4 values for control (with oil) cheeses were significantly lower than those of PAC6.1 (with oil) cheeses and were similar to a^4 values for PAC5.9 (with oil) cheeses. Also the a^4 values for PAC6.1 (with oil) cheeses were similar to those for PAC5.9 (with oil) cheeses. The a^4 values for PAC6.3 (no oil), PAC6.1 (no oil) and PAC5.9 (no oil) cheeses were similar and significantly higher than the a^4 values of control (no oil) cheeses. Thus upon baking the pre-acidified cheeses did not

show any improvement in the redness or burning compared to control. Increase in pre-acidification from pH 6.1 to 5.9 did not markedly affect the cheese characteristics when baked and PAC6.3 cheeses showed increased browning which is not desirable.

The b^2 values of control (with oil) cheeses were similar to those for the PAC6.3 (with oil), PAC6.1 (with oil) and PAC5.9 (with oil) cheeses. Among the pre-acidified cheeses PAC6.3 (with oil) cheeses had the least b^2 values compared to PAC6.1 (with oil) and PAC5.9 (with oil) cheeses and the latter cheeses had similar b^2 values to each other. The b^2 values for control (no oil) cheeses were similar to those for the PAC6.3 (no oil), PAC6.1 (no oil) and PAC5.9 (no oil) cheeses. Among the pre-acidified cheeses that were not applied with oil, the b^2 values of PAC6.3 cheeses were significantly lower than those for PAC6.1 and PAC5.9 cheeses and the latter cheeses had similar b^2 values. The b^3 values of PAC6.3 (with oil) cheeses were significantly lower than those for control (with oil), PAC6.1 (with oil) and PAC5.9 (with oil) cheeses. The b^3 values of PAC6.1 (with oil) and PAC5.9 (with oil) cheeses were similar to each other and were significantly higher than those for control (with oil) cheeses indicating more yellow colour after the cheeses were baked. The b^3 values of control (no oil), PAC6.1 (no oil) and PAC5.9 (no oil) cheeses were similar and significantly higher than the b^3 values for PAC6.3 (no oil) cheeses. The b^4 values of PAC6.1 (with oil) and PAC5.9 (with oil) cheeses were similar and these values were significantly higher than for control (with oil) and PAC6.3 (with oil) cheeses. The control (with oil) cheeses had significantly higher b^4 values compared to PAC6.3 (with oil) cheeses. The b^4 values of PAC5.9 (no oil) cheeses were similar to those for PAC6.1 (no oil) cheeses but significantly higher than those for the control (no oil) and PAC6.3 (no oil) cheeses. Thus yellowness of PAC6.1 cheeses was significantly higher compared to PAC6.3 and PAC5.9 cheeses, which is desirable.

8.4.3.1.3 *Effects of application of oil on the shredded low fat pre-acidified cheeses on the Hunter L, a, and b values.*

Application of oil did not show any significant effect on L-values of fresh control, PAC6.3, PAC6.1 and PAC5.9 cheeses. After baking and cooling the L-values (L^3 and L^4) were not significantly different for control, PAC6.3, PAC6.1 and PAC5.9 cheeses that were either applied with oil or had no oil applied. The a^2 values for the fresh control, PAC6.3, PAC6.1 and PAC5.9 cheeses were not significantly affected by application of oil but after baking PAC6.1 (no oil) cheeses showed significantly higher a^3 values compared to PAC6.1 (with oil) cheeses. Thus application of oil decreased the burning of PAC6.1 cheeses. Control, PAC6.3 and PAC5.9 cheeses that were either applied with oil or were without oil showed similar a^3 values. Cooling after baking caused only significant decrease in a^4 values for PAC5.9 (with oil) cheeses compared to PAC5.9 (no oil) cheeses, which indicates less burning. There was no significant effect due to application of oil on control, PAC6.3 and PAC6.1 (no oil) cheeses. The b^2 , b^3 and b^4 values for the control, PAC6.3, PAC6.1 and PAC5.9 cheeses that were either applied with oil or were without oil were similar (statistically not different). Thus application of oil caused reduction in burning and browning of some cheeses like PAC6.1 whereas it did not affect the other cheeses.

8.4.3.1.4 *Effect of baking and cooling on Hunter L, a, and b values*

Cheeses that were applied with oil namely, control, PAC6.3, PAC6.1 and PAC5.9 cheeses showed increased L^3 values upon baking compared to their respective L^2 values and the L^4 values remained significantly higher (compared to their L^2 values) even after cooling the pizzas. The L^2 values of control (no oil) cheeses significantly increased upon baking (L^3 values) but significantly decreased upon cooling (L^4 values). The L^3 and L^4 values of PAC6.3 (no oil) cheeses were similar and significantly higher than their L^2 values. The PAC6.1 and PAC5.9 (no oil) cheeses did not show any significant change upon baking and upon cooling and their L^2 , L^3 and L^4 values remained similar. Thus baking improved the whiteness of

cheeses, which were applied with oil but showed an indefinite trend for cheeses that were not applied with oil. The a^2 values of control cheeses did not significantly change upon baking but were significantly higher (a^4 values) upon cooling. The a^4 values were significantly higher for the control (with oil) cheeses compared to a^2 and a^3 values of these cheeses. The a^2 values for control (no oil), PAC6.3 (with/no oil), PAC6.1 (with/no oil) and PAC5.9 (with/no oil) cheeses showed increased a^3 values due to baking and remained higher even after cooling. The a^3 values for control (no oil), PAC6.3 (with/no oil), PAC6.1 (with/no oil) and PAC5.9 with/no oil) cheeses were not significantly different to their a^4 values. Thus the pre-acidified (with/no oil) cheeses showed excessive browning after they were baked compared to fresh cheeses.

The b^2 values of control (with/no oil) cheeses increased upon baking (b^3 values) and the b -values increased (b^4 values) upon cooling for control (with oil) cheeses while the b^3 values remained similar to the b^4 values for the control (no oil) cheeses. The b^2 , b^3 and b^4 values for the PAC6.3 (with/no oil) cheeses were similar. The b -values for PAC6.1 (with/no oil) and PAC5.9 (with/no oil) cheeses showed similar trends upon baking and upon cooling as that for control (no oil) cheeses, with an increase due to baking and no further increase in b -values with cooling. Hence, yellowness of PAC6.1 and PAC5.9 cheeses, which is desirable, was increased after baking.

8.5 Conclusions

The control and pre-acidified cheeses had similar yield and the level of pre-acidification did not seem to affect the yield significantly ($p > 0.05$). PAC6.3 cheeses showed the lowest moisture, FDM and M:P contents and had a significantly higher protein content compared to control, PAC6.1 and PAC5.9 cheeses. PAC6.1 cheeses showed greater melt than control or PAC5.9 cheeses. PAC5.9 cheeses showed lower hardness values compared to PAC6.3 and PAC6.1 cheeses. Control cheeses were less hard than the pre-acidified cheeses except PAC5.9

cheeses at 2 d and 15 d, probably due to increase in time of starter culture activity during cheese making. The pre-acidified cheeses required prolonged storage to soften up. Pre-acidification was found to increase the cohesive forces within the cheese matrix while storage did not show a marked effect on this characteristic. Springiness values for all the cheeses except PAC6.1 cheeses increased with increase in storage time although a definite correlation could not be established between the level of pre-acidification and springiness values of the cheeses.

PAC6.1 cheeses showed good melt, shred fusion and flow of the melted cheeses while control and PAC6.3 cheeses did not show shred fusion or good melt and flow characteristics. Increase in pre-acidification to pH 5.9 did not markedly improve the melt and flow characteristics of PAC5.9 cheeses but only improved the whiteness of the cheese. PAC5.9 cheeses showed reduced melt and flow characteristics than PAC6.1 cheeses. Application of oil was necessary for PAC6.1 cheeses to achieve the desired pizza baked characteristics. PAC6.3 cheeses showed increased burning (a^4 values) and formed large brown blisters. These cheeses also had the least b values before baking and after baking while PAC6.1 and PAC5.9 cheeses had higher b values indicating more yellow coloured shreds. The L values of all cheeses were higher after they were baked and cooled than when measured fresh. The a and b values of cooled cheeses were not markedly different to hot baked and fresh cheeses which indicated that there was no undesirable browning during cooling. Application of oil reduced the a values measured for baked cheeses indicating that oil layer on the surface of the cheese shreds had a protective function. This enabled the cheeses applied with oil to show whiteness, melt and flow better than the no oil cheeses.

Overall, PAC6.1 cheeses showed improved melt, shred fusion and flow after baking on a pizza without any significant decrease in their yield. Thus controlled pre-acidification has been observed to be desirable and beneficial for the manufacture of ULFM cheeses.

Table 8.4.1 Yield, composition and meltability of control cheese and citric acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1, PAC5.9) cheeses, (n = 9).

	Control cheese	PAC6.3 cheese	PAC6.1 cheese	PAC5.9 cheese	
	Mean ± SE ¹	Mean ± SE ¹	Mean ± SE ¹	Mean ± SE ¹	p-value ²
Yield ³ (g/ 50 Kg)	4275.0 ± 175.0 ^a	4000.0 ± 11.5 ^a	4125.0 ± 25.0 ^a	4112.5 ± 12.5 ^a	0.1853
Moisture (%)	57.050 ± 0.48 ^a	53.174 ± 0.13 ^b	56.582 ± 0.08 ^a	56.154 ± 0.29 ^a	0.0000 [*]
Protein (%)	31.107 ± 0.51 ^b	35.093 ± 0.07 ^a	31.551 ± 0.72 ^b	31.832 ± 0.28 ^b	0.0000 [*]
FDM ⁴ (%)	13.978 ± 0.15 ^a	12.814 ± 0.04 ^b	13.820 ± 0.03 ^a	13.687 ± 0.09 ^a	0.0000 [*]
M:P ⁵	1.851 ± 0.04 ^a	1.515 ± 0.00 ^b	1.795 ± 0.04 ^a	1.753 ± 0.02 ^a	0.0000 [*]
Melt at 11d (mm)	51.885 ± 0.97 ^c	53.370 ± 0.59 ^{bc}	95.427 ± 5.13 ^a	40.970 ± 0.41 ^d	0.0000 [*]

¹Mean ± Standard error; ²ANOVA of means; ³n= 3; ⁴FDM = Fat in dry matter; ⁵M:P = Moisture: Protein; ^{a, b, c, d} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 8.4.2 Hardness values measured as Newton force (Mean \pm SE¹) for control and citric acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1, PAC5.9) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAC6.3 cheese	PAC6.1 cheese	PAC5.9 cheese	p-value ²
2	63.18 \pm 2.64 ^{a, D}	117.29 \pm 5.36 ^{a, A}	96.83 \pm 2.52 ^{a, BC}	81.23 \pm 4.70 ^{a, CD}	0.0000 [*]
8	27.32 \pm 3.34 ^{bc, D}	124.80 \pm 5.01 ^{a, A}	72.90 \pm 4.12 ^{bc, B}	47.82 \pm 4.17 ^{b, C}	0.0000 [*]
15	22.23 \pm 4.00 ^{c, C}	62.46 \pm 2.20 ^{b, A}	68.88 \pm 5.02 ^{c, A}	32.40 \pm 2.24 ^{c, BC}	0.0000 [*]
p-value ³	0.0000 [*]	0.0000 [*]	0.0004 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 8.4.3 Cohesiveness values (Mean \pm SE¹) for control cheese and citric acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1, PAC5.9) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAC6.3 cheese	PAC6.1 cheese	PAC5.9 cheese	p-value ²
2	0.687 \pm 0.02 ^{a, AB}	0.696 \pm 0.01 ^{a, AB}	0.642 \pm 0.02 ^{b, B}	0.722 \pm 0.01 ^{a, A}	0.0305 [*]
8	0.708 \pm 0.01 ^{a, A}	0.717 \pm 0.00 ^{a, A}	0.727 \pm 0.01 ^{a, A}	0.702 \pm 0.01 ^{a, A}	0.1182
15	0.690 \pm 0.00 ^{a, B}	0.717 \pm 0.01 ^{a, A}	0.710 \pm 0.00 ^{a, AB}	0.714 \pm 0.00 ^{a, A}	0.0038 [*]
p-value ³	0.0996	0.1934	0.0010 [*]	0.2614	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05).

Table 8.4.4 Springiness values (Mean \pm SE¹) measured in mm for control cheese and citric acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1, PAC5.9) cheeses, (n = 9) during refrigerated storage.

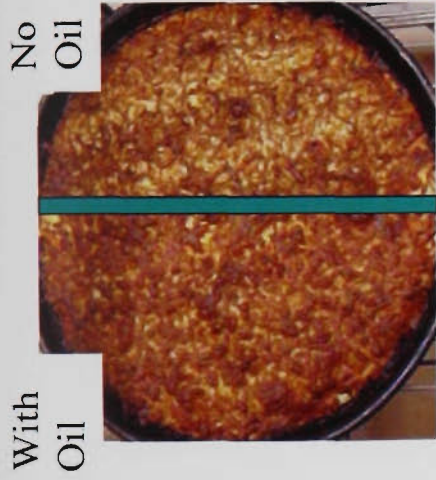
Storage (d)	Control cheese	PAC6.3 cheese	PAC6.1 cheese	PAC5.9 cheese	p-value ²
2	2.017 \pm 0.07 ^{b, AB}	2.089 \pm 0.06 ^{b, A}	1.800 \pm 0.10 ^{a, B}	1.833 \pm 0.06 ^{b, AB}	0.0157 [*]
8	2.317 \pm 0.07 ^{a, A}	1.578 \pm 0.06 ^{c, BC}	1.467 \pm 0.15 ^{a, C}	2.083 \pm 0.05 ^{ab, A}	0.0000 [*]
15	2.517 \pm 0.04 ^{a, A}	2.456 \pm 0.08 ^{a, A}	1.750 \pm 0.08 ^{a, B}	2.214 \pm 0.09 ^{a, A}	0.0000 [*]
p-value ³	0.0001 [*]	0.0000 [*]	0.1203	0.0044 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 8.4.5 Mean \pm SE¹ (n = 9) Hunter L a b-values of control cheese and citric acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAC6.3, PAC6.1, PAC5.9) cheeses, applied with or without canola oil and stored for 20 d at refrigerated temperature, measured fresh² (before baking), after baking³ and after cooling⁴ to room temperature.

Control cheese		PAC6.3 cheese			PAC6.1 cheese			PAC5.9 cheese		
Hunter	With oil	No Oil	With oil	No Oil	With oil	No Oil	With oil	With oil	No Oil	p-value ⁵
L ²	59.53 \pm 1.74 ^{b, A}	62.48 \pm 0.50 ^{c, A}	60.71 \pm 0.64 ^{b, A}	61.65 \pm 0.65 ^{b, A}	59.74 \pm 0.48 ^{b, A}	59.96 \pm 1.14 ^{a, A}	59.24 \pm 1.11 ^{b, A}	62.14 \pm 0.67 ^{a, A}	62.14 \pm 0.67 ^{a, A}	0.1138
L ³	73.68 \pm 0.36 ^{a, A}	72.49 \pm 0.26 ^{a, CDEF}	97.44 \pm 2.15 ^{a, A}	91.92 3.10 ^{a, A}	63.69 \pm 1.04 ^{a, FG}	56.63 \pm 2.39 ^{a, G}	69.08 \pm 1.20 ^{a, A}	65.37 \pm 1.18 ^{a, A}	65.37 \pm 1.18 ^{a, A}	0.0000*
L ⁴	71.08 \pm 0.51 ^{a, A}	69.98 \pm 0.67 ^{b, CDEF}	94.00 \pm 2.20 ^{a, A}	87.71 \pm 2.23 ^{a, A}	61.19 \pm 1.05 ^{ab, A}	59.41 \pm 1.28 ^{a, G}	67.65 \pm 1.15 ^{a, A}	63.86 \pm 1.65 ^{a, A}	63.86 \pm 1.65 ^{a, A}	0.0000*
p-value ⁶	0.0000*	0.0000*	0.0000*	0.0000*	0.0222*	0.3548	0.0000*	0.2136	0.2136	
a ²	0.06 \pm 0.18 ^{bc, A}	-0.40 \pm 0.15 ^{b, ABC}	-1.00 \pm 0.12 ^{b, A}	-1.30 \pm 0.09 ^{b, C}	-0.29 \pm 0.28 ^{b, A}	0.02 \pm 0.32 ^{b, A}	-0.33 \pm 0.17 ^{b, A}	-0.81 \pm 0.24 ^{b, A}	-0.81 \pm 0.24 ^{b, A}	0.0000*
a ³	-0.02 \pm 0.12 ^{c, E}	3.00 \pm 0.45 ^{a, DE}	14.68 \pm 0.82 ^{a, A}	13.64 \pm 0.45 ^{a, A}	7.53 \pm 1.26 ^{a, BCD}	14.93 \pm 1.78 ^{a, A}	4.90 \pm 1.33 ^{a, CDE}	10.45 \pm 1.07 ^{a, A}	10.45 \pm 1.07 ^{a, A}	0.0000*
a ⁴	1.36 \pm 0.35 ^{a, E}	2.69 \pm 0.72 ^{a, DE}	14.58 \pm 0.29 ^{a, A}	12.47 \pm 0.44 ^{a, A}	9.58 \pm 0.81 ^{a, BC}	11.34 \pm 1.66 ^{a, AB}	5.79 \pm 1.21 ^{a, CDE}	11.52 \pm 1.35 ^{a, A}	11.52 \pm 1.35 ^{a, A}	0.0000*
p-value ⁶	0.0006*	0.0003*	0.0000*	0.0000*	0.0000*	0.0000*	0.0017*	0.0000*	0.0000*	
b ²	17.11 \pm 0.60 ^{c, BCD}	18.41 \pm 0.36 ^{b, ABC}	15.14 \pm 0.27 ^{a, D}	16.69 \pm 0.54 ^{a, A}	17.92 \pm 0.29 ^{b, A}	20.24 \pm 0.31 ^{b, A}	18.27 \pm 0.56 ^{b, A}	18.84 \pm 0.41 ^{b, A}	18.84 \pm 0.41 ^{b, A}	0.0000*
b ³	25.59 \pm 0.59 ^{b, B}	30.87 \pm 1.16 ^{a, AB}	16.69 \pm 1.87 ^{a, A}	14.03 0.74 ^{a, D}	35.88 \pm 1.39 ^{a, A}	31.59 \pm 2.22 ^{a, AB}	34.97 \pm 1.78 ^{a, A}	36.32 \pm 0.65 ^{a, A}	36.32 \pm 0.65 ^{a, A}	0.0000*
b ⁴	28.30 \pm 0.67 ^{a, C}	29.25 \pm 1.01 ^{a, BC}	15.33 \pm 0.51 ^{a, E}	15.73 \pm 0.90 ^{a, A}	34.63 \pm 1.36 ^{a, AB}	32.01 \pm 0.88 ^{a, A}	34.40 \pm 0.73 ^{a, AB}	36.47 \pm 0.57 ^{a, A}	36.47 \pm 0.57 ^{a, A}	0.0000*
p-value ⁶	0.0000*	0.0000*	0.5779	0.0768	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	

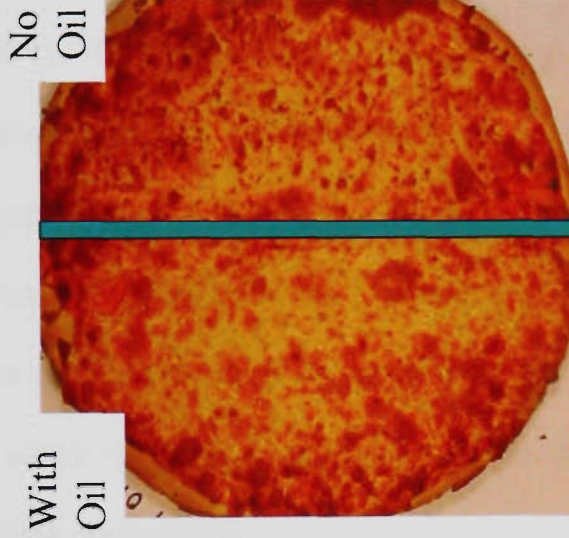
¹Mean \pm Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D, E, F, G} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).



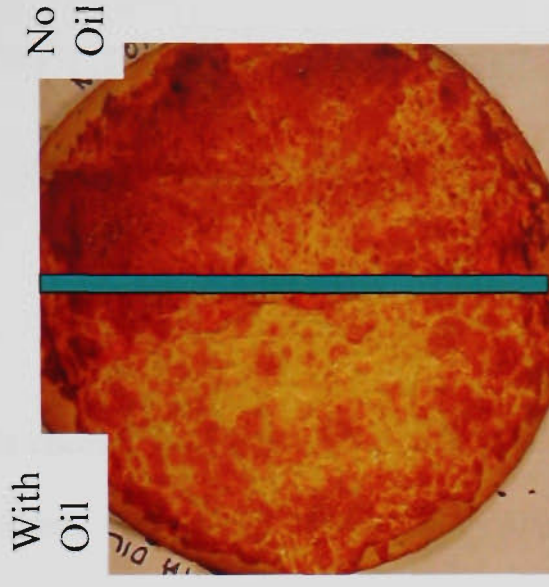
Control cheese



PAC6.3 cheese



PAC6.1 cheese



PAC5.9 cheese

Figure 8.4.1 Pizzas made from control cheese and PAC6.3, PAC6.1 and PAC5.9 cheeses after storing for 20 d.

9.0 EFFECT OF PRE-ACIDIFICATION OF MILK USING ACETIC ACID ON ULTRA LOW FAT MOZZARELLA CHEESE CHARACTERISTICS

9.1 Introduction

Low moisture part-skim mozzarella cheeses have been extensively studied for improving the functional characteristics of the cheeses through use of modified manufacturing techniques such as pre-acidification (Metzger *et al.*, 2000), and more recently through post-manufacture change in pH (Kindstedt *et al.*, 2001). The conditions of pH and the high temperatures during stretching have been shown to cause aggregation of para-casein (Kimura *et al.*, 1992). Such para-casein aggregates when subjected to the shear forces during stretching, show stringiness and upon baking show good melt and flow characteristics. The extent to which these functional characteristics are developed in the mozzarella cheeses is dictated by the amount of hydration of para-casein (Creamer, 1985), which depends on the extent of solubilisation of colloidal calcium (Guinee *et al.*, 2000) from the casein micelles.

Previous studies in our laboratory (Bhaskaracharya and Shah, 2001b) showed that ULFM cheeses had a significantly higher protein content than full fat mozzarella cheese. The increase in protein content also causes an increase in the amount of calcium that is bound to casein. Thus ULFM cheeses have higher colloidal calcium content. Pre-acidification helps to remove some of the colloidal calcium, thus allowing the para-casein to become susceptible to hydration. Creamer and Waugh, (1966) showed that with an increase in concentration of calcium there was an increase in bonding of α_s -casein monomers along with a decrease in the solvation characteristic. Hydration of casein was found to increase at reduced calcium concentrations. Also studies by Zittle (1957) have shown that the divalent form of phosphates

are mainly bound to casein and increase in phosphated casein helps in binding of calcium to the casein and thus increases stability of casein in the milk system.

Studies have shown (Keller *et al.*, 1974; Kindstedt and Guo, 1997) that direct acidified curd has lower total calcium content and can be stretched at higher pH ~5.6 whereas curd formed by lactose fermentation using starter bacteria has higher total calcium content and requires lower pH <~5.3 to obtain smooth curd when subjected to high temperatures during stretching. Whey draining has been studied extensively by several researchers including Yun *et al.* (1995) and Holsinger *et al.* (1995), to ascertain the effect of pH at whey draining on the textural and functional characteristics of the mozzarella cheeses. The calcium content of the cheeses was reduced when the pH of whey draining was lowered to ~6.0. Another method to reduce the total calcium was developed by Nilson and LaClair (1976) by stirring the curd after whey draining in warm water until the pH decreased to 5.55- 5.60 after which the water was drained and curd stretched. This method has been reported to increase the moisture retained in the cheese compared to a normal dry cheddared mozzarella cheese. This could be due to increased solubilized calcium leaching into the water (Barbano, 1999).

Glucono- δ -lactone (GdL) (Joshi *et al.*, 2002) lowered the calcium content of the mozzarella cheese and decreased the elastic and viscous moduli. GdL is a white powder, which when added to aqueous solution dissolves rapidly and subsequently slowly hydrolyses into gluconic acid. This produces a gentle acidification similar to that produced by lactic acid bacteria by fermentation of lactose to lactic acid. The GdL exists in the solution at an equilibrium state with the gluconic acid and this equilibrium is affected by concentration of GdL and temperature and pH of solution. Mozzarella cheese made from milk containing 2-4% fat which were given direct acidification with either acetic acid or hydrochloric acid showed that acetic acid based cheeses had three times higher melt and better stretch quality than hydrochloric acid

based cheeses (Shukla and Ladkani, 1989a). Further the manufacturing steps were optimised to make direct acid mozzarella cheese from buffalo milk (Shukla and Ladkani, 1989b) using acetic acid or hydrochloric acid. When mozzarella cheeses were made by direct acidification with acetic acid from the ultrafiltered whole milk retentates, Fernandez and Kosikowski (1986) found that the moisture content was decreased in such cheeses and their composition was similar to that of low moisture mozzarella (Fernandez, 1985).

9.2 Aims

The aims of this study were to manufacture ULFM cheeses from cheese milk pre-acidified with acetic acid to pH of 6.3, 6.1 or 5.9 and further using *Lactobacillus helveticus* and *Streptococcus thermophilus* to ripen the milk for mozzarella cheese making, to analyse the cheeses for their composition, textural and functional characteristics, and to determine the pH of pre-acidification using acetic acid which provides the best textural and functional characteristics.

The previous experiments showed that acetic acid or citric acid might be used as an acidifying agent to improve characteristics of ULFM cheeses. In this study acetic acid pre-acidification to pH 6.1 and 5.9 were carried out and characteristics of ULFM cheeses were observed.

9.3 Materials and methods

9.3.1 Cheese making

Cheese milk was standardised to contain 0.5% fat and 3.0% casein as described in section 3.2.6.1. The milk mixed with *Streptococcus thermophilus* (TS2000) and *Lactobacillus helveticus* (LB1) starter cultures and further pre-acidified using acetic acid to pH 6.3, 6.1 or 5.9 as described in section 3.2.6.3. ULFM cheeses containing ~6.0% fat were manufactured by

following the steps described in section 3.2.6.2. Three batches were made of control (without any pre-acidification) and pre-acidified cheeses using dry salting method and the cheeses were stored at 4 °C.

9.3.2 Analysis of cheeses

The yield of cheeses was measured and the cheese samples were prepared as described in section 3.2.9 for estimation of composition, melt, texture characteristics (section 3.2.8) and for pizza bake characteristics (section 3.2.7 and 3.2.24). The composition including moisture/total solids was estimated using the method described in section 3.2.20, fat content (section 3.2.22) and protein content (section 3.2.25) and M: P were determined. Textural characteristics including hardness, cohesiveness and springiness were measured by compressing cylindrical cheese samples to 50% height as described in section 3.2.23 using an Instron according to the method of Bhaskaracharya (2000) after storing the cheeses for 2, 8, 15, 22 and 29 d. Meltability test was carried out for cheeses stored for 11, 18 and 25 d as described in section 3.2.15 using 10.0 g of grated cheese sample heated in glass tube at 110°C for 100 min similar to the method used by Poduval and Mistry (1999). Pizza-bake test was carried out (section 3.2.24) at 20 d of storage and the colour, size of blisters and their distribution upon baking were assessed.

9.3.3 Statistical analysis

Statistical analysis of the results was carried out using StatPro[®] software on Microsoft[®] excel. The sample sizes, sample means, sample standard deviations and standard error were calculated. The means from at least two populations were compared. The analysis of variance table was prepared and two sources of variation: the variation *within* each population and variation *among* sample means from the different populations was compared. If the latter variation was large relative to the former, as was measured using an F test, this was deemed to

be evidence of differences between the population means. The p-value obtained from this table and the confidence intervals (at 95% confidence level) for all the differences between pairs of means were considered for describing the statistical differences and their significance in the results tables. A small p-value was a result of large differences in population means and the confidence intervals that did not include zero were taken to indicate that the means that were compared were not equal.

9.4 Results and discussion

9.4.1 Yield and composition

The yield and composition including moisture, protein, FDM and M:P contents for control, PAA6.3, PAA6.1 and PAA5.9 cheeses are shown in Table 9.4.1. The yield of the cheeses decreased ($p<0.05$) with increase in level of pre-acidification i.e. control cheeses made without pre-acidification had the highest yield and was followed by PAA6.3, PAA6.1 and PAA5.9 cheeses in decreasing order. The moisture content of control, PAA6.3 and PAA6.1 cheeses were similar (statistically not different) and significantly higher than PAA5.9 cheeses. The protein content of PAA5.9 cheeses was significantly higher than control, PAA6.3 and PAA6.1 cheeses. Similarly, the FDM and M:P contents of control, PAA6.3 and PAA6.1 cheeses were statistically similar and significantly higher ($p<0.05$) than those for PAA5.9 cheeses. These results indicate that increase in level of pre-acidification with acetic acid caused a decrease in moisture retained per unit of protein.

9.4.2 Texture characteristics

Table 9.4.2 shows the hardness values measured at 2, 8, 15, 22 and 29 d of storage for control, PAA6.3, PAA6.1 and PAA5.9 cheeses. The hardness values for control, PAA6.3, PAA6.1 and PAA5.9 cheeses were similar at 2, 22 and 29 d of refrigerated storage. But PAA5.9 cheeses

showed excessive hardness ($p<0.05$) at 8 and 15 d of storage compared to control, PAA6.3 and PAA6.1 cheeses, which had similar (statistically not different) hardness values. The hardness values for control cheeses decreased during storage and values at 2 d were significantly higher compared to those at 8, 15, 22 or 29 d of storage. The hardness values did not significantly change after 8 d for control cheeses. The pre-acidified cheeses showed decreased hardness with storage. The PAA6.3 cheeses had similar hardness values at 2 d and 8 d and the values decreased at 15 d and were the least at 22 d of storage. The PAA6.1 cheeses had similar hardness values at 2 and 8 d of storage and lower values at 15, 22 and 29 d. The lowest hardness values for PAA6.1 cheeses were observed at 29 d of storage. The PAA5.9 cheeses had the highest hardness values at 8 d, which decreased significantly after 22 d with the lowest values, recorded at 29 d of storage. The delay in the decrease of hardness seemed to be affected by the level of pre-acidification. Increased level of pre-acidification caused a decrease in ripening time that was available for the starter cultures to grow and cause proteolysis in the cheeses. Also increased pre-acidification causes reduction in total calcium content of the cheeses as discussed in 6.2.4 and suggested by Metzger *et al.* (2000). The decreased calcium content could have reduced the measured hardness.

Table 9.4.3 shows the cohesiveness values for control, PAA6.3, PAA6.1 and PAA5.9 cheeses measured at 2, 8, 15, 22 and 29 d of refrigerated storage. The cohesiveness values for control cheeses were significantly higher compared to those for PAA5.9 cheeses while PAA6.3 and PAA6.1 cheeses had similar values as control and PAA5.9 cheeses at 2 d of storage. The cohesiveness measured at 8, 15, 22 and 29 d for control, PAA6.3, PAA6.1 and PAA5.9 cheeses were similar (statistically not different). Control, PAA6.3 and PAA6.1 cheeses did not show any significant change in cohesiveness values due to storage, while PAA5.9 cheeses had significantly lower cohesiveness values at 2 d, which increased after 8 d of storage and remained similar till 29 d.

The mean and standard error for springiness values of control, PAA6.3, PAA6.1 and PAA5.9 cheeses measured at 2, 8, 15, 22 and 29 d of refrigerated storage are shown in Table 9.4.4. The control cheeses had similar springiness values as PAA6.3 and PAA6.1 cheeses while the PAA5.9 cheeses had significantly higher ($p<0.05$) springiness possibly due to their higher protein and lower moisture contents compared to those of control and PAA6.1 cheeses after 2 d. At 8, 15, 22 and 29 d of storage the control and pre-acidified cheeses showed similar springiness. During storage control cheeses showed similar springiness values at 2 d and 8 d followed by an increase when measured at 15 d and the values remained similar after 15 d till 29 d. PAA6.3 cheeses did not show any significant effect due to storage. Initially, at 2 d the PAA6.1 cheeses had low springiness values, which became significantly higher at 22 d but at 8, 15 and 29 d the springiness values were intermediate between those at 2 d and 22 d and were statistically similar. The PAA5.9 cheeses showed higher springiness values at 2 d, which increased during storage at 8, 15 and 22 d. The springiness values measured for PAA5.9 cheeses at 8 d was similar to that measured at 15, 22 and 29 d but the values were least at 15 d. At 29 d the springiness values of PAA5.9 cheeses were similar to the values measured at 2, 8, 15 or 22 d.

The springiness values of control and pre-acidified cheeses increased up to 15 or 22 d followed by a decrease at 29 d. The variation in springiness values could be due to the dynamic interactions within the cheeses. These dynamic interactions would comprise changes leading to stabilisation of the cheese constituents during the first 22 d after cheese manufacture. During this period there is a transfer of intact caseins and calcium to the serum phase while moisture and sodium are transferred to the casein micelles as described by Kindstedt and Guo (1997). These interactions cause modifications in the cheese microstructure such as swelling of the protein strands and reduction in diameter of serum channels within the cheese matrix

(McMahon *et al.*, 1999; Guo *et al.*, 1998). These changes at the micro-structural level in ULFM cheeses could reduce brittleness similar to that observed in a dry product and the serum being available to act as a shock absorber in the cheeses. Further studies need to be carried out to understand the dynamics of micro-structural changes to the textural characteristics of mozzarella cheeses.

9.4.3 Functional characteristics

9.4.3.1 Meltability

In Table 9.4.5 the results from the meltability test conducted on control, PAA6.3, PAA6.1 and PAA5.9 cheeses at 11, 18 and 25 d of refrigerated storage are shown. Control cheeses showed the highest ($p<0.05$) melt distance at 11, 18 and 25 d compared to the pre-acidified (PAA6.3, PAA6.1 and PAA5.9) cheeses, while PAA5.9 cheeses showed the least ($p<0.05$) melt distance at 11, 18 and 25 d. The meltability of the cheeses seemed to decrease with increase in pre-acidification at 11 and 25 d. The control cheeses showed significantly more melt than all pre-acidified cheeses at 11 d. PAA6.3 cheeses had similar meltability as PAA6.1 cheeses while PAA6.1 cheeses had higher meltability than PAA5.9 cheeses at 11 d. The control cheeses showed higher meltability than the pre-acidified cheeses at 18 d. Among the pre-acidified cheeses PAA6.1 cheeses had significantly higher melt compared to PAA6.3 and PAA5.9 cheeses at 18 d. The PAA6.3 cheeses had similar melt as PAA5.9 cheeses and the latter had the least meltability among all cheeses at 18 d. At 25 d, the control cheeses showed the highest melt compared to PAA6.3, PAA6.1 and PAA5.9 cheeses. The pre-acidified (PAA6.3, PAA6.1 and PAA5.9) cheeses showed similar meltability at 25 d of storage.

During storage, control and PAA6.1 cheeses showed similar meltability values at 11, 18 and 25 d while PAA6.3 cheeses had higher meltability at 11 d, which decreased by 18 d. The PAA6.3 cheeses had intermediate meltability at 25 d, which was similar to that measured at 11 and 18

d. The PAA5.9 cheeses showed an initial low melt characteristic at 11 d, which remained similar at 18 d but had increased at 25 d. Only the meltability measured for PAA5.9 cheeses at 11 d and 25 d were significantly different.

9.4.3.2 Pizza bake analysis

9.4.3.2.1 Physical properties of blister formation and burning as observed with the naked eye on 20 d old ULFM cheeses made from milk pre-acidified using acetic acid to pH of 6.3, 6.1 and 5.9 and control cheeses.

The control (no oil) cheeses showed (Figure 9.4.1) most of the cheese shreds as intact and had not melted during baking. These cheese shreds were brown, had burnt appearance and the cheese did not melt and flow on the pizza base. Very few blisters were formed and these were small in size. The control (with oil) cheeses showed almost complete shred fusion and some melting of the cheese. There were a few large blisters (burnt) and many small blisters (also burnt) of the control (with oil) cheeses. Overall, the control (with oil) cheeses showed some improvement in shred fusion due to application of oil, but both control (with oil) and control (no oil) cheeses did not show any whiteness, rather they were burnt and had not melted and flowed on the pizza base during baking.

PAA6.3 (no oil) cheeses had melted on the pizza base during baking and most of the cheese shreds had disintegrated. The cheeses also showed some flow but due to scorching most of the melted cheese surface was burnt. The cheese formed large blisters at the periphery of the pizza base while small blisters were formed in the centre. The differences in the blister sizes could be due to the variations in air temperature and air current in the conventional oven during baking. In comparison, PAA6.3 (with oil) cheeses showed excellent shred fusion, melted completely and started to flow. The cheese showed some blister formation and these blisters were darker at the periphery compared to the ones in the middle. The cheese showed glossiness and whiteness but due to the browning of the blisters it was not considered to be acceptable.

PAA6.1 (no oil) cheeses had melted completely and flowed on the pizza base. Very large blisters were formed at the edges of the pizza base, which were scorched. In the middle of the pizza the cheese showed whiteness with speckles of brown blisters. The shreds of PAA6.1 (with oil) cheese had completely melted, fused and there was a very good flow of the cheese on the pizza base. The edges of the cheese formed large blisters, which showed burning. In the middle of the pizzas the cheeses showed a shiny surface similar to a full fat mozzarella cheese but was whiter due to lack of fat.

PAA5.9 (no oil) cheeses had melted on the pizza base during baking and most of the cheese shreds had disintegrated. The cheeses showed scorching of the melted cheese although some flow characteristics could be observed. The cheese formed large blisters at the periphery of the pizza base while small blisters were formed in the centre. The differences in the blister sizes could be due to the variations in air temperature and air current in the conventional oven during baking as stated earlier. PAA5.9 (with oil) cheeses showed some browning of the blisters that were formed during baking. The cheese shreds had completely melted and fused. The cheese showed good flow characteristics on the pizza base. PAA5.9 (with oil) cheeses showed slightly increased browning (more uniform through out the melted cheese) compared to PAA6.1 (with oil) cheeses and the edges were also burnt.

Overall, PAA6.1 cheeses whether applied with or not, showed the best pizza bake characteristics of a good melt, shred fusion, flow and fewer brown blisters in comparison with PAA6.3 and PAA5.9 cheeses.

Table 9.4.6 shows the Hunter L, a, and b values for fresh, baked and cooled control, PAA6.3, PAA6.1 and PAA5.9 cheeses applied with or without oil and stored for 20 d. Table 9.4.6 shows

the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

9.4.3.2.2 *Effect of acetic acid pre-acidification on Hunter L, a, and b values*

The L^2 values of control (with/no oil), PAA6.3 (with/no oil), PAA6.1 (with/no oil) and PAA5.9 (with/no oil) cheeses were similar and showed that pre-acidification did not affect the whiteness of fresh cheeses. The L^3 and L^4 values of control (with/no oil), PAA6.3 (with/no oil) and PAA6.1 (with/no oil) and PAA5.9 (with oil) cheeses were similar and higher to the respective values for PAA5.9 (no oil) cheeses. The a^2 values for control (with oil) cheeses were highest while those for PAA6.3 (no oil) were the least. The a^2 values for the control (with oil) and PAA6.1 (with oil) cheeses were similar (statistically not different) but the formers values were significantly higher than those for PAA6.3 (with oil) and PAA5.9 (with oil) cheeses. Similarly the PAA6.3 (with oil) cheeses had similar a^2 values as that for PAA5.9 (with oil) cheeses. Among the cheeses that were not applied with oil control, PAA6.1 and PAA5.9 cheeses had similar a^2 values, which were significantly higher than those for PAA6.3 cheeses.

Upon baking the a^3 values for control (with oil) cheeses were found to be similar to those for PAA6.1 (with oil) and PAA5.9 (with oil) cheeses. Only the PAA6.3 (with oil) cheeses showed lower a^3 values compared to PAA5.9 (with oil) cheeses. Among the cheeses the PAA5.9 (no oil) cheeses showed significantly higher a^3 values compared to the control (no oil), PAA6.3 (no oil) and PAA6.1 (no oil) cheeses. The latter cheeses had similar a^3 values. Among the cooled cheeses PAA6.3 (with oil) cheeses had the least ($p < 0.05$) a^4 values and the PAA5.9 (no oil) cheeses had the highest a^4 values. The PAA5.9 (no oil) cheeses had significantly higher a^4 values compared to the other cheeses including control and pre-acidified cheeses applied with or without oil.

The b^2 values for control and pre-acidified cheeses were similar. After baking, control (with oil) cheeses showed the least b^3 values while the PAA5.9 (with/no oil) cheeses had the highest b^3 values. The control (with oil) cheeses had significantly lower b^3 values compared to PAA5.9 (with oil) cheeses but were similar to the b^3 values of PAA6.3 (with oil) and PAA6.1 (with oil) cheeses. The PAA6.3 (with oil) cheeses had similar b^3 values as the control (with oil), PAA6.1 (with oil) and PAA5.9 (with oil) cheeses. The PAA6.1 (with oil) cheeses had significantly lower b^3 values compared to those for PAA5.9 (with oil) cheeses. All the pre-acidified and control cheeses baked on the pizza bases that were without oil, showed similar b^3 values. Among the cheeses that were applied with oil, control and pre-acidified cheeses showed similar b^4 values and only PAA6.1 cheeses had significantly lower values compared to PAA5.9 cheeses. The control (no oil) and all the pre-acidified cheeses that were without oil had similar b^4 values.

9.4.3.2.3 *Effect of application of oil on the Hunter L, a, and b values of pre-acidified cheeses*

The L^2 values for fresh control, PAA6.3, PAA6.1 and PAA5.9 cheeses were similar showing no effect of application of oil. The L^3 and L^4 values for control, PAA6.3 and PAA6.1 cheeses were also similar and had no effect of application of oil. Only the PAA5.9 (no oil) cheeses showed significantly lower L^3 and L^4 values compared to PAA5.9 (with oil) cheeses. The a^2 values for fresh control, PAA6.3, PAA6.1 and PAA5.9 cheeses were similar showing no effect of application of oil. The a^3 and a^4 values for control, PAA6.3 and PAA6.1 cheeses were also similar and had no effect of application of oil. Only the PAA5.9 (no oil) cheeses showed significantly higher a^3 and a^4 values compared to PAA5.9 (with oil) cheeses. The b-values for fresh, baked and cooled control, PAA6.3, PAA6.1 and PAA5.9 cheeses did not show a significant effect due to application of oil.

9.4.3.2.4 Effect of baking and cooling on the Hunter L, a, and b values

The L^2 values showed an increase ($p < 0.05$) upon baking for control (with/no oil), PAA6.3 (with/no oil), PAA6.1 (with/no oil) and PAA5.9 (with oil) cheeses and these L^3 values remained similar to the L^4 values for the respective cheeses. The control (no oil) cheeses showed a significant decrease in L^4 values compared to their L^3 values. PAA5.9 (no oil) cheeses showed no effect of baking or cooling and the L^2 , L^3 and L^4 values were similar. The a^2 values increased upon baking and further increased with cooling for control (with/no oil), PAA6.3 (with/no oil), PAA6.1 (no oil) and PAA5.9 (with/no oil) cheeses. Only PAA6.1 (with oil) cheeses showed similar values and thus no effect of baking on the a^2 , a^3 and a^4 values. The b-values also significantly increased due to baking and further upon cooling the b^4 values remained similar to the b^3 values for control (no oil), PAA6.3 (with/no oil), PAA6.1 (with/no oil) and PAA5.9 (with/no oil) cheeses. Only the control (with oil) cheeses showed increase in b^4 values due to cooling compared to b^3 values.

9.5 Conclusions

Pre-acidification using acetic acid decreased the yield of the cheeses and the yield also decreased with increase in level of pre-acidification. PAA5.9 cheeses had the lowest moisture content, the highest protein content, and showed higher hardness values (except at 2 d) compared to control, PAA6.3 and PAA6.1 cheeses. All the cheeses showed a decrease in hardness values during storage. The hardness values of ULFM cheeses showed an inverse relationship to their level of pre-acidification. Cohesiveness values for control, PAA6.3 and PAA6.1 cheeses were similar while PAA5.9 cheeses had lower cohesiveness values compared to control cheeses. Storage did not seem to affect this characteristic for control, PAA6.3 and PAA6.1 cheeses but increased during storage for PAA5.9 cheeses. The control and pre-acidified cheeses showed similar springiness values. During storage the springiness values of

the cheeses increased up to 15 or 22 d and then decreased which could be due to the dynamic interactions within the cheese matrix.

Control cheeses had higher meltability compared to the pre-acidified cheeses. Meltability of pre-acidified cheeses showed a negative correlation with the level of pre-acidification and storage period. All the pre-acidified cheeses showed proper shred fusion, melt and flow characteristics when baked. PAA6.1 cheeses showed increased whiteness during baking with very little browning and were better than a control cheese. PAA6.3 and PAA5.9 cheeses showed more browning than PAA6.1 cheeses. Control cheeses did not show proper melt and flow characteristics and such cheeses had increased browning. Application of oil improved functionality of all the cheeses.

Overall, there was an improvement in some of the characteristics of the ULFM cheeses due to pre-acidification in comparison with control cheeses. At the same time the yield of pre-acidified cheeses decreased which is a concern. Application of oil to ULFM cheese shreds improves their pizza bake performance. PAA6.1 cheeses were observed to have the improvements desired.

Table 9.4.1 Yield and composition (Mean \pm SE¹) of control cheese and acetic acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAA6.3, PAA6.1, PAA5.9) cheeses, (n = 9).

	Control cheese	PAA6.3 cheese	PAA6.1 cheese	PAA5.9 cheese	p-value ²
Yield ³ (g/ 50 Kg)	4275.0 \pm 175.0 ^a	4012.5 \pm 12.5 ^{ab}	3932.5 \pm 127.5 ^{ab}	3522.5 \pm 52.5 ^b	0.0379 [*]
Moisture (%)	57.050 \pm 0.48 ^a	57.590 \pm 0.14 ^a	56.410 \pm 0.48 ^a	54.635 \pm 0.11 ^b	0.0001 [*]
Protein (%)	31.107 \pm 0.51 ^b	31.430 \pm 0.11 ^b	31.782 \pm 0.28 ^b	34.561 \pm 0.24 ^a	0.0000 [*]
FDM ⁴ (%)	13.978 \pm 0.15 ^a	14.148 \pm 0.05 ^a	13.773 \pm 0.15 ^a	13.226 \pm 0.03 ^b	0.0001 [*]
M:P ⁵	1.851 \pm 0.04 ^a	1.832 \pm 0.01 ^a	1.790 \pm 0.03 ^a	1.582 \pm 0.01 ^b	0.0000 [*]

¹Mean \pm Standard error; ²ANOVA of means; ³n= 3; ⁴FDM = Fat in dry matter; ⁵M:P = Moisture: Protein; ^{a, b} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 9.4.2 Hardness values (Mean \pm SE¹) measured in Newton force for control cheese and acetic acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAA6.3, PAA6.1, PAA5.9) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAA6.3 cheese	PAA6.1 cheese	PAA5.9 cheese	p-value ²
2	63.18 \pm 2.64 ^{a, A}	51.04 \pm 2.87 ^{a, A}	59.83 \pm 5.92 ^{a, A}	47.23 \pm 5.24 ^{bcd, A}	0.0803
8	27.32 \pm 3.34 ^{cde, D}	48.00 \pm 4.08 ^{a, BCD}	44.65 \pm 1.84 ^{abc, CD}	77.55 \pm 6.37 ^{a, A}	0.0000*
15	22.23 \pm 4.00 ^{de, D}	31.32 \pm 4.73 ^{bcd, BCD}	30.82 \pm 2.45 ^{cd, CD}	62.28 \pm 7.20 ^{ab, A}	0.0001*
22	32.90 \pm 3.90 ^{bcd, A}	18.17 \pm 1.41 ^{d, A}	32.30 \pm 5.36 ^{bcd, A}	33.17 \pm 3.35 ^{cd, A}	0.1880
29	20.88 \pm 2.16 ^{e, A}	19.77 \pm 3.24 ^{cd, A}	22.80 \pm 3.47 ^{d, A}	27.40 \pm 2.67 ^{d, A}	0.3388
p-value ³	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

Table 9.4.3 Cohesiveness values (Mean \pm SE¹) for control cheese and acetic acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAA6.3, PAA6.1, PAA5.9) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAA6.3 cheese	PAA6.1 cheese	PAA5.9 cheese	p-value ²
2	0.687 \pm 0.02 ^{a, A}	0.670 \pm 0.01 ^{a, AB}	0.657 \pm 0.03 ^{a, AB}	0.578 \pm 0.04 ^{b, B}	0.0348 [*]
8	0.708 \pm 0.01 ^{a, A}	0.660 \pm 0.01 ^{a, A}	0.690 \pm 0.01 ^{a, A}	0.660 \pm 0.02 ^{ab, A}	0.2150
15	0.690 \pm 0.00 ^{a, A}	0.683 \pm 0.01 ^{a, A}	0.693 \pm 0.01 ^{a, A}	0.698 \pm 0.00 ^{a, A}	0.4310
22	0.668 \pm 0.01 ^{a, A}	0.682 \pm 0.01 ^{a, A}	0.696 \pm 0.00 ^{a, A}	0.697 \pm 0.01 ^{a, A}	0.0601
29	0.678 \pm 0.01 ^{a, A}	0.672 \pm 0.01 ^{a, A}	0.698 \pm 0.00 ^{a, A}	0.698 \pm 0.00 ^{a, A}	0.1003
p-value ³	0.0996	0.5292	0.2824	0.0002 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{^, B} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 9.4.4 Springiness values (Mean \pm SE¹) measured in mm for control cheese and acetic acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAA6.3, PAA6.1, PAA5.9) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAA6.3 cheese	PAA6.1 cheese	PAA5.9 cheese	p-value ²
2	2.017 \pm 0.07 ^{b, BC}	2.620 \pm 0.14 ^{a, ABC}	1.980 \pm 0.10 ^{b, C}	3.560 \pm 0.41 ^{a, A}	0.0002 [*]
8	2.317 \pm 0.07 ^{ab, A}	2.233 \pm 0.07 ^{a, A}	2.200 \pm 0.07 ^{ab, A}	2.267 \pm 0.20 ^{cd, A}	0.9226
15	2.517 \pm 0.04 ^{a, A}	2.533 \pm 0.13 ^{a, A}	2.450 \pm 0.15 ^{ab, A}	2.250 \pm 0.10 ^{d, A}	0.2832
22	2.500 \pm 0.07 ^{a, A}	2.433 \pm 0.06 ^{a, A}	2.560 \pm 0.13 ^{a, A}	2.383 \pm 0.09 ^{bcd, A}	0.5556
29	2.467 \pm 0.08 ^{a, A}	2.600 \pm 0.23 ^{a, A}	2.425 \pm 0.09 ^{ab, A}	2.540 \pm 0.14 ^{abcd, A}	0.8745
p-value ³	0.0001 [*]	0.3109	0.0166 [*]	0.0011 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c, d} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05);

^{*}Significant (p<0.05).

Table 9.4.5 Mean \pm SE¹ of melt distance measured (mm) for control cheese and acetic acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAA6.3, PAA6.1, PAA5.9) cheeses, (n = 9) during refrigerated storage.

Storage (d)	Control cheese	PAA6.3 cheese	PAA6.1 cheese	PAA5.9 cheese	p-value ²
11	51.885 \pm 0.97 ^{a, A}	43.310 \pm 2.69 ^{a, BC}	41.760 \pm 3.21 ^{a, C}	25.116 \pm 0.23 ^{b, D}	0.0000 [*]
18	52.195 \pm 2.59 ^{a, A}	34.070 \pm 0.94 ^{b, CD}	42.840 \pm 2.28 ^{a, B}	32.310 \pm 1.29 ^{ab, D}	0.0000 [*]
25	53.483 \pm 3.09 ^{a, A}	37.000 \pm 1.14 ^{ab, BC}	39.370 \pm 2.37 ^{a, BC}	35.295 \pm 2.76 ^{a, C}	0.0011 [*]
p-value ³	0.9515	0.0141 [*]	0.2402	0.0029 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05);

^{*} Significant (p<0.05).

Table 9.4.6 Mean \pm SE¹ (n = 9) Hunter L a b-values measured for control cheese and acetic acid pre-acidified to pH 6.3, 6.1 and 5.9 (PAA6.3, PAA6.1, PAA5.9) cheeses, applied with or without canola oil and stored for 20 d at refrigerated temperature, fresh² (before baking), after baking³ and after cooling⁴ to room temperature.

Hunter	Control cheese		PAA6.3 cheese		PAA6.1 cheese		PAA5.9 cheese		p-value ⁵
	With oil	No Oil	With oil	No Oil	With oil	No Oil	With oil	No Oil	
L ²	59.53 \pm 1.74 ^{b, A}	62.48 \pm 0.50 ^{c, A}	60.53 \pm 1.05 ^{b, A}	63.08 \pm 1.26 ^{b, A}	59.38 \pm 1.25 ^{b, A}	60.56 \pm 0.46 ^{b, A}	59.69 \pm 0.64 ^{b, A}	61.49 \pm 1.76 ^{a, A}	0.2107
L ³	73.68 \pm 0.36 ^{a, A}	72.49 \pm 0.26 ^{a, A}	74.85 \pm 0.33 ^{a, A}	72.05 \pm 1.64 ^{a, A}	73.72 \pm 0.45 ^{a, A}	72.83 \pm 1.20 ^{a, A}	68.94 \pm 0.57 ^{a, A}	59.75 \pm 2.13 ^{a, B}	0.0000*
L ⁴	71.08 \pm 0.51 ^{a, A}	69.98 \pm 0.67 ^{b, A}	73.68 \pm 0.30 ^{a, A}	69.89 \pm 1.60 ^{a, A}	72.02 \pm 0.65 ^{a, A}	69.90 \pm 1.55 ^{a, A}	66.41 \pm 1.26 ^{a, A}	55.49 \pm 2.41 ^{a, B}	0.0000*
p-value ⁶	0.0000*	0.0000*	0.0000*	0.0020*	0.0222*	0.0354*	0.0000*	0.1691	
a ²	0.06 \pm 0.18 ^{bc, A}	-0.40 \pm 0.15 ^{b,}	-1.82 \pm 0.09 ^{c, EF}	-2.15 \pm 0.10 ^{b, F}	-0.62 \pm 0.18 ^{a,}	-1.04 \pm 0.16 ^{b,}	-0.99 \pm 0.13 ^{b,}	-1.19 \pm 0.25 ^{b,}	0.0000*
a ³	-0.02 \pm 0.12 ^{c,}	3.00 \pm 0.45 ^{a,}	-1.37 \pm 0.18 ^{bc, H}	2.39 \pm 1.36 ^{a,}	-0.64 \pm 0.22 ^{a, GH}	1.80 \pm 0.80 ^{ab,}	3.91 \pm 1.23 ^{a,}	12.15 \pm 1.58 ^{a, A}	0.0000*
a ⁴	1.36 \pm 0.35 ^{a, FGH}	2.69 \pm 0.72 ^{a,}	-0.71 \pm 0.18 ^{a, H}	1.78 \pm 0.94 ^{a,}	-0.32 \pm 0.18 ^{a, GH}	1.86 \pm 1.01 ^{a,}	4.32 \pm 1.16 ^{a,}	12.30 \pm 1.89 ^{a, A}	0.0000*
p-value ⁶	0.0006*	0.0003*	0.0006*	0.0085*	0.4449	0.0230*	0.0025*	0.0000*	
b ²	17.11 \pm 0.60 ^{c, A}	18.41 \pm 0.36 ^{b, A}	18.94 \pm 0.38 ^{b, A}	19.35 \pm 0.37 ^{b, A}	17.67 \pm 0.44 ^{b, A}	18.50 \pm 0.36 ^{b, A}	17.04 \pm 0.54 ^{b, A}	18.08 \pm 0.99 ^{b, A}	0.2640
b ³	25.59 \pm 0.59 ^{b, C}	30.87 \pm 1.16 ^{a,}	27.36 \pm 0.38 ^{a,}	30.59 \pm 1.13 ^{a,}	25.82 \pm 0.39 ^{a, BC}	28.80 \pm 0.65 ^{a,}	33.04 \pm 2.02 ^{a, A}	32.42 \pm 0.85 ^{a, A}	0.0000*
b ⁴	28.30 \pm 0.67 ^{a, AB}	29.25 \pm 1.01 ^{a, AB}	28.69 \pm 0.38 ^{a, AB}	30.02 \pm 0.72 ^{a, AB}	26.79 \pm 0.56 ^{a, B}	29.56 \pm 0.45 ^{a, AB}	31.84 \pm 0.50 ^{a, A}	29.50 \pm 0.8 ^{a, AB}	0.0007*
p-value ⁶	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D, E, F, G, H} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

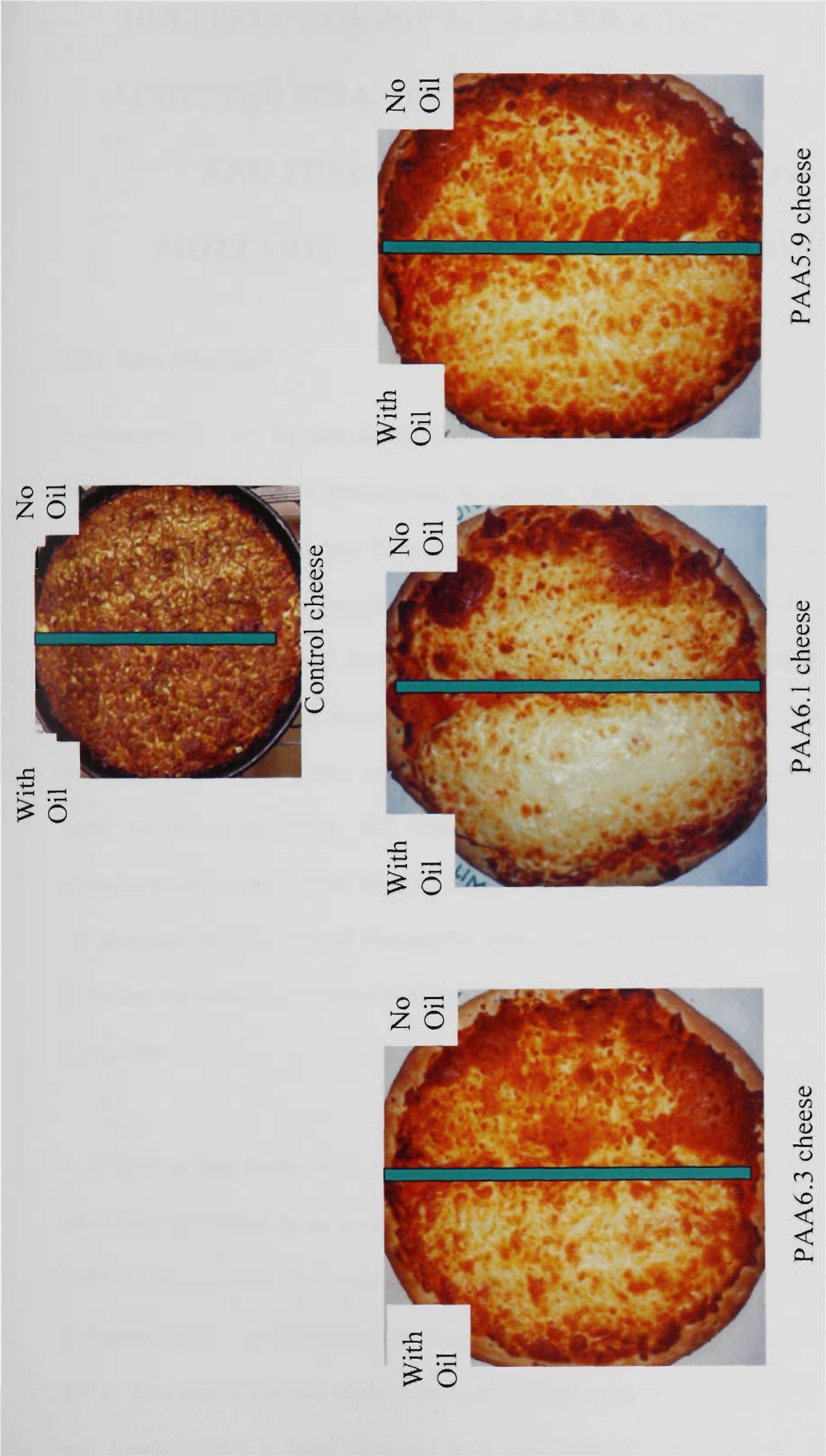


Figure 9.4.1 Pizzas made from control cheese and PAA6.3, PAA6.1 and PAA5.9 cheeses after storing for 20 d.

10.0 EFFECT OF FAT REPLACER ADDITION TO PRE- ACIDIFIED MILK ON YIELD, COMPOSITION, TEXTURE AND FUNCTIONALITY OF ULTRA LOW FAT MOZZARELLA CHEESES MADE AT PILOT-SCALE

10.1 Introduction

Fat content in full fat mozzarella cheese contributes to the taste, reduces hardness, and increases meltability and stretchability of cheeses. Due to improved melt, shredded cheeses when baked fuse together and flow upon the pizza base. Presence of fat in the cheese during baking allows free oil formation, which provides a protective coating to the cheese shreds and prevents from burning and browning also improved heat transfer. Smaller size blisters are produced and the cheese shows glossiness and is white after baking (Rudan *et al.*, 1999; Rowney *et al.*, 1999; Rudan and Barbano, 1998). However, the full fat mozzarella cheeses show an excessive oiling off when baked, which is unappealing to the consumer (Bhaskaracharya 2000). Also, the high fat content is not considered to be healthy. Reduction in fat increases the hardness of mozzarella cheeses due to compactness of the protein matrix, decreases the moisture retention and such cheeses show poor meltability and functionality (Fife *et al.*, 1996).

L. helveticus was found to increase the proteolytic activity during the initial storage period, which was attributed to an aminopeptidase produced by the bacterium (Ardo and Pettersson, 1988). This increase in peptidolysis along with availability of galactose, which was not fermented by *S. thermophilus* was suggested to increase maillard browning (Merrill *et al.*, 1994). Mozzarella cheeses made from buffalo milk using starter culture were reported (Athar and Anwar, 1992) to have increased total solids, protein content, ash content and reduced

lactose and fat contents compared to cheeses made using direct acidification technique with lactic acid.

Casein in its native state in milk is able to remain dispersed as colloidal calcium phosphate complex. At higher pH, close to neutral, casein is closer to its native state of conformation. Pre-acidification depletes calcium from the colloidal calcium phosphate complex and the casein changes its conformation. The altered state of casein could either increase the water holding capacity (decrease the levels of expressible serum) depending on the availability of hydrophilic sites on the caseins. Pre-acidification causes a loss in calcium leading to a negatively charged casein micelle (Kindstedt and Guo 1997). Availability of hydrogen ions helps association of casein micelles with water. Addition of salt increases sodium ions, which partially neutralise the residual negative charge on the casein micelles. The divalent calcium ions are replaced by the monovalent sodium and hydrogen ions on the casein micelle. The ratio of calcium: protein is decreased by small changes in pH, salt concentration and temperature induced openness of the casein micelles. At lower pH of pre-acidification the ratio of calcium: protein decreases and association of hydrogen and sodium ions increases the hydrophilic properties of casein. Moreover, sodium ions being highly hydrophilic, increase the hydrophilic property of caseins.

Pizza cheeses were manufactured (Breene *et al.*, 1964) from either whole or part skim milk using lactic, acetic or hydrochloric acid for acidifying cheese-milk to pH 5.6. This method of manufacturing cheese using direct acidification produced a bland mozzarella cheese with improved meltability and stringiness properties without prolonged storage. The bland flavour was overcome by acidifying the pizza sauce (tomato sauce) with lactic acid. An imitation mozzarella cheese product was made using casein, water, citric acid, yoghurt, cream and curd. These ingredients were mixed along with an emulsifying salt, melted, stretched and extruded

into plastic tubes (Toppino *et al.*, 1988). The recipe for making imitation cheese shows that pH plays an important role in stretchability and extrusion of cheese. Addition of citric acid would possibly remove some of the calcium phosphate complex from casein and allow the para casein to stretch. But such cheese-like product contained ~ 35% fat.

Whelan and Conant (1979) developed a cheese food product by mixing mozzarella, provolone and romano cheeses with citric acid, sodium citrate, sucrose, vegetable oil and gums such as xanthan and locust bean gum to produce a cheese product similar to mozzarella. This cheese food product contained 12- 16% fat content and was reported to be of acceptable quality. Similarly, Kim *et al.* (1995) developed a 23% reduced fat mozzarella cheese using a blend of soymilk and cow milk and acidifying it with citric, lactic or phosphoric acids.

Addition of fat replacers was reported to increase the fat dispersibility in cheeses. The fat globules were observed to be smaller and better dispersed in imitation cheeses as observed by Mounsey and O’Riordan (2001) under an electron microscope. Fat replacers play an important role by behaving like milk fat, by increasing the moisture retention in cheeses thereby increasing the yield, and by reducing the calories of the cheese through replacement of milk fat. Thus addition of fat replacers should improve the functionality of low-fat mozzarella cheeses without detrimental effect on the sliceability and shreddability of such cheeses. Previous studies in our laboratory (Bhaskaracharya and Shah, 2001b and 2001c) showed that the protein matrix is disrupted due to addition of native or modified starches in skim milk mozzarella cheeses. Mounsey and O’Riordan (2001) also reported similar results for imitation cheeses. Thus some of the fat replacers could also act as a filler material.

10.2 Aims

The aims of this experiment were to manufacture ULFM cheeses using milk pre-acidified to pH 6.1 with citric acid and with or without addition of either Maltrin M100 or Versagel. The pre-acidification with citric acid to pH 6.1 was chosen as it gave the best functional characteristics without a significant decrease in yield as compared to PAC6.3, PAC5.9 and the acetic acid based cheeses. The pilot scale trials were conducted to obtain sufficient sample to estimate the composition, yield, water holding capacity, proteolysis, texture characteristics and functional characteristics. Another aim of this experiment was to study the effect of oil application to shredded fresh cheese on their functional characteristics over a prolonged storage period.

10.3 Materials and methods

10.3.1 Cheese making

Cheese milk was standardised to 0.49% fat and 3.05% protein contents and was randomly allotted to three cheese vats (200 L per vat). The milk was warmed to 30°C in two cheese vats, mixed with either Maltrin or Versagel (section 3.2.3) at 0.25%, warmed to 33°C, inoculated with *Streptococcus thermophilus* and *Lactobacillus helveticus* starter cultures, mixed thoroughly and pre-acidified using citric acid to pH 6.1. The milk was warmed to 35°C and rennet was added. Four cheese batches (each from 200 L milk) were made of each variety on four separate days using the procedures described in section 3.2.6.4.

10.3.2 Analysis of cheeses

The yield of cheeses was measured and the cheese samples were prepared as described in section 3.2.9 for estimation of composition, melt, proteolysis, texture characteristics (section 3.2.8) and pizza bake characteristics (section 3.2.7 and 3.2.24). The moisture/total solids was

estimated as described in section 3.2.20, fat as in section 3.2.22. and protein as in section 3.2.25 contents were determined. The starch content in Maltrin based cheeses was estimated using HPLC methods as described in subsections 3.2.18. Texture characteristics including hardness, cohesiveness and springiness were measured for cheeses stored for 15, 29, 44, 56 and 80 d by compressing cylindrical cheese samples to 50% of their height as described in section 3.2.23 using an Instron according to the method of Bhaskaracharya (2000). Meltability test was carried out as described in section 3.2.15 at 22, 32, 45, 60 and 85 d of storage using 10.0 g of grated cheese sample heated in a glass tube at 110°C for 100 min. Pizza-bake test was carried out (section 3.2.24) at 29, 44, 56 and 80 d of storage and the colour, size of blisters and their distribution upon baking were assessed. Stretchability of the cheeses was measured objectively using an Instron machine as described in section 3.2.16. The cheeses stored for 29, 40, 55 and 75 d were evaluated for their distance of stretch. The expressible serum content, which is a measure of the water holding capacity of the cheeses was measured as described in section 3.2.14 on 22, 35 and 50 d of storage. Samples of cheeses were analysed for determining the TCA soluble nitrogen contents on 16, 30, 45, 60 and 90 d of storage as described in section 3.2.17.

10.3.3 Statistical analysis

Statistical analysis of the results was carried out using StatPro[®] software on Microsoft[®] excel. The means, standard deviations and standard error were calculated. The means from at least two samples were compared. The analysis of variance table was prepared and two sources of variation: the variation *within* each population and variation *among* sample means from the different populations was compared. If the latter variation was large relative to the former, as was measured using an F test, this was deemed to be evidence of differences between the population means. The p-value obtained from this table and the confidence intervals (at 95% confidence level) for all the differences between pairs of means were considered for describing

the statistical differences and their significance. A small p-value was a result of large differences in sample means and the confidence intervals that did not include zero were taken to indicate that the means that were compared were not equal.

10.4 Results and discussion

10.4.1 Yield and composition

The mean and standard error for yield, moisture, protein, FDM, and M:P contents of Maltrin based, control and Versagel based cheeses are shown in Table 10.4.1. The control and fat replaced cheeses had similar (statistically not different) yield. The moisture, protein, FDM and M:P contents of Maltrin based cheeses and control cheeses were similar and significantly different to Versagel based cheeses. The moisture and FDM contents of Maltrin based cheeses were the lowest while those for Versagel based cheeses were the highest. Similarly the M:P content of the control cheeses was the lowest while that for Versagel based cheeses was the highest. The protein content of Maltrin based cheeses and control cheeses were significantly higher than that for Versagel based cheeses. The Versagel based cheeses showed lower protein content but these cheeses were able to retain more moisture (M:P) compared to control and Maltrin based cheeses probably due to the moisture retention ability of Versagel. The increased moisture content of the Versagel based cheeses could have caused the decrease in protein content of these cheeses. The Maltrin based cheeses did not show a significant improvement in yield or compositional characteristics due to addition of Maltrin. The differences in moisture and protein contents compensated to each other and the yield of control and fat replaced cheeses was similar.

10.4.2 Starch partitioning in cheese, whey and stretch water

Figure 10.4.1 indicates the peak area response versus concentration plotted for DP7 (maltoheptaose). The equation for calculating concentration is shown below:

$$y = 0.0371 x.$$

Maltrin based samples were only analysed for starch content. During sample preparation lactose from the products interfered with the peaks obtained for starch. Hence, samples were treated with lactase for several hours and milk and cheese samples also defatted before injecting the samples of appropriate dilutions into the HPLC column. Initial studies were carried out with several standards based on their degree of polymerisation (DP) such as glucose, galactose, sucrose, lactose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose. The HPLC column showed good repeatability in eluting the standards with proper separation. During preliminary analysis, milk, whey and cheese samples showed only DP7 as a means to approximately correlate the amount of Maltrin remaining in the samples. The HPLC analysis showed maltoheptaose (DP7) to elute in all the samples. The sample preparation procedure was developed based on personal communications with the HPLC column suppliers (Andrew Cruickshank, Altech Associates, Melbourne), with a view to eliminate the milk proteins and fat from interfering with the elution of oligosaccharides.

Initial trials were carried out to determine the solubility of Maltrin M100 in O-phosphoric acid and after successful trials a procedure was developed. Proteins and fat should be removed during sample preparation to avoid their deposition on the column and subsequent reduction in column efficiency (Scott, 1992). Purification of starch from samples required precipitation of proteins using acid according to Euber and Brunner (1978), and as the mobile phase required for the column was 0.1% O-phosphoric acid, the samples could be prepared using this acid. An ultra turrax homogeniser was used to extract starch from cheese samples while for liquids such as milk, whey and stretch water inversion of the falcon tubes containing the sample and acid

was sufficient. The precipitated proteins were filtered out and the extract was collected. The precipitate along with the filter paper was given three washings to remove any traces of Maltrin from the protein precipitates and the washings were collected with the initial extract. The extract was cooled to refrigeration temperature ($\sim 4^{\circ}\text{C}$) to allow for any fat present in the extracts to separate due to gravity (personal communication with Dale Every, Crop and Food Research Institute, New Zealand). The collected extract was treated with hexane (Richmond *et al.*, 1982) and the hexane layer was separated to de-fat the samples according to Okada *et al.* (1983) and Scott (1992). The de-fatted solutions were neutralised with 4M sodium hydroxide solution to pH 6.6 at which the lactase shows maximum activity. Lactase was added and milk samples were incubated for 5 h while whey samples were incubated for 6 h at 20- 25°C (optimum temperature of lactase) to breakdown lactose. The solutions were finally diluted and injected into HPLC column to obtain peak area response.

Table 10.4.2 shows the amount of maltoheptaose measured in Maltrin based cheeses made on different days. Literature obtained from the supplier showed that Maltrin M100 contained about 80% oligosaccharides with DP greater than 10. The HPLC column used to analyse the cheese samples was able to detect only DP7 and lower oligosaccharides. A few peaks were observed having an elution time of 6.5 min but such peaks were not identified in all samples probably due to very low concentration. Thus DP7 peak area responses were used to estimate the starch content in the Maltrin based cheeses. The mean DP7 concentration of 2.7 $\mu\text{g/g}$ of sample was obtained for the Maltrin based cheese samples. Literature provided by Grain Processing Corp., USA (suppliers for Maltrin M100) indicated that M100 had about 6.8% of DP7. Addition of 0.25% M100 to milk would indicate about 2.5 mg of M100 should be present per g of milk. During cheese making a ten-fold concentration of milk solids is expected. Thus we expected to obtain higher values of DP7. But theoretical estimation will not be possible because saccharides with DP numbers 1- 10 are small in size and could be lost in whey while

the higher DP number i.e. greater than DP10 would possibly be remaining trapped in the curd. Thus, the results show that most of the oligosaccharides having DP7 or lower were lost into whey while there is the possibility that DP10 and above oligosaccharides could remain in the cheese trapped within the protein matrix (Bhaskaracharya and Shah, 2001c).

10.4.3 Water holding capacity

Figure 10.4.2 shows the quantity of serum expressed from the control, Maltrin based and Versagel based cheeses measured after storing them for 22, 35 and 50 d. The three varieties of cheeses expressed a noticeable amount of serum after 22 d of storage with the least quantity expressed by Maltrin based cheeses. Versagel based cheeses expressed the most amount of serum at 22 d and 35 d compared to the other cheeses. Both Maltrin based and control cheeses had negligible amounts of expressible serum after storing for 35 d. Considering the decreasing trend in expressible serum content of the three cheeses, studies were completed at 50 d to quantify the amounts of serum expressed. Thus Versagel based cheeses required longer time to absorb the moisture completely while Maltrin based and control cheeses were able to retain most of their moisture contents by 35 d. The cheeses showed a decreasing trend in expressible serum with increase in storage. Similar observations were made by Kindstedt and Guo (1997) for mozzarella cheeses during storage.

10.4.4 TCA soluble nitrogen content

Figure 10.4.3 shows the TCA soluble nitrogen content of the control, Maltrin and Versagel based cheeses estimated over the storage period of 90 d. The extent of proteolysis in ULFM cheeses was measured by estimating the 12% TCA soluble nitrogen content. The Maltrin based cheeses showed higher proteolysis at 45 d of storage while control and Versagel based cheeses had lower proteolysis. At 60 d both control and Maltrin based cheeses had similar extents of proteolysis and Versagel based cheeses showed lower proteolysis. At 90 d the Maltrin based

cheeses had the highest proteolysis compared to control and Versagel based cheeses. Versagel based cheeses consistently showed the least amount of proteolysis throughout storage compared to Maltrin based and control cheeses. The reduction in water holding capacity and the lower levels of TCA soluble nitrogen indicate that Versagel based cheeses had reduced proteolysis during storage which could be due to β -lactoglobulin from Versagel forming complex with κ -caseins and reducing the sites available for the proteolytic enzymes.

10.4.5 Texture characteristics

Hardness values for Maltrin based, control and Versagel based cheeses are shown in Table 10.4.3. Their mean and standard error values for hardness measured at 15, 29, 44, 56 and 80 d of storage are shown. Control cheeses had significantly higher hardness compared to both Maltrin based and Versagel based cheeses at 15 d. Between the two fat replaced cheeses, Maltrin based cheeses were significantly harder than Versagel based cheeses at 15 d of refrigerated storage. Upon storage for 29 d the hardness values for control and Maltrin based cheeses were similar (statistically not different) while Versagel based cheeses had lower hardness values compared to control cheeses. The Maltrin based and Versagel based cheeses had statistically similar hardness values. The three varieties of cheeses showed similar hardness values at 44 d and 56 d. Prolonged storage up to 80 d caused Maltrin based cheeses and control cheeses to continue to show similar hardness but at the same time their hardness values were significantly higher than those for Versagel based cheeses. The higher hardness values for Maltrin based cheeses indicate that the fat replacer behaves more as a filler and had not hydrated completely.

During the 80-d storage study of Maltrin based cheeses, their hardness values did not change (statistically similar values). The control cheeses were initially hard at 15 d and their hardness values significantly reduced at 29, 44 and 80 d. The control cheeses showed similar hardness

values from 29 d to 56 d, and had the least hardness values at 80 d of storage. The Versagel based cheese showed similar hardness values at 15, 29, 44 and 56 d and these values decreased significantly after storing the cheeses for 80 d. Similar observations for high amylose (potato starch) containing cheeses were made by Mounsey and O’Riordan (2001).

Cohesiveness is dependent upon the strength of the internal bonds between the various constituents of cheese (Szczesniak, 1963). In Table 10.4.4, the cohesiveness values measured during refrigerated storage of Maltrin based, control and Versagel based cheeses are shown. All three cheeses showed similar cohesiveness at 15 d. At 29 and 44 d, only control cheeses had significantly higher cohesiveness than Maltrin based cheeses, while the fat replaced cheeses had similar cohesiveness values. All three varieties of cheeses had similar cohesiveness values at 56 and 80 d. Maltrin based cheeses showed similar cohesiveness values at 15, 29 and 44 d and these values were significantly lower than those at 56 and 80 d. Maltrin based cheeses showed similar cohesiveness at 56 and 80 d of storage. The control cheeses had similar cohesiveness at 15 and 29 d, the values increased at 44 d and remained higher at 56 and 80 d of storage. Versagel based cheeses had the least cohesiveness at 15 d but the values increased at 29 d. Versagel appeared to have undergone limited hydration. The Versagel particles require higher temperature to cause the κ -carageenan and xanthan gums to swell. Decreased swelling of Versagel particles could cause them to act similarly to rice starch (Mounsey and O’Riordan, 2001) and possibly cause little disruption to the cheese matrix resulting in lower cohesiveness values. These cheeses showed similar cohesiveness at 29, 44, 56 and 80 d of storage.

The springiness values measured at 15, 29, 44, 56 and 80 d of storage for Maltrin based, control and Versagel based cheeses are presented in Table 10.4.5. Versagel based cheeses were significantly more springy than only Maltrin based cheeses and the latter cheeses had

statistically similar springiness values as for control cheeses at 15 d of storage. At 29 d the three cheeses showed significantly different springiness values and Versagel based cheeses had the highest springiness while Maltrin based cheeses were least springy. Further, upon storage of cheeses for 44 d, the control cheeses showed similar springiness values as for the fat replaced cheeses but a comparison between the latter cheeses showed that the Versagel based cheeses had significantly higher springiness than that for the Maltrin based cheeses. At 56 and 80 d of storage, the control cheeses had significantly lower springiness values compared to Versagel based cheeses but similar values as those for Maltrin based cheeses. The Versagel based cheeses had similar springiness values at 56 d but the values were significantly higher at 80 d to Maltrin based cheeses.

10.4.6 Functional Characteristics

10.4.6.1 Meltability results

The mean and standard error for meltability of Maltrin based, control and Versagel based cheeses measured at 22, 32, 45, 60 and 85 d of refrigerated storage are given in Table 10.4.6. A typical melt test is shown in Figure 10.4.4 for Maltrin based cheeses stored for 32 d. The Maltrin based and control cheeses (Figure 10.4.5) showed similar meltability at 22 and 32 d while the Versagel based cheeses (Figure 10.4.6) had significantly reduced meltability compared to other two cheeses. At 45 and 60 d of storage, Maltrin based cheeses had significantly higher meltability compared to control and Versagel based cheeses and the latter cheeses had similar meltability. Further, cheeses stored up to 85 d showed Maltrin based and control cheeses to have similar meltability while Versagel based cheeses had significantly lower meltability than the former cheeses. Thus throughout storage Maltrin based cheeses had greater meltability than Versagel based cheeses and at 45 and 60 d the former cheeses showed higher melt than control cheeses as well. Versagel based cheeses showed the least melt compared to Maltrin based and control cheeses.

During storage Maltrin based cheeses had the least meltability at 45 d and significantly higher melt at 85 d. Control cheeses had similar meltability at 22 and 32 d but significantly reduced melt at 45 d and 60 d. The control cheeses showed the greatest melt at 85 d. Versagel based cheeses had similar (statistically not different) melt throughout storage and showed no effect of prolonged storage. The poor meltability of Versagel based cheeses could be due to insufficient hydration of Versagel. Thus addition of maltodextrin appeared to improve the melt while protein based fat replacer (Versagel) had a negative impact of this characteristic of the fat replaced cheeses.

10.4.6.2 Pizza bake results for cheeses stored for 29 d

10.4.6.2.1 Physical properties as observed with the naked eye of 20 d old ULFM cheeses made from milk pre-acidified using citric acid to pH of 6.1 and mixed with or without fat replacer.

Figure 10.4.7 shows the pizzas made using 29 d old Maltrin based, control and Versagel based cheeses. The shredded cheeses of each variety were prepared as explained in section 3.2.7. Maltrin based (no oil) cheeses showed incomplete shred fusion and although most of the cheese was melted some of the shreds had changed shape and swollen due to baking while a few shreds remained intact. The intact and swollen cheese shreds showed burning and very few small blisters were observed on the surface of the cheese. In contrast, Maltrin based (with oil) cheeses showed almost complete shred fusion with a few shreds intact that submerged below the melted cheese. Due to being covered by the melted cheese the shreds may not have reached the right temperature and thus did not melt. The surfaces of such cheeses when melted showed brown patches and the edges of such cheeses were slightly burnt. The surface of Maltrin based (with oil) cheeses did not show glistening due to application of oil, which could be due to absorption of the oil into the cheese shreds and not being liberated when cheeses were heated.

Control (no oil) cheeses showed many intact cheese shreds that had excessive browning. Due to the intact shreds the cheeses although melted, appeared to be incompletely melted and not sufficiently fused together. Hence the cheeses were not acceptable on pizza. The control (with oil) cheeses showed good shred fusion probably due to the pre-acidification, which reduced the calcium content, thus softening the cheese shreds and during heating. A lot of brown regions were observed on the surface of the melted cheese and some blister formation was also evident. Scorching of the cheese caused the brown color of the patches and because such patches were widespread on the surface of the melted control (with oil) cheeses, they were not of acceptable pizza bake quality.

Versagel based (no oil) cheeses showed excessive burning compared to control and Maltrin based cheeses. The Versagel based (no oil) cheeses had a lot of unmelted cheese shreds that were improperly fused. Scorching and browning of the cheese shreds was evident from their pizza bake analysis. Even the Versagel based (with oil) cheeses showed incomplete shred fusion and the cheeses had not melted. This reduced the flow characteristics as well and the Versagel based (with/no oil) cheeses performed the poorest among the three varieties of cheeses at 29 d of storage.

Table 10.4.7 shows the Hunter L, a, and b values for fresh, baked and cooled Maltrin based cheese, control cheese and Versagel based cheese, applied with or without oil and stored for 29 d. Table 10.4.7 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

10.4.6.2.2 Effect of addition of fat replacers to cheeses on their Hunter L, a, and b values

The fresh Maltrin based, control and Versagel based cheeses had similar (statistically not different) Hunter L^2 , a^2 and b^2 values, which indicates that the addition of fat replacers did not

alter the colour of the fresh cheeses. When Maltrin based, control and Versagel based cheeses were baked on the pizza bases, the Hunter L^3 , a^3 , and b^3 values for the white areas on the pizza were similar for the cheeses and the L^4 , a^4 , and b^4 values for the brown areas were similar for the three varieties of cheeses. The Maltrin based and control cheeses that were applied with oil, had significantly higher b^4 values than those for Versagel based (no oil) cheeses. The white areas on the cooled pizzas showed control (no oil) cheeses to have lower L^5 values compared to Maltrin based (with oil) and Versagel based (with/no oil) cheeses. The Maltrin based (no oil) cheeses and control (with oil) cheeses had similar L^5 values as the rest of the (with/no oil) applied cheeses. The a^5 and b^5 values were statistically similar for the three varieties of cheeses that were either treated or not treated with oil. The brown areas on cooled pizzas showed Maltrin based (with oil) cheeses and Versagel based (with oil) cheeses had significantly higher L^6 values compared to those for control (no oil) cheeses.

10.4.6.2.3 Effect of application of oil on Hunter L, a, and b values for Maltrin based, control and Versagel based cheeses

The Hunter L^2 , a^2 , and b^2 values for fresh samples of the three varieties of cheeses showed no effect due to oil application. Upon baking the Hunter L^3 , a^3 , and b^3 values for the white areas on pizzas had no significant effect of application of oil. Similarly, the brown areas on the pizzas also did not show any significant effect of oil application on the Hunter L^4 , a^4 , and b^4 values for the three varieties of cheeses. The white areas of cooled Maltrin based, control and Versagel based cheeses had similar L^5 , a^5 , and b^5 values as their respective cheeses that were not applied with oil and hence no effect due to application of oil was evident. Application of oil significantly increased the L^6 values for Maltrin based cheeses indicating that the Maltrin based cheeses, which were not applied with oil, were comparatively browner. The L^6 values of control (with oil) and Versagel based (with oil) cheeses did not show any improvement compared to their respective cheeses that were not applied with oil. Also the a^6 and b^6 values

for the three varieties of cheeses were similar and the cheeses did not show a significant effect due to application of oil.

10.4.6.2.4 Effect of baking and cooling on the Hunter L, a, and b values of Maltrin based, control and Versagel based cheeses applied with/no oil

Fresh Maltrin based (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. Even the brown areas were comparatively more white (L^4 values) when pizzas were hot than when they were cold (L^6 values). The cooled cheeses lost their whiteness to a degree and showed translucency. The extent of translucency would be indicated by L, a and b values. The Maltrin based (no oil) cheeses had similar (statistically not different) L^2 values when fresh as compared to L^5 values after the pizzas were cooled. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values, which indicate colour change from green to yellow showed an increase after baking (b^3 and b^4 values) for the Maltrin based (no oil) cheeses and the b^3 values were significantly higher (more yellow) than the b^5 values measured after cooling the pizzas. The white areas on the cooled pizzas made with Maltrin based (no oil) cheeses were more yellow (b^5 values) compared to the b^6 values of the brown areas on the cooled pizzas. The fresh cheeses showed the least b^2 values, which indicates that the cheeses improved on their b values after being baked or after baking and cooling.

Fresh Maltrin based (with oil) cheeses showed increases in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The Maltrin based (with oil) cheeses had similar (statistically not different) L^2 values when fresh as compared to their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for Maltrin based (with oil) cheeses showed increased b^3 and b^4 values (yellowness) after baking (white and brown areas) and they were similar to b^5 and b^6 values for white and brown areas on cooled cheeses. The fresh cheeses had b^2 values significantly lower than after the cheeses were baked or baked and cooled.

Fresh control (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas upon cooling. Even the brown areas were comparatively more white (L^4 values) when pizzas were hot than when they were cold (L^6 values). The control (no oil) cheeses had similar (statistically not different) L^2 values when fresh as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values of these cheeses showed that baking increased browning (a^4 values) which were significantly higher than the a^6 values for brown areas after cooling the pizzas. The a^6 values were also significantly higher than a^5 values showing that

some parts of the cheeses were extensively red while the white areas on pizzas were significantly less red. The white areas on baked pizzas showed similar a^3 values as after cooling (a^5 values). The fresh control (no oil) cheeses had the least red colouration (a^2 values). The yellowness (b^3 values) of the cheeses after baking (white areas) were significantly greater than for the cheeses after cooling (b^5 values), but both the b^3 and b^5 values were similar to b^4 and b^6 values. The fresh cheeses showed the least yellowness (b^2 values).

Fresh control (with oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. Even the brown areas were comparatively more white (L^4 values) when pizzas were hot than when they were cold (L^6 values). The control (with oil) cheeses had similar (statistically not different) L^2 values when fresh as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for the control (with oil) cheeses showed an increase after baking (b^3 and b^4 values) and the b^3 values were significantly higher (more yellow) than the b^5 values measured after cooling the pizzas. The b^5 values of white areas and the b^6 values of brown areas on the cooled pizzas made with control (with oil) cheeses were similar. The fresh cheeses showed the least b^2 values, which indicates that the cheeses improved on their b values after being baked or after baking and cooling.

The fresh Versagel based (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. Even the brown areas were comparatively more white (L^4 values) when pizzas were hot than when they were cold (L^6 values). The Versagel based (no oil) cheeses had similar (statistically not different) L^2 values when fresh as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The yellowness (b^3 values) of baked Versagel based (no oil) cheeses (white areas on pizzas) was similar to b^4 values (brown areas on pizzas) and b^5 values (white areas on cooled pizzas). But the b^3 values were significantly higher than the b^6 values for the brown areas of cooled pizzas. The b -values measured after baking or after baking and cooling were significantly higher than the b^2 values similar to the trend shown by other cheeses.

Fresh Versagel based (with oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The Versagel based (with oil) cheeses had similar (statistically not different) L^2 values when fresh as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values of these cheeses showed that baking

increased browning (a^4 values) which were significantly higher than the a^6 values for brown areas after cooling the pizzas. The a^6 values were also significantly higher than a^5 values showing that some parts of the cheeses were extensively brown while the white areas on pizzas were significantly less red. The white areas on baked pizzas showed similar a^3 values as after cooling (a^5 values). The fresh Versagel based (with oil) cheeses had the least red colouration (a^2 values). The yellowness (b^3 values) of baked Versagel based (with oil) cheeses (white areas on pizzas) was similar to b^4 values (brown areas on pizzas) and b^5 values (white areas on cooled pizzas). But the b^4 values were significantly higher than the b^6 values for the brown areas of cooled pizzas. The b-values measured after baking or after baking and cooling were significantly higher than the b^2 values similar to the trend shown by other cheeses.

10.4.6.3 Pizza bake results for Maltrin based, control and Versagel based cheeses stored for 44 d

10.4.6.3.1 Physical properties as observed with the naked eye of 44 d old ULFM cheeses made from milk pre-acidified using citric acid to pH of 6.1 and mixed with or without fat replacer.

The pizza bake characteristics of Maltrin based, control and Versagel based cheeses were similar at 44 d (Figure 10.4.8) to those at 29 d. The Versagel based (no oil) cheeses after 44 d of storage showed decrease in number of intact shreds upon baking and had reduced burning. Similarly the Versagel based (with oil) cheeses showed increased whiteness due to reduction in number and size of brown patches on melted cheese surface. Thus after storing for 44 d also the three varieties of cheeses were not sufficiently functional on the pizza and were unacceptable.

Table 10.4.8 shows the Hunter L, a, and b values for fresh, baked and cooled Maltrin based cheese, control cheese and Versagel based cheese, applied with oil or not applied with oil and

stored for 44 d. Table 10.4.8 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

10.4.6.3.2 Effect of addition of fat replacer to cheeses on their Hunter L, a, and b values

Fresh cheeses made with or without addition of fat replacers had similar Hunter L^2 , a^2 and b^2 values. The cheeses made with the addition of either Maltrin or Versagel fat replacer showed similar whiteness (L^3 values) as that of control cheeses upon baking and at the same time the Maltrin based (with oil) cheeses had significantly higher red colour (a^3 values) compared to control (with oil) cheeses while the rest of the cheeses applied with or without oil showed similar a^3 values. The b^3 values showing yellowness of the baked pizzas, showed marked differences. The Maltrin based (with oil) cheeses had significantly higher b^3 values compared to Maltrin based (no oil), control (with/no oil) and Versagel based (no oil) cheeses. The control (no oil) cheeses had the least b^3 values. The L^4 values showing whiteness of the pizzas baked, showed marked differences. The Maltrin based (with oil) cheeses had significantly higher L^4 values compared to Maltrin based (no oil), control (with/no oil) and Versagel based (no oil) cheeses. The control (no oil) cheeses had the least L^4 values. The a^4 values of all the cheeses were similar. The brown areas of pizzas made with control (with oil) cheeses had significantly higher yellow colouration (b^4 values) compared to those of Versagel based (no oil) cheeses while the rest of the cheeses had similar (statistically not different) b^4 values. After cooling the pizzas the control (with oil) cheeses showed significantly more whiteness (L^5 values) while those made with Maltrin based (with oil) cheeses had significantly lower whiteness (L^5 values). The L^5 values for the other cheeses were similar and so were the a^5 and b^5 values for all the cheeses. The brown areas on the pizzas after cooling showed similar L^6 and a^6 values but the b^6 values of Maltrin based (with oil) cheeses were significantly higher compared to those for control (no oil) cheeses.

10.4.6.3.3 Effect of application of oil to Maltrin based, control and Versagel based cheeses on the Hunter L, a, and b values

The application of oil did not have a significant effect on the Hunter L^2 , a^2 , and b^2 values for fresh cheeses. Also baked cheeses did not show an effect of oil application on L^3 and a^3 values, but the b^3 values of Maltrin based (with oil) cheeses were significantly higher than for Maltrin based (no oil) cheeses. The b^3 values for control (with/no oil) and Versagel based (with/no oil) cheeses were similar. The brown areas of the pizzas with Maltrin based (with oil) and control (with oil) cheeses had significantly higher L^4 values than their respective (no oil) cheeses. Control (with oil) cheeses showed significantly higher b^4 values compared to the b^4 values of control (no oil) cheeses. The L^5 , a^5 and b^5 values were similar for Maltrin based (with oil) and Maltrin based (no oil) cheeses; for control (with oil) and control (no oil) cheeses and for Versagel based (with oil) and Versagel based (no oil) cheeses. Similar observations were obtained for L^6 , a^6 , and b^6 values for the control and fat replaced cheeses that were either applied with oil or not applied with oil.

10.4.6.3.4 Effect of baking and cooling on the Hunter L, a, and b values of Maltrin based, control and Versagel based cheeses

The fresh Maltrin based (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. Even the brown areas were comparatively more white (L^4 values) when pizzas were hot than when they were cold (L^6 values). The Maltrin based (no oil) cheeses had significantly higher L^5 (white areas of cooled cheeses) values compared to fresh L^2 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the redness/brown colouration of the cheeses significantly

compared to the fresh cheeses. The yellowness (b^3 values) of baked Maltrin based (no oil) cheeses (white areas on pizzas) was significantly lower to b^4 values (brown areas on pizzas) and similar to b^5 values (white areas on cooled pizzas). But the b^3 and b^5 values were significantly higher than the b^6 values for the brown areas of cooled pizzas. The b-values measured after baking or after baking and cooling were significantly higher than the b^2 values similar to the trend shown by other cheeses.

Maltrin based (with oil) cheeses showed similar whiteness for baked white (L^3 values), baked brown (L^4 values) and baked and cooled white areas (L^5 values) while the baked brown areas after cooling the pizzas had the least L^6 values. The baked white and baked brown areas had more whiteness compared to the fresh Maltrin based (with oil) cheeses. But due to increased browning the fresh cheeses had significantly higher L^2 values than after baking and cooling (L^6 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values) and a^2 values for fresh cheeses. Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for Maltrin based (with oil) cheeses showed increased b^3 and b^4 values (yellowness) after baking (white and brown areas) and the b^3 values were similar to b^5 and b^6 values for white and brown areas respectively on cooled cheeses. The fresh cheeses had b^2 values significantly lower than after the cheeses were baked or baked and cooled.

Fresh control (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The control (no oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5

values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values) and a^2 values for fresh cheeses. Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for control (no oil) cheeses showed increased b^4 values (yellowness) after baking (brown areas) than b^3 values. The b^3 values (baked white areas on pizzas) were similar to b^5 and b^6 values for white and brown areas respectively on cooled cheeses but significantly higher than b^2 values for fresh control (no oil) cheeses.

Fresh Control (with oil) cheeses showed similar L^3 and L^5 values (white areas on pizzas). The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The control (with oil) cheeses had significantly lower L^2 values (fresh cheeses) compared to L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than L^3 values (white areas on the baked pizzas) or the L^5 values (white areas on cooled pizzas). The a values for the control (with oil) cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values) and a^2 values for fresh cheeses. Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for control (with oil) cheeses showed increased b^4 values (yellowness) after baking (brown areas) compared to b^3 , b^5 and b^6 values of these cheeses. The b^3 values were similar to b^5 and b^6 values for white and brown areas on cooled cheeses. The fresh cheeses had b^2 values significantly lower than after the cheeses were baked or baked and cooled.

Versagel based (no oil) cheeses showed similar L^3 and L^5 values (white areas on pizzas). The L^4 values of baked brown areas on pizzas, which were hot, were significantly higher to their L^6 values after the pizzas were cooled. The Versagel based (no oil) cheeses had similar L^2 values (fresh cheeses) as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than L^2 values, L^3 values (white areas on the baked pizzas) or the L^5 values (white areas on cooled pizzas). The a values for the Versagel based (no oil) cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). The a^2 values for fresh cheeses were significantly lower than their a^5 values. Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for fresh baked (white and brown areas) and cooled (white and brown areas) on pizzas of Versagel based (no oil) cheeses were similar except that the b^5 values were significantly higher (more yellow) than the b^2 values.

Versagel based (with oil) cheeses showed similar L^3 and L^5 values (white areas on pizzas). The L^4 values of baked brown areas on pizzas, which were hot, were similar to their L^6 values after the pizzas were cooled and L^2 values (fresh cheeses). The Versagel based (with oil) cheeses had similar L^2 values (fresh cheeses) as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than L^3 values (white areas on the baked pizzas) or the L^5 values (white areas on cooled pizzas). The a values for the Versagel based (no oil) cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). The a^2 values for fresh cheeses were similar to their a^3 and a^5 values. Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for fresh baked (white and brown areas) and

cooled (white and brown areas) on pizzas of Versagel based (no oil) cheeses were similar except that the b^4 values were significantly higher (more yellow) than the b^2 values.

10.4.6.4 Pizza bake results for Maltrin based, control and Versagel based cheeses stored for 56 d

10.4.6.4.1 Physical properties as observed with the naked eye of 20 d old ULFM cheeses made from milk pre-acidified using citric acid to pH of 6.1 and mixed with or without fat replacer.

Figure 10.4.9 shows the pizza bake characteristics of Maltrin based, control and Versagel based cheeses after storing for 56 d. The Maltrin based (no oil) cheeses showed increased melt and shred fusion but also had increased browning. Similarly the Maltrin based (with oil) cheeses had completely melted and fused cheeses shreds. The cheeses showed good flow characteristic but were brown due to scorching. The control (with/no oil) cheeses continued to show characteristics similar to those for 29 d and 44 d old cheeses except for the increased browning of the cheeses. The Versagel based (with oil) cheeses showed increased whiteness compared to similar cheeses stored and analysed after 29 d and after 44 d. This could probably be due to re-adsorption of moisture as explained in the expressible serum section, which could make the cheese shreds softer and easier to melt and fuse especially with the added protection of application of oil. The Versagel based (no oil) cheeses that were baked after 56 d showed similar characteristics to similar cheeses when analysed after storing for 44 d.

Table 10.4.9 shows the Hunter L, a, and b values for fresh, baked and cooled Maltrin based, control and Versagel based cheeses applied with oil or not applied with oil and stored for 56 d. Table 10.4.9 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

10.4.6.4.2 Effect of addition of fat replacers to mozzarella cheeses on their Hunter L, a, and b values

The three varieties of cheeses showed similar L^2 , a^2 and b^2 values when examined fresh. After baking the L^3 and a^3 values (white areas on pizzas) for the Maltrin based, control and Versagel based cheeses were similar (statistically not different). The Maltrin based (with oil) and control (with oil) cheeses had significantly higher b^3 values compared to both Maltrin based (no oil) and control (no oil) cheeses. The brown areas on pizzas baked with the three varieties of cheeses showed that control (with oil) cheeses had significantly higher L^4 values than did control (no oil), Maltrin based (no oil) and Versagel based (no oil) cheeses, but were similar to the L^4 values of fat replaced (with oil) cheeses. Also the three varieties of cheeses not applied with oil when examined showed similar L^4 values. The Maltrin based (with oil) cheeses had significantly higher a^4 values compared to control and fat replaced cheeses that were not applied with oil. The control (with oil) cheeses had significantly higher b^4 values than Maltrin based (no oil) and Versagel based (no oil) cheeses. Cooling of pizzas caused Versagel based (with oil) cheeses to show significantly higher L^5 values (white areas on cooled pizzas) than for control (no oil) cheeses. The latter cheeses showed significantly lower L^6 values than the three varieties of cheeses that were applied with oil. The redness (a^5 values) and yellowness (b^5 values) for all cheeses baked and cooled were similar. The a^6 values for Maltrin based (with oil) and Versagel based (with oil) cheeses were significantly higher than for Versagel based (no oil) cheeses. The b^6 values for all three cheeses (with/no oil) were similar.

10.4.6.4.3 Effect of application of oil on the Hunter L, a, and b values of Maltrin based, control and Versagel based cheeses

The fresh Maltrin based, control and Versagel based cheeses showed no effect of application of oil on their Hunter L^2 , a^2 and b^2 values and after baking the cheeses had similar L^3 and a^3 values. The Maltrin based and control (with oil) cheeses had significantly higher b^3 values than their (no oil) cheeses. The brown areas on pizzas showed that control (with oil) cheeses had significantly higher L^4 values than control (no oil) cheeses while Maltrin based and Versagel

based cheeses showed no effect of oil on their L^4 values. The fat replaced cheeses showed increased a^4 values due to application of oil compared to their no oil cheeses. The b^4 values of the three cheeses showed no effect of application of oil. The pizzas when cooled showed no significant effect due to oiling on their L^5 , a^5 and b^5 values (white areas on the pizzas). Control (with oil) cheeses had significantly higher L^6 values than their (no oil) cheeses while the fat replaced cheeses showed no effect on L^6 values due to oil application. The Versagel based (with oil) cheeses had significantly higher a^6 values than Versagel based (no oil) cheeses while Maltrin based and control cheeses had no significant effect on their a^6 values due to oil application. None of the three cheeses showed any effect due to oil application on their b^6 values.

10.4.6.4.4 Effect of baking and cooling pizzas made using Maltrin based, control and Versagel based cheeses on their Hunter L, a, and b values

The fresh Maltrin based (no oil) cheeses showed whiteness (L^2 values) similar to their L^3 values upon baking (white areas on the pizza) and their L^5 values after cooling (white areas on pizzas). The brown areas were statistically less brown (L^4 values) when pizzas were hot than when they were cold (L^6 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and the a^3 values were significantly higher than those for cooled pizzas (a^5 values). Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The white areas on Maltrin based (no oil) cheeses had similar b^3 and b^5 values and these values were significantly higher than b^2 values (fresh), b^4 and b^6 values (brown areas on baked cheeses and cooled cheeses). The yellowness (b -values) of the brown areas and the fresh cheeses were similar.

Fresh Maltrin based (with oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the cheese, which were also significantly higher than L^5 values of the white areas on cooled cheeses. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The Maltrin based (with oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on cooled pizzas (L^5 values). The a values of these cheeses showed that baking increased browning (a^4 values) which were significantly higher than the a^6 values for brown areas after cooling the pizzas. The a^6 values were also significantly higher than a^5 values showing that some parts of the cheeses were extensively red while the white areas on pizzas were significantly less red. The white areas on baked pizzas showed similar a^3 values as after cooling (a^5 values). The fresh Maltrin based (with oil) cheeses had the least red colouration (a^2 values). The white areas on pizzas applied with Maltrin based (with oil) cheeses had significantly higher b^3 values (baked) than their b^5 values (cooled). The b^5 values were significantly higher than b^4 values (brown areas on baked pizzas), the b^4 values significantly reduced after the pizzas were cooled (b^6 values). The yellowness (b-values) of the fresh cheeses was the least and similar to their b^6 values.

The fresh control (no oil) cheeses showed whiteness (L^2 values) similar to their L^3 values upon baking (white areas on the pizza) and their L^5 values after cooling (white areas on pizzas). The brown areas were statistically less brown (L^4 values) when pizzas were hot than when they were cold (L^6 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on cooled pizzas (L^5 values). The a values for the cheeses also

showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the redness/brown colouration of the cheeses significantly compared to the fresh cheeses. The white areas on pizzas applied with control (no oil) cheeses had similar b^3 and b^5 values and these values were significantly higher than b^2 values (fresh), b^4 and b^6 values (brown areas on baked pizzas and cooled pizzas). The yellowness (b-values) of the brown areas and the fresh cheeses were similar.

Fresh control (with oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The control (with oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on cooled pizzas (L^5 values). The a values of these cheeses showed that baking increased browning (a^4 values) which were significantly higher than the a^6 values for brown areas after cooling the pizzas. The a^6 values were also significantly higher than a^5 values showing that some parts of the cheeses were extensively red while the white areas on pizzas were significantly less red. The white areas on baked pizzas showed similar a^3 values as after cooling (a^5 values). The fresh control (with oil) cheeses had the least red colouration (a^2 values). The white areas on pizzas applied with control (with oil) cheeses had significantly higher b^3 values (baked) than their b^5 values (cooled). The b^5 values were similar to b^4 values (brown areas on baked pizzas), the b^4 values significantly reduced after the pizzas were cooled (b^6 values). The yellowness (b-values) of the fresh cheeses was the least and similar to their b^6 values.

Fresh Versagel based (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The Versagel based (no oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on cooled pizzas (L^5 values). The a values of these cheeses showed that baking increased browning (a^4 values) which were significantly higher than the a^6 values for brown areas after cooling the pizzas. The a^6 values were also significantly higher than a^5 values showing that some parts of the cheeses were extensively red while the white areas on pizzas were significantly less red. The white areas on baked pizzas showed similar a^3 values as after cooling (a^5 values). The fresh Versagel based (no oil) cheeses had the least red colouration (a^2 values). The white areas on pizzas applied with Versagel based (no oil) cheeses had similar b^3 and b^5 values and these values were significantly higher than b^2 values (fresh), b^4 and b^6 values (brown areas on baked pizzas and cooled pizzas). The yellowness (b-values) of the brown areas and the fresh cheeses were similar.

Fresh Versagel based (with oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The Versagel based (with oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked

pizzas (L^3 values) or the white areas on cooled pizzas (L^5 values). The a values of these cheeses showed that baking increased browning (a^4 values) which were significantly higher than the a^6 values for brown areas after cooling the pizzas. The a^6 values were also significantly higher than a^5 values showing that some parts of the cheeses were extensively red while the white areas on pizzas were significantly less red. The white areas on baked pizzas showed similar a^3 values as after cooling (a^5 values). The fresh Versagel based (with oil) cheeses had the least red colouration (a^2 values). The white areas on pizzas applied with Versagel based (with oil) cheeses had similar b^3 and b^5 values and these values were significantly higher than b^4 values (brown areas on baked pizzas). The b^4 and b^6 values (brown areas on baked pizzas and cooled pizzas) were similar. The b -values of the brown areas of cooled pizzas (b^6 values) and the fresh cheeses (b^2 values) were similar.

10.4.6.5 Pizza bake results for cheeses stored for 80 d

10.4.6.5.1 Physical properties as observed with the naked eye of 20 d old ULFM cheeses made from milk pre-acidified using citric acid to pH of 6.1 and mixed with or without fat replacer.

Figure 10.4.10 shows pizza bake characteristics for Maltrin based, control and Versagel based cheeses stored for 80 d. The Maltrin based (no oil) cheeses showed almost complete shred fusion and melted with a few pieces of shreds that were left swollen and distorted due to baking. No blister formation was evident except for the incompletely fused shreds, which had swollen. The cheese had a good flow characteristic but showed excessive browning (although not burnt). In comparison Maltrin based (with oil) cheeses showed excellent melt, shred fusion and had good flow properties. Again these cheeses also showed browning, which is not desirable. The control (no oil) cheeses had incompletely fused shreds and showed browning. The control (with oil) cheeses had completely melted shreds and no intact shreds could be observed. But the cheeses turned brown during baking and were unacceptable. Versagel based

(no oil) cheeses had incompletely melted and fused cheese shreds. These cheese shreds were swollen and slightly burnt. The cheeses did not show desired flow characteristics.

Unlike the other cheeses, Versagel based (with oil) cheeses seemed to have better pizza bake characteristics after prolonged storage of 80 d. The cheeses when baked showed no intact shreds, were white in color, melted and flowed beyond the pizza bases. Some localized browning was also observed but it was not extensive. Thus the fat replaced and pre-acidified ULFM cheeses did not show improved pizza bake characteristics compared to the pre-acidified ULFM cheeses (control cheeses) except for Versagel based (with oil) cheeses that were stored for 80 d. The prolonged storage was helpful for the Versagel based cheeses to reabsorb the free moisture and with the application of oil had enough protective effect to perform similar to a full fat mozzarella cheese.

Table 10.4.10 shows the Hunter L, a, and b values for fresh, baked and cooled Maltrin based, control, Versagel based cheeses applied with oil or not applied with oil and stored for 80 d. Table 10.4.10 shows the Hunter L^2 , a^2 , b^2 values for fresh cheeses; L^3 , a^3 , b^3 values for baked cheeses and L^4 , a^4 , b^4 values for cooled cheeses.

10.4.6.5.2 Effect of fat replacer addition to low-fat mozzarella cheeses on their Hunter L, a, and b values

The fresh L^2 , a^2 , and b^2 values for the three varieties of cheeses were similar indicating no adverse effect of fat replacer addition to mozzarella cheeses over the prolonged storage period of 80 d. The baked pizzas showed Versagel based (with oil) cheeses had significantly higher L^3 values and lower a^3 values compared to control (no oil) cheeses. The b^3 values of all the cheeses applied with/without oil were similar. The L^4 values (brown areas on baked) of Maltrin based (with oil) and Versagel based (with oil) cheeses were significantly higher than the L^4 values of Versagel based (no oil) cheeses. The a^4 and b^4 values of control and fat replaced

cheeses applied with/without oil were similar. The Versagel based (with oil) cheeses had significantly higher L^5 values compared to Maltrin based (no oil) and control (no oil) cheeses. The a^5 and b^5 values of control and fat replaced cheeses applied with/without oil were similar. The L^6 , a^6 and b^6 values for the Maltrin based, control and Versagel based (with/no oil) were similar (statistically not different).

10.4.6.5.3 Effect of application of oil to Maltrin based, control and Versagel based cheeses on their Hunter L, a, and b values

The effect of oil application to the cheeses was only significantly different after the cheeses were baked. The Versagel based (with oil) cheeses had significantly higher L^4 values compared to Versagel based (no oil) cheeses. The fresh, baked and cooled pizzas showed no effect due to application of oil on the Hunter a and b values. The L values also were similar except for the Versagel based cheeses (as mentioned above).

10.4.6.5.4 Effect of baking and cooling of pizzas covered with Maltrin based, control and Versagel based cheeses on their Hunter L, a, and b values

Fresh Maltrin based (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The Maltrin based (no oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the redness/brown colouration of the cheeses significantly compared to the fresh cheeses. The white areas on pizzas applied with Maltrin

based (no oil) cheeses had similar b^3 , b^4 , b^5 and b^6 values and these values were significantly higher than b^2 values of fresh cheeses.

The fresh Maltrin based (with oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. Even the brown areas were comparatively more white (L^4 values) when pizzas were hot than when they were cold (L^6 values). The Maltrin based (with oil) cheeses had significantly higher L^5 (white areas of cooled cheeses) values compared to fresh L^2 values. These cheeses also showed increased browning and the L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The L^2 values were similar the L^4 values of these cheeses. The a values for the Maltrin based (with oil) cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). The a^2 values for fresh cheeses were significantly lower than their a^5 values. Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The yellowness (b^3 values) of baked Maltrin based (with oil) cheeses (white areas on pizzas) was significantly lower to b^4 values (brown areas on pizzas) and similar to b^5 and b^6 values (white and brown areas on cooled pizzas). The b -values measured after baking or after baking and cooling were significantly higher than the b^2 values for fresh cheeses.

Fresh control (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The control (no oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5

values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values of these cheeses showed that baking increased browning (a^4 values) which were significantly higher than the a^6 values for brown areas after cooling the pizzas. The a^6 values were also significantly higher than a^5 values showing that some parts of the cheeses were extensively red while the white areas on pizzas were significantly less red. The white areas on baked pizzas showed similar a^3 values as after cooling (a^5 values). The fresh control (no oil) cheeses had the least red colouration (a^2 values). The yellowness (b^3 values) of baked control (no oil) cheeses (white areas on pizzas) was similar to b^4 values (brown areas on pizzas) and b^5 values (white areas on cooled pizzas). But the b^3 values were significantly higher than the b^6 values for the brown areas of cooled pizzas. The b -values measured after baking or after baking and cooling were significantly higher than the b^2 values similar to the trend shown by other cheeses.

The fresh control (with oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. Even the brown areas were comparatively more white (L^4 values) when pizzas were hot than when they were cold (L^6 values). The control (with oil) cheeses had similar (statistically not different) L^2 values when fresh as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the redness/brown colouration of the cheeses significantly compared to the fresh cheeses. The b values for control (with oil) cheeses showed similar b^3 and b^4 values

(yellowness) after baking (white and brown areas) and the b^3 values were similar to b^5 values but significantly higher than b^6 values for brown areas on cooled cheeses. The b^4 values of baked brown areas were also significantly higher than their b^5 values. The fresh cheeses had b^2 values significantly lower than after the cheeses were baked or baked and cooled.

Fresh Versagel based (no oil) cheeses showed increase in whiteness (L^3 values) upon baking for the white areas on the pizza, which were also significantly higher than L^5 values of the white areas on pizzas cooled. The L^4 values of baked brown areas when pizzas were hot were similar to their L^6 values after the pizzas were cooled. The Versagel based (no oil) cheeses had similar (statistically not different) L values when fresh (L^2 values) as after the cheeses were cooled (L^5 values). These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than fresh cheeses (L^2 values) and white areas on the baked pizzas (L^3 values) or the white areas on baked and cooled pizzas (L^5 values). The a values for the cheeses also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values). Baking increased the redness/brown colouration of the cheeses significantly compared to the fresh cheeses. The white areas on pizzas applied with Versagel based (no oil) cheeses had similar b^3 , b^4 , b^5 and b^6 values and these values were significantly higher than b^2 values of fresh cheeses.

Versagel based (with oil) cheeses showed similar L^3 and L^5 values (white areas on pizzas). The L^4 values of baked brown areas on pizzas, which were hot, were similar to their L^6 values after the pizzas were cooled and L^2 values (fresh cheeses). The Versagel based (with oil) cheeses had similar L^2 values (fresh cheeses) as their L^5 values. These cheeses also showed increased browning and the L^4 and L^6 values were significantly less white than L^3 values (white areas on the baked pizzas) or the L^5 values (white areas on cooled pizzas). The a values for the cheeses

also showed that the brown areas of the baked pizzas (a^4 values) and cooled pizzas (a^6 values) were significantly more red than those for the white areas of baked pizzas (a^3 values) and cooled pizzas (a^5 values) and a^2 values for fresh cheeses. Baking increased the red/brown colouration of the cheeses significantly compared to the fresh cheeses. The white areas on pizzas applied with Versagel based (with oil) cheeses had similar b^3 , b^4 , b^5 and b^6 values and these values were significantly higher than b^2 values of fresh cheeses.

10.4.6.6 Stretch test observations for Maltrin based, control and Versagel based mozzarella cheeses stored for 29 d

Maltrin based cheeses showed thick strands, which were smooth and had surface sheen. No surface drying was observed for these cheeses. Among 12 samples analysed, 5 stretched to 300 mm or greater length, 4 samples stretched from 250- 295 mm and 3 samples stretched below 250 mm as shown in Table 10.4.11. A typical stretched sample is shown in Figure 10.4.11. Some samples showed separation of very little moisture prior to performing the stretch test, and this moisture was not reabsorbed. The characteristics of the strand were web-like, thick, shiny surface, smooth without any lumps of unfused or unmelted cheese.

Control cheeses showed similar stretch characteristics to that of Maltrin based cheeses. The strands were thick web-like, smooth and no lumps were seen. The cheese samples had completely melted and were fused together with very little moisture separation. These cheeses showed more surface sheen than the Maltrin based cheeses as shown in Figure 10.4.12. Among 12 samples 5 samples stretched to 300 mm or longer, 5 samples stretched for 250- 295 mm while 2 samples stretched below 250 mm as shown in Table 10.4.12.

Versagel based cheeses showed better stretch property than control and Maltrin based cheeses. Among 12 samples that were analysed 7 of them stretched to 300 mm or longer, 3 samples

stretched to 250- 295 mm and 2 samples showed stretch length of below 250 mm as shown in Table 10.4.13. There were some differences in terms of stretch characteristics, which clearly differentiated these cheeses from the Control and Maltrin based cheeses. One notable difference was that small lumps or raised portions of the cheese could be seen on the strands during stretching. These lumps were very small and solid to touch (so no air bubbles or water trapped pockets as observed by squeezing between the fingers). The other common and important observation for these cheeses was that the cheese tended to lift off from the beaker sides and very little was left in the beaker at the start of the stretch test. Thus as soon as the stretch test was started the cheese fell apart from the beaker sides and then continued to stretch. There was some water separated which was not measured but seemed to be more in quantity compared to control or Maltrin based cheeses.

10.4.6.7 Stretch test observations for Maltrin based, control and Versagel based mozzarella cheeses stored for 40 d.

Maltrin based mozzarella cheeses showed a thick web-like stretched cheese. Most of these samples had little or no moisture separation in the beaker and broke at approximately 300 mm or just after. The cheeses showed some variation in smoothness due to differences between the batches of cheeses but within the same batch the samples behaved similarly. Some of the cheeses showed separation of cheese strings into several thin strands and large holes could be seen within the stretched cheeses. Such strings produced during stretching were not smooth and contained dried cheese particles. Among 12 samples, 10 of them stretched to 300 mm and then broke while the remaining 2 samples had 250- 270 mm stretch as shown in Table 10.4.11. These variations in stretch distances for the few samples could be due to slight variations within the cheese samples or during the conduction of the stretch test.

Control mozzarella cheeses showed good melt and stretched smoothly. The stretched cheese mostly was web-like with few holes. Among 12 samples analysed for control cheeses 10 samples stretched to 300 mm or longer while the remaining 2 samples broke just before 300 mm i.e. at 290 and 298 mm as shown in Table 10.4.12. The control samples seemed to have more gloss or shine and were smoother than Maltrin based cheeses.

Versagel based cheeses showed good stretch with all the 12 samples stretching to a minimum length of 300 mm as shown in Table 10.4.13. All the samples showed string formation and tended to have many voids while being stretched. These samples were more stringy than control cheeses. Also, before the cheeses were stretched, moisture separation was observed on top of the melted cheeses in the beakers. The amount of moisture separated seemed more than that for control and Maltrin based cheeses. A few samples could be stretched to 900 mm (3 times the normal stretch distance) as shown in Figure 10.4.13. The strands that had been formed were broken at approximately 890 mm. The Versagel cheeses stretched exceptionally well and had good gloss and surface shine.

10.4.6.8 Stretch test observations for Maltrin based, control and Versagel based mozzarella cheeses stored for 55 d.

Maltrin based mozzarella cheeses showed a thick web-like stretched cheese. The samples did not show any moisture separation in the beaker and stretched to more than 300 mm. The cheeses showed some variation in smoothness due to differences between the batches of cheeses but within the same batch the samples behaved similarly. The cheeses showed very little separation of stretched cheese into strings. The stretched cheese had a glossy/shiny surface characteristic. Only 1 sample stretched to 250 mm, which could be because the sample may not have been uniformly warmed to the desired temperature (Table 10.4.11).

Control cheeses when stretched formed into thin strings. The melted cheeses did not show any moisture separation in the beakers. The cheese samples seemed to have melted uniformly but when stretched the cheese showed lumps similar to particles embedded in the strands formed. Although, the cheeses had the characteristic stretch of a web like membrane, the formation of strings was obvious. All the samples stretched to 300 mm (Table 10.4.12) but in all the samples most of the strings broke before reaching 300 mm extension. Only the remaining strings that were formed during stretching continued to resist the external force exerted by the Instron.

Versagel based cheeses melted uniformly in the beaker and some moisture separation was observed in all the samples, although measurement was not possible due to the meagre amount of moisture that was visible on the surface. The cheeses when stretched formed a web like membrane, which seemed to have particles or lumps in the interior of the stretched cheeses. Among 12 samples analysed for stretch test, 8 of them stretched to greater than 300 mm while the remaining 4 samples stretched to 250- 295 mm as shown in Table 10.4.13.

10.4.6.9 Stretch test observations for Maltrin based, control and Versagel based mozzarella cheeses stored for 75 d.

Maltrin based cheeses had similar characteristics of stretchability at 75 d to that at 55 d. The stretch distances decreased after the prolonged storage and 5 samples stretched to 300 mm or longer while 4 of the samples had 250- 295 mm stretch and the remaining one sample had a reduced stretch of 230 mm as shown in Table 10.4.11.

Control cheeses showed poor stretch characteristics compared to Maltrin based cheeses at 75 d. Four samples showed greater than 300 mm stretch while 6 of the samples had below 250 mm of stretch as shown in Table 10.4.12. Although the cheeses stretched smoothly, they had reduced stretch length.

Versagel based cheeses continued to have better stretchability than Maltrin based and control cheeses. Seven samples showed greater than 300 mm of stretch, two had stretched to 250- 295 mm and remaining two stretched to below 250 mm as shown in Table 10.4.13. Thus Versagel based cheeses showed better stretch distance but had small lumps embedded within the stretched strands.

10.5 Conclusions

The fat replaced cheeses did not show any increase in yield due to addition of Maltrin M100 or Versagel. The Maltrin based and control cheeses were similar in composition including moisture, FDM and M:P contents and were lower than for Versagel based cheeses. The protein contents of Maltrin based and control cheeses were higher than that for Versagel based cheeses. HPLC analysis was unable to determine the amount of Maltrin remaining in the curd although estimation of DP7 (maltoheptaose) showed similar oligosaccharides to be very low in concentration in the Maltrin based cheeses. All the cheeses expressed measurable amounts of serum after 22 d of manufacture. Maltrin based and control cheeses showed negligible amounts of expressible serum at 35 d while Versagel continued to show proportionally higher amounts. During storage all cheeses showed a decreasing trend in the amount of expressible serum. Versagel based cheeses showed the least amount of proteolysis through out storage compared to Maltrin based and control cheeses. Versagel based cheeses showed reduced hardness compared to control and Maltrin based cheeses through out storage and the hardness values for the three cheeses decreased with storage. Cohesiveness values for Maltrin based, control and Versagel based cheeses were similar and these values increased with storage period for the three varieties of cheeses. Springiness values for Versagel based cheeses were higher than those for control and Maltrin based cheeses. Maltrin based cheeses showed greater meltability

throughout the storage than Versagel based cheeses and the former cheeses also had greater meltability than control cheeses at 45 and 60 d of storage.

Pizza bake characteristics showed Maltrin based cheeses to have better melt, shred fusion and flow characteristics compared to control and Versagel based cheeses. The latter cheeses showed burning and had several brown blisters on their surface at 29 d. Application of oil was found to be necessary for proper shred fusion. Versagel based cheeses showed the least shred fusion at 29 d and the shreds did not melt completely even by 44 d. Maltrin and control cheeses also showed browning although to a lesser extent compared to Versagel based cheeses at 44 d. Storage seemed to improve the pizza bake characteristics of Versagel based cheeses and these cheeses showed better functionality at 56 d. The control and Maltrin based cheeses did not show any improvement due to prolonged storage up to 56 d. Application of oil did not produce any defects in the stored cheeses. Prolonged storage to 80 d improved the melt and shred fusion characteristics of Maltrin based cheeses but the cheeses showed undesirable browning. Control cheeses did not show sufficient shred fusion and melt and intact shreds caused scorching of the cheese. Unlike control and Maltrin based cheeses, the Versagel based cheeses that were applied with oil had improved melt and shred fusion. The cheeses had improved flow and showed increased whiteness after 80 d of storage. Versagel based cheeses required prolonged storage of 80 d along with oil application to show improved cheese characteristics.

Maltrin based cheeses showed reduced stretch distance at 29 d and this characteristic was improved by 40 d. The Maltrin based cheeses had excellent stretch at 55 d of storage, which decreased with further increase in storage. Control cheeses also showed a similar stretchability as Maltrin based cheeses and had the highest stretch ability at 55d of storage which decreased at 75 d. Versagel based cheeses showed reduced stretch distance at 29 d but the cheeses

achieved maximum stretchability as early as 40 d. These cheeses showed reduced in stretchability at 55 and 75 d of storage.

Overall both the fat replacers showed improved functional characteristics in the ULFM cheeses. The results showed that improvements could be made in the characteristics of fat replaced ULFM cheeses by adding fat replacers. The rate of addition of fat replacer will be modified to further improve the characteristics of fat replaced ULFM cheeses.

Table 10.4.1 Yield and composition of Maltrin based cheese, control cheese and Versagel based cheese (n = 12).

Characteristic	Maltrin based cheese	Control cheese	Versagel based cheese	
	Mean ± SE ¹	Mean ± SE ¹	Mean ± SE ¹	p-value ²
Yield ³ (Kg/200 L)	14.203 ± 0.351 ^a	14.342 ± 0.302 ^a	15.192 ± 0.454 ^a	0.1857
Moisture (%)	52.22 ± 0.18 ^c	52.41 ± 0.07 ^{bc}	54.65 ± 0.15 ^a	0.0000 [*]
Protein (%)	36.99 ± 0.29 ^a	37.37 ± 0.21 ^a	35.30 ± 0.19 ^b	0.0000 [*]
FDM ⁴ (%)	12.56 ± 0.05 ^c	12.61 ± 0.02 ^{bc}	13.23 ± 0.04 ^a	0.0000 [*]
M:P ⁵	1.41 ± 0.01 ^{bc}	1.40 ± 0.01 ^c	1.55 ± 0.01 ^a	0.0000 [*]

¹Mean ± Standard error; ²ANOVA of means; ³n= 4 ; ⁴FDM= Fat in dry matter; ⁵M:P= Moisture: Protein; ^{a, b, c} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 10.4.2 Residual maltoheptaose (DP7) in Maltrin based cheeses (n = 8).

Cheese replicates	Mean quantity of DP7 (µg/g of cheese)	Standard error
04V1	2.70	0.27
05V2	2.73	0.05
06V3	2.70	0.14
07V1	2.34	0.18

Table 10.4.3 Hardness values (Mean \pm SE¹) measured in Newton force for Maltrin based cheese, control cheese and Versagel based cheese measured (n = 12) during refrigerated storage.

Storage (d)	Maltrin based cheese	Control cheese	Versagel based cheese	p-value ²
15	98.96 \pm 6.45 ^{a, B}	118.76 \pm 5.08 ^{a, A}	74.25 \pm 4.24 ^{ab, C}	0.0000 [*]
29	80.93 \pm 4.32 ^{a, AB}	92.07 \pm 6.12 ^{bcd, A}	69.78 \pm 4.77 ^{ab, B}	0.0157 [*]
44	87.97 \pm 8.85 ^{a, A}	91.72 \pm 3.38 ^{cd, A}	79.92 \pm 4.34 ^{a, A}	0.3773
56	105.00 \pm 8.21 ^{a, A}	101.67 \pm 5.11 ^{abc, A}	83.65 \pm 3.38 ^{a, A}	0.3460
80	79.20 \pm 3.89 ^{a, A}	76.72 \pm 5.27 ^{d, A}	61.07 \pm 2.74 ^{b, B}	0.0072 [*]
p-value ³	0.3190	0.0000 [*]	0.0020 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c, d} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05).

Table 10.4.4 Cohesiveness values (Mean \pm SE¹) for Maltrin based cheese, control cheese and Versagel based cheese measured (n = 12) during refrigerated storage.

Storage (d)	Maltrin based cheese	Control cheese	Versagel based cheese	p-value ²
15	0.52 \pm 0.04 ^{b, A}	0.61 \pm 0.03 ^{b, A}	0.55 \pm 0.03 ^{b, A}	0.1323
29	0.60 \pm 0.02 ^{ab, B}	0.68 \pm 0.02 ^{ab, A}	0.66 \pm 0.01 ^{a, AB}	0.0125 [*]
44	0.61 \pm 0.04 ^{ab, B}	0.71 \pm 0.01 ^{a, A}	0.67 \pm 0.01 ^{a, AB}	0.0165 [*]
56	0.69 \pm 0.01 ^{a, A}	0.70 \pm 0.01 ^{a, A}	0.69 \pm 0.01 ^{a, A}	0.5487
80	0.72 \pm 0.01 ^{a, A}	0.72 \pm 0.01 ^{a, A}	0.71 \pm 0.01 ^{a, A}	0.6482
p-value ³	0.0000 [*]	0.0001 [*]	0.0000 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B} Means within same row not sharing common superscripts differ (p<0.05);

^{*} Significant (p<0.05).

Table 10.4.5 Springiness values (Mean \pm SE¹) measured in mm for Maltrin based cheese, control cheese and Versagel based cheese measured (n = 12) during refrigerated storage.

Storage (d)	Maltrin based cheese	Control cheese	Versagel based cheese	p-value ²
15	1.31 \pm 0.10 ^{c, B}	1.50 \pm 0.11 ^{a, AB}	1.73 \pm 0.10 ^{ab, A}	0.0238 [*]
29	1.39 \pm 0.05 ^{bc, C}	1.56 \pm 0.04 ^{a, B}	1.72 \pm 0.04 ^{b, A}	0.0000 [*]
44	1.51 \pm 0.08 ^{abc, B}	1.64 \pm 0.05 ^{a, AB}	1.84 \pm 0.05 ^{ab, A}	0.0022 [*]
56	1.69 \pm 0.07 ^{ab, AB}	1.46 \pm 0.06 ^{a, B}	1.82 \pm 0.05 ^{ab, A}	0.0014 [*]
80	1.80 \pm 0.05 ^{a, BC}	1.75 \pm 0.06 ^{a, C}	2.01 \pm 0.05 ^{a, A}	0.0029 [*]
p-value ³	0.0000 [*]	0.3720	0.0164 [*]	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05).

Table 10.4.6 Meltability (Mean \pm SE¹) measured in mm for Maltrin based cheese, control cheese and Versagel based cheese measured (n = 12) during refrigerated storage.

Storage (d)	Maltrin based cheese	Control cheese	Versagel based cheese	p-value ²
22	57.88 \pm 1.29 ^{bcd, A}	55.74 \pm 1.39 ^{c, A}	43.68 \pm 1.57 ^{a, B}	0.0000 [*]
32	56.74 \pm 0.99 ^{cd, A}	57.18 \pm 1.09 ^{bc, A}	44.64 \pm 1.17 ^{a, B}	0.0000 [*]
45	52.99 \pm 2.66 ^{d, A}	43.55 \pm 1.51 ^{e, C}	45.68 \pm 1.42 ^{a, BC}	0.0044 [*]
60	59.85 \pm 0.79 ^{abc, A}	48.42 \pm 2.33 ^{de, BC}	46.29 \pm 1.87 ^{a, C}	0.0000 [*]
85	65.62 \pm 1.11 ^{a, A}	67.16 \pm 1.05 ^{a, A}	48.74 \pm 2.14 ^{a, B}	0.0000 [*]
p-value ³	0.0000 [*]	0.0000 [*]	0.2747	

¹Mean \pm Standard error; ²ANOVA of means arranged within the same row; ³ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05);

^{*} Significant (p<0.05).

Table 10.4.7 Mean \pm SE¹ (n = 12) Hunter L a b-values of Maltrin based cheese, control cheese and Versagel based cheese, applied with or without canola oil and stored for 29 d at refrigerated temperature, measured fresh² after baking (white³ and brown⁴) and after cooling (white⁵ and brown⁶) to room temperature.

Hunter L a b	Maltrin based cheese		Control cheese		Versagel based cheese		p-value ⁷
	No oil	With Oil	No oil	With Oil	No Oil	With Oil	
L ²	59.58 \pm 0.79 ^{bc, A}	59.93 \pm 0.87 ^{c, A}	59.20 \pm 1.14 ^{bc, A}	59.95 \pm 1.37 ^{bc, A}	59.87 \pm 0.87 ^{c, A}	61.52 \pm 0.60 ^{c, A}	0.6502
L ³	70.25 \pm 0.99 ^{a, A}	69.13 \pm 0.98 ^{a, A}	66.55 \pm 0.80 ^{a, A}	66.99 \pm 1.26 ^{a, A}	70.97 \pm 1.07 ^{a, A}	69.54 \pm 0.86 ^{a, A}	0.1370
L ⁴	50.33 \pm 1.94 ^{d, A}	51.07 \pm 2.01 ^{de, A}	48.58 \pm 1.73 ^{d, A}	49.81 \pm 2.05 ^{d, A}	47.53 \pm 1.81 ^{d, A}	49.43 \pm 2.11 ^{de, A}	0.8319
L ⁵	57.22 \pm 0.99 ^{c, AB}	61.86 \pm 0.66 ^{bc, A}	54.78 \pm 0.91 ^{c, B}	58.44 \pm 1.14 ^{c, AB}	60.63 \pm 1.19 ^{bc, A}	62.04 \pm 1.32 ^{bc, A}	0.0000*
L ⁶	39.89 \pm 0.93 ^{c, BC}	45.54 \pm 1.28 ^{e, A}	38.88 \pm 1.18 ^{e, C}	42.12 \pm 0.66 ^{e, ABC}	41.08 \pm 0.92 ^{e, ABC}	44.81 \pm 1.69 ^{e, AB}	0.0004*
a ²	-4.29 \pm 0.18 ^{d, A}	-4.21 \pm 0.11 ^{d, A}	-4.50 \pm 0.09 ^{e, A}	-4.54 \pm 0.11 ^{d, A}	-4.32 \pm 0.19 ^{d, A}	-4.54 \pm 0.09 ^{e, A}	0.3337
a ³	2.01 \pm 0.98 ^{bc, A}	0.74 \pm 1.03 ^{bc, A}	5.14 \pm 0.99 ^{cd, A}	2.72 \pm 1.12 ^{bc, A}	0.96 \pm 0.82 ^{bc, A}	1.32 \pm 0.88 ^{d, A}	0.2310
a ⁴	13.95 \pm 1.41 ^{a, A}	17.04 \pm 1.40 ^{a, A}	15.25 \pm 0.61 ^{a, A}	17.27 \pm 1.63 ^{a, A}	14.81 \pm 0.87 ^{a, A}	16.88 \pm 1.16 ^{at, A}	0.2898
a ⁵	1.96 \pm 0.71 ^{c, A}	0.08 \pm 0.53 ^{c, A}	2.98 \pm 0.65 ^{d, A}	2.24 \pm 0.78 ^{c, A}	0.94 \pm 0.66 ^{c, A}	1.95 \pm 0.92 ^{cd, A}	0.0844
a ⁶	12.78 \pm 0.80 ^{a, A}	13.60 \pm 0.74 ^{a, A}	11.84 \pm 0.42 ^{b, A}	14.19 \pm 0.23 ^{a, A}	12.01 \pm 0.54 ^{a, A}	12.69 \pm 0.88 ^{b, A}	0.0927
b ²	15.86 \pm 0.45 ^{d, A}	14.11 \pm 0.48 ^{d, A}	15.31 \pm 0.33 ^{d, A}	15.06 \pm 0.37 ^{d, A}	14.78 \pm 0.41 ^{c, A}	15.30 \pm 0.39 ^{c, A}	0.0741
b ³	29.98 \pm 1.25 ^{a, A}	31.21 \pm 1.36 ^{ab, A}	31.40 \pm 0.76 ^{a, A}	33.73 \pm 1.27 ^{a, A}	28.67 \pm 0.82 ^{a, A}	29.93 \pm 1.14 ^{ab, A}	0.4240
b ⁴	26.80 \pm 1.17 ^{ab, AB}	37.13 \pm 2.45 ^{a, A}	27.78 \pm 1.87 ^{ab, AB}	33.64 \pm 2.50 ^{ab, A}	25.98 \pm 1.90 ^{ab, B}	32.29 \pm 2.84 ^{a, AB}	0.0026*
b ⁵	25.71 \pm 0.76 ^{b, A}	26.41 \pm 0.88 ^{bc, A}	25.06 \pm 0.74 ^{bc, A}	26.78 \pm 0.99 ^{bc, A}	26.62 \pm 0.74 ^{ab, A}	28.30 \pm 0.76 ^{ab, A}	0.1218
b ⁶	20.66 \pm 0.58 ^{c, A}	24.55 \pm 1.07 ^{c, A}	21.51 \pm 1.23 ^{c, A}	21.50 \pm 0.95 ^{c, A}	22.09 \pm 0.82 ^{b, A}	24.30 \pm 1.51 ^{b, A}	0.0574
L a b ²³⁴⁵⁶ (p-value ⁸)	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked white regions; ⁴Baked brown regions; ⁵Cooled white regions; ⁶Cooled brown regions; ⁷ANOVA of means arranged within the same row; ⁸ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

Table 10.4.8 Mean \pm SE¹ (n = 12) Hunter L a b-values of Maltrin based cheese, control cheese and Versagel based cheese, applied with or without canola oil and stored for 44 d at refrigerated temperature, measured fresh², after baking (white³ and brown⁴) and after cooling (white⁵ and brown⁶) to room temperature.

Hunter L a b	Maltrin based cheese		Control cheese		Versagel based cheese		p-value ⁷
	No oil	With Oil	No oil	With Oil	No Oil	With Oil	
L ²	57.81 \pm 0.77 ^{c, A}	57.88 \pm 0.73 ^{b, A}	57.51 \pm 0.87 ^{c, A}	57.51 \pm 0.90 ^{bcd, A}	60.75 \pm 1.10 ^{b, A}	59.60 \pm 1.04 ^{bc, A}	0.0658
L ³	72.59 \pm 0.82 ^{a, A}	65.12 \pm 1.80 ^{a, A}	70.94 \pm 1.20 ^{a, A}	66.64 \pm 2.21 ^{a, A}	72.87 \pm 1.60 ^{a, A}	70.04 \pm 1.60 ^{a, A}	0.6600
L ⁴	52.27 \pm 1.61 ^{d, CDE}	65.12 \pm 1.80 ^{a, A}	40.24 \pm 0.68 ^{e, E}	57.33 \pm 3.26 ^{cd, ABCD}	49.83 \pm 2.52 ^{cd, DE}	54.46 \pm 1.40 ^{cd, BCDE}	0.0000*
L ⁵	64.16 \pm 0.54 ^{b, AB}	61.92 \pm 1.34 ^{ab, B}	62.08 \pm 1.35 ^{bc, AB}	68.02 \pm 0.74 ^{a, A}	66.43 \pm 0.85 ^{ab, AB}	65.53 \pm 1.94 ^{ab, AB}	0.0044*
L ⁶	44.44 \pm 1.13 ^{e, A}	47.80 \pm 1.96 ^{c, A}	41.83 \pm 2.68 ^{de, A}	50.38 \pm 2.98 ^{d, A}	45.57 \pm 4.76 ^{d, A}	47.84 \pm 1.90 ^{d, A}	0.2639
a ²	-4.39 \pm 0.11 ^{d, A}	-4.38 \pm 0.12 ^{d, A}	-4.46 \pm 0.14 ^{d, A}	-4.46 \pm 0.15 ^{d, A}	-4.40 \pm 0.11 ^{d, A}	-4.45 \pm 0.15 ^{d, A}	0.9959
a ³	-1.38 \pm 0.33 ^{c, AB}	1.48 \pm 1.15 ^{bcd, A}	-2.81 \pm 0.45 ^{cd, AB}	-3.68 \pm 0.50 ^{cd, B}	-1.48 \pm 0.74 ^{cd, AB}	-1.69 \pm 1.01 ^{bcd, AB}	0.0066*
a ⁴	10.49 \pm 2.32 ^{a, A}	10.30 \pm 2.33 ^{a, A}	9.04 \pm 2.44 ^{a, A}	10.81 \pm 1.74 ^{a, A}	11.46 \pm 1.21 ^{a, A}	12.80 \pm 1.36 ^{a, A}	0.7632
a ⁵	-0.95 \pm 0.55 ^{bc, A}	-2.57 \pm 0.96 ^{cd, A}	-0.83 \pm 0.50 ^{bcd, A}	-2.36 \pm 0.69 ^{bcd, A}	-0.70 \pm 0.61 ^{bc, A}	-2.59 \pm 0.70 ^{cd, A}	0.1214
a ⁶	10.29 \pm 1.10 ^{a, A}	9.30 \pm 2.69 ^{a, A}	11.10 \pm 1.12 ^{a, A}	11.78 \pm 2.37 ^{a, A}	14.05 \pm 1.76 ^{a, A}	12.10 \pm 0.88 ^{a, A}	0.4447
b ²	15.22 \pm 0.43 ^{e, A}	14.92 \pm 0.43 ^{c, A}	15.63 \pm 0.46 ^{d, A}	15.25 \pm 0.39 ^{e, A}	15.65 \pm 0.36 ^{b, A}	15.34 \pm 0.48 ^{b, A}	0.8376
b ³	27.67 \pm 0.51 ^{bc, C'DE}	33.68 \pm 1.66 ^{ab, A}	23.26 \pm 0.90 ^{bc, E}	27.41 \pm 1.21 ^{d, DE}	28.04 \pm 0.91 ^{ab, BCDE}	29.26 \pm 1.86 ^{ab, ABCDE}	0.0000*
b ⁴	39.70 \pm 1.17 ^{a, AB}	38.75 \pm 1.99 ^{a, AB}	30.58 \pm 1.24 ^{a, BC}	40.69 \pm 1.74 ^{a, A}	25.75 \pm 2.38 ^{ab, C}	33.37 \pm 1.44 ^{a, ABC}	0.0000*
b ⁵	27.16 \pm 0.77 ^{c, A}	30.24 \pm 1.18 ^{b, A}	26.77 \pm 0.66 ^{ab, A}	27.52 \pm 0.82 ^{cd, A}	28.05 \pm 0.81 ^{a, A}	29.28 \pm 1.04 ^{ab, A}	0.0734
b ⁶	22.76 \pm 1.01 ^{d, AB}	31.92 \pm 1.67 ^{ab, A}	18.58 \pm 1.88 ^{cd, B}	29.55 \pm 1.73 ^{bcd, AB}	26.79 \pm 2.22 ^{ab, AB}	28.48 \pm 1.52 ^{ab, AB}	0.0032*
L a b ^{2,4,5,6}	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked white regions; ⁴Baked brown regions; ⁵Cooled white regions; ⁶Cooled brown regions; ⁷ANOVA of means arranged within the same row; ⁸ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D, E} Means within same row not sharing common superscripts differ (p<0.05); *Significant (p<0.05).

Table 10.4.9 Mean \pm SE¹ (n = 12) Hunter L a b-values of Maltrin based cheese, control cheese and Versagel based cheese, applied with or without canola oil and stored for 56 d at refrigerated temperature, measured fresh², after baking (white³ and brown⁴) and after cooling (white⁵ and brown⁶) to room temperature.

Hunter	Maltrin based cheese		Control cheese		Versagel based cheese		p-value ⁷
	No oil	With Oil	No oil	With Oil	No Oil	With Oil	
L a b							
L ²	57.11 \pm 0.69 ^{a, A}	57.03 \pm 0.97 ^{bc, A}	57.05 \pm 1.33 ^{a, A}	55.75 \pm 1.00 ^{c, A}	59.62 \pm 0.79 ^{bc, A}	59.48 \pm 0.67 ^{c, A}	0.2760
L ³	59.81 \pm 2.38 ^{a, A}	61.91 \pm 1.65 ^{a, A}	60.27 \pm 3.39 ^{a, A}	62.80 \pm 1.54 ^{a, A}	67.31 \pm 1.38 ^{a, A}	67.89 \pm 1.50 ^{a, A}	0.2490
L ⁴	38.77 \pm 0.92 ^{bc, BCD}	42.20 \pm 0.89 ^{de, ABCD}	38.75 \pm 0.98 ^{bc, CD}	44.66 \pm 0.89 ^{de, A}	37.87 \pm 1.20 ^{de, D}	42.67 \pm 1.28 ^{de, ABCD}	0.0000*
L ⁵	56.09 \pm 1.36 ^{a, CD}	56.40 \pm 0.96 ^{c, BCD}	53.15 \pm 1.29 ^{a, D}	56.90 \pm 1.10 ^{bc, ABCD}	57.44 \pm 1.28 ^{c, ABCD}	61.85 \pm 1.27 ^{bc, A}	0.0008*
L ⁶	36.73 \pm 0.57 ^{c, BCD}	40.58 \pm 0.41 ^{e, ABC}	35.80 \pm 0.81 ^{c, D}	41.38 \pm 0.73 ^{e, A}	36.41 \pm 1.60 ^{c, CD}	40.45 \pm 0.83 ^{c, ABC}	0.0000*
a ²	-4.39 \pm 0.17 ^{d, A}	-4.31 \pm 0.10 ^{e, A}	-4.29 \pm 0.21 ^{d, A}	-4.41 \pm 0.18 ^{e, A}	-4.41 \pm 0.13 ^{e, A}	-4.67 \pm 0.11 ^{e, A}	0.5659
a ³	9.78 \pm 1.76 ^{b, A}	7.94 \pm 1.60 ^{cd, A}	6.90 \pm 2.51 ^{bc, A}	6.37 \pm 1.49 ^{cd, A}	3.58 \pm 1.01 ^{d, A}	2.24 \pm 1.43 ^{d, A}	0.3130
a ⁴	16.16 \pm 0.40 ^{a, BCD}	19.10 \pm 0.43 ^{a, A}	15.97 \pm 0.43 ^{a, D}	18.36 \pm 0.51 ^{a, ABC}	16.15 \pm 0.58 ^{a, CD}	17.66 \pm 0.47 ^{a, ABCD}	0.0000*
a ⁵	4.70 \pm 0.74 ^{c, A}	5.84 \pm 0.89 ^{d, A}	5.10 \pm 1.81 ^{c, A}	5.65 \pm 0.86 ^{d, A}	4.11 \pm 0.98 ^{cd, A}	2.84 \pm 1.35 ^{cd, A}	0.3613
a ⁶	13.33 \pm 0.31 ^{a, AB}	14.54 \pm 0.19 ^{b, A}	13.17 \pm 0.33 ^{a, AB}	14.21 \pm 0.26 ^{b, AB}	12.88 \pm 0.35 ^{b, B}	14.32 \pm 0.23 ^{b, A}	0.0001*
b ²	15.20 \pm 0.57 ^{d, A}	14.13 \pm 0.29 ^{e, A}	14.41 \pm 0.46 ^{d, A}	14.59 \pm 0.41 ^{e, A}	14.74 \pm 0.34 ^{d, A}	15.33 \pm 0.41 ^{d, A}	0.3255
b ³	31.19 \pm 1.14 ^{a, BC}	38.95 \pm 1.43 ^{a, A}	29.94 \pm 1.62 ^{a, C}	39.62 \pm 1.56 ^{a, A}	33.01 \pm 1.08 ^{a, ABC}	34.33 \pm 2.04 ^{a, ABC}	0.0000*
b ⁴	18.12 \pm 1.15 ^{bcd, BC}	22.74 \pm 1.57 ^{c, ABC}	18.84 \pm 1.03 ^{bcd, ABC}	26.07 \pm 2.03 ^{c, A}	18.04 \pm 1.33 ^{bcd, C}	21.84 \pm 2.10 ^{bc, ABC}	0.0028*
b ⁵	29.43 \pm 1.22 ^{a, A}	30.71 \pm 1.14 ^{b, A}	29.93 \pm 0.98 ^{a, A}	31.36 \pm 0.63 ^{bc, A}	29.52 \pm 0.93 ^{a, A}	29.95 \pm 0.71 ^{a, A}	0.6769
b ⁶	17.69 \pm 0.79 ^{cd, A}	17.73 \pm 0.71 ^{de, A}	15.45 \pm 1.10 ^{cd, A}	18.50 \pm 1.06 ^{de, A}	17.18 \pm 1.76 ^{cd, A}	18.90 \pm 1.20 ^{cd, A}	0.3718
L a b ²³⁴⁵⁶	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked white regions; ⁴Baked brown regions; ⁵Cooled white regions; ⁶Cooled brown regions; ⁷ANOVA of means arranged within the same row; ⁸ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C, D} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

Table 10.4.10 Mean \pm SE¹ (n = 12) Hunter L a b-values of Maltrin based cheese, control cheese and Versagel based cheese, applied with or without canola oil and stored for 80 d at refrigerated temperature, measured fresh² (before baking), after baking (white³ and brown⁴) and after cooling (white⁵ and brown⁶) to room temperature.

Hunter L a b	Maltrin based cheese		Control cheese		Versagel based cheese		p-value ⁷
	No oil	With Oil	No oil	With Oil	No Oil	With Oil	
L ²	57.87 \pm 1.05 ^{c, A}	58.47 \pm 0.81 ^{cd, A}	59.34 \pm 1.04 ^{bc, A}	60.49 \pm 1.02 ^{c, A}	60.06 \pm 1.20 ^{c, A}	60.07 \pm 0.85 ^{bc, A}	0.3979
L ³	68.51 \pm 0.90 ^{a, AB}	70.64 \pm 0.80 ^{a, AB}	67.33 \pm 1.26 ^{a, B}	68.06 \pm 0.95 ^{a, AB}	71.67 \pm 1.48 ^{a, AB}	72.76 \pm 0.77 ^{a, A}	0.0028*
L ⁴	49.47 \pm 2.45 ^{de, AB}	56.09 \pm 1.11 ^{d, A}	47.98 \pm 1.22 ^{de, AB}	52.75 \pm 0.74 ^{d, AB}	44.51 \pm 1.43 ^{de, B}	54.43 \pm 3.20 ^{cd, A}	0.0012*
L ⁵	58.79 \pm 1.38 ^{bc, BC}	62.89 \pm 1.06 ^{b, ABC}	58.07 \pm 1.07 ^{c, C}	62.39 \pm 0.68 ^{bc, ABC}	62.94 \pm 1.90 ^{bc, ABC}	65.15 \pm 1.44 ^{ab, A}	0.0011*
L ⁶	45.45 \pm 1.59 ^{e, A}	47.97 \pm 1.06 ^{e, A}	44.92 \pm 1.20 ^{e, A}	46.97 \pm 0.68 ^{e, A}	43.07 \pm 1.97 ^{e, A}	48.18 \pm 2.45 ^{d, A}	0.1568
a ²	-4.38 \pm 0.19 ^{d, A}	-4.18 \pm 0.16 ^{d, A}	-4.54 \pm 0.17 ^{c, A}	-4.66 \pm 0.13 ^{d, A}	-4.37 \pm 0.16 ^{d, A}	-4.35 \pm 0.14 ^{d, A}	0.3631
a ³	2.91 \pm 0.86 ^{bc, AB}	-0.25 \pm 0.93 ^{cd, AB}	3.15 \pm 1.05 ^{cd, A}	1.84 \pm 1.00 ^{bc, AB}	0.52 \pm 1.10 ^{c, AB}	-1.75 \pm 0.75 ^{cd, B}	0.0052*
a ⁴	14.20 \pm 1.16 ^{a, A}	11.32 \pm 2.13 ^{a, A}	16.94 \pm 0.42 ^{a, A}	16.48 \pm 0.82 ^{a, A}	16.23 \pm 0.89 ^{a, A}	13.95 \pm 1.83 ^{a, A}	0.0394*
a ⁵	2.41 \pm 0.75 ^{c, A}	3.75 \pm 1.69 ^{bc, A}	2.91 \pm 0.77 ^{d, A}	1.21 \pm 0.79 ^{c, A}	0.63 \pm 1.00 ^{bc, A}	-0.18 \pm 1.12 ^{bcd, A}	0.1290
a ⁶	12.88 \pm 0.64 ^{a, A}	13.71 \pm 0.90 ^{a, A}	12.93 \pm 0.59 ^{b, A}	13.81 \pm 0.55 ^{a, A}	13.19 \pm 0.59 ^{a, A}	11.69 \pm 0.87 ^{a, A}	0.3532
b ²	16.02 \pm 0.42 ^{b, A}	14.42 \pm 0.44 ^{c, A}	15.76 \pm 0.37 ^{c, A}	15.43 \pm 0.45 ^{d, A}	15.58 \pm 0.40 ^{b, A}	15.84 \pm 0.40 ^{b, A}	0.1070
b ³	31.99 \pm 1.24 ^{a, A}	31.52 \pm 1.11 ^{bcd, A}	32.76 \pm 0.82 ^{a, A}	33.55 \pm 1.37 ^{ab, A}	29.74 \pm 0.64 ^{a, A}	28.78 \pm 0.93 ^{a, A}	0.3530
b ⁴	29.57 \pm 1.70 ^{a, AB}	37.42 \pm 1.49 ^{a, A}	28.85 \pm 1.25 ^{ab, AB}	35.63 \pm 1.30 ^{a, A}	25.47 \pm 2.10 ^{a, B}	31.47 \pm 2.92 ^{a, AB}	0.0004*
b ⁵	29.33 \pm 0.54 ^{a, A}	28.35 \pm 0.76 ^{cd, A}	29.57 \pm 0.75 ^{ab, A}	29.79 \pm 0.92 ^{bc, A}	28.42 \pm 0.54 ^{a, A}	28.87 \pm 0.52 ^{a, A}	0.6124
b ⁶	26.23 \pm 1.66 ^{a, A}	28.28 \pm 0.90 ^{d, A}	26.91 \pm 1.14 ^{b, A}	28.46 \pm 0.97 ^{c, A}	25.61 \pm 2.07 ^{a, A}	27.58 \pm 2.42 ^{a, A}	0.7664
L a b ²³⁴⁵⁶	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	

¹Mean \pm Standard error; ²Fresh cheeses; ³Baked white regions; ⁴Baked brown regions; ⁵Cooled white regions; ⁶Cooled brown regions; ⁷ANOVA of means arranged within the same row; ⁸ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); * Significant (p<0.05).

Table 10.4.11 Stretch test measured (length in mm) for Maltrin based mozzarella cheeses during storage.

Cheese ID	Sample No.	29 d	40 d	55 d	75 d
04V1	1	176	>300	>300	>300
	2	300	>300	>300	>300
	3	250	>300	>300	295
05V2	13	>300	250	>300	>300
	14	295	267	>300	270
	15	276	>300	>300	NA
06V3	25	300	>300	>300	>300
	26	300	>300	>300	258
	27	300	>300	>300	NA
07V1	28	245	>300	>300	260
	29	195	>300	>300	230
	30	290	>300	250	>300

Table 10.4.12 Stretch test measurements (length in mm) for control cheeses during storage.

Cheese ID	Sample No.	29 d	40 d	55 d	75 d
04V2	4	300	>300	>300	>300
	5	230	>300	>300	>300
	6	268	>300	>300	238
05V3	16	>300	>300	>300	245
	17	260	>300	>300	235
	18	280	298	>300	237
	19	>300	>300	>300	>300
06V1	20	280	300	>300	>300
	21	272	>300	>300	NA
	31	239	>300	>300	245
07V2	32	300	>300	>300	250
	33	300	290	>300	NA

Table 10.4.13 Stretch test measurements (length in mm) for Versagel based mozzarella cheeses during storage.

Cheese ID	Sample No.	29 d	40 d	55 d	75 d
04V3	7	265	>300	>300	>300
	8	268	>300	>300	>300
	9	>300	>300	>300	249
	10	>300	>300	295	>300
05V1	11	240	>300	265	>300
	12	245	>300	295	>300
	22	>300	>300	>300	>300
	23	285	>300	>300	>300
06V2	24	>300	>300	>300	NA
	34	300	>300	>300	280
	35	300	>300	>300	295
	36	300	>300	285	230

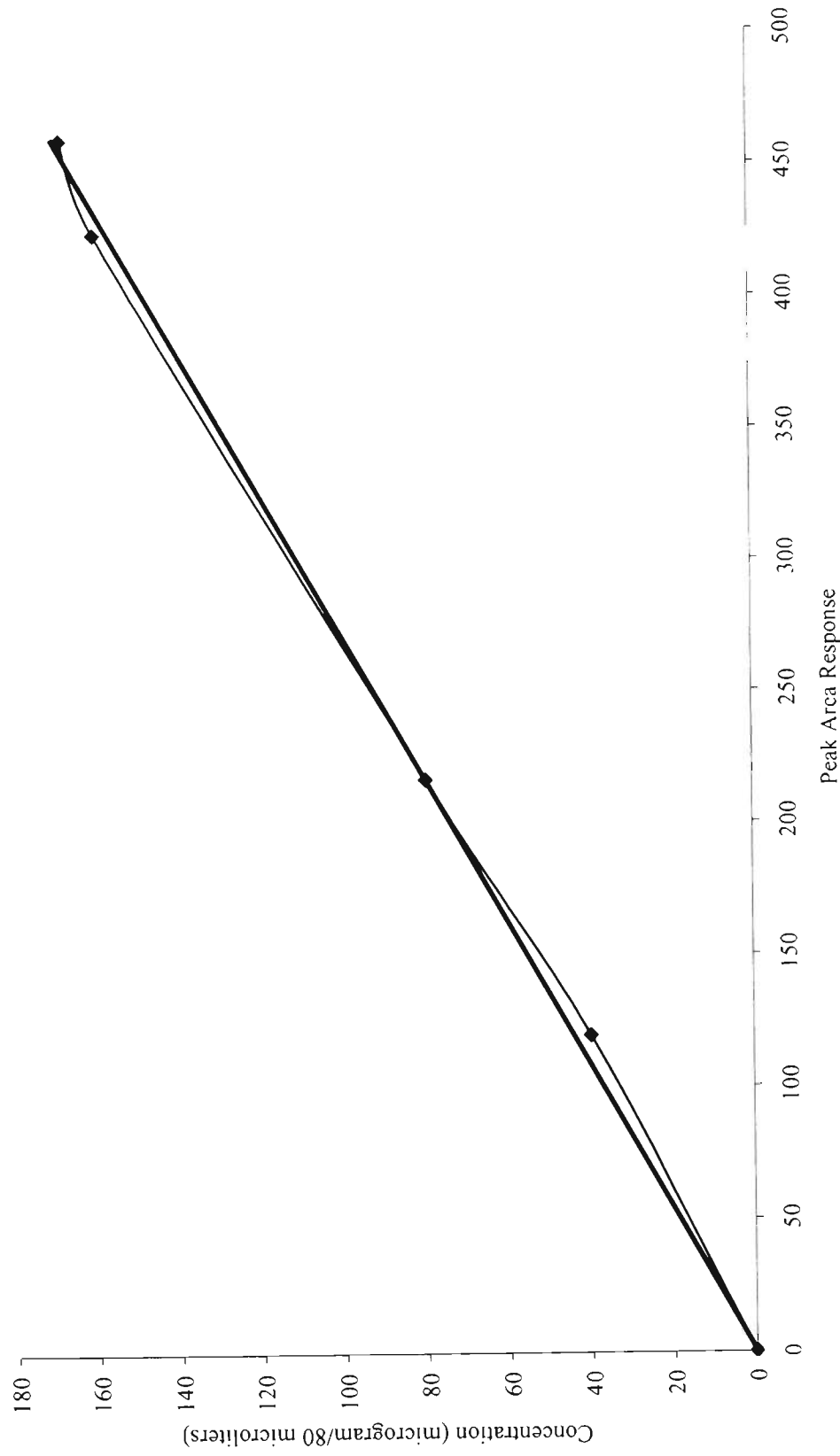


Figure 10.4.1 Peak area response for DP7 standards with elution time of 7.1 min and showing $y = 0.0371x$ and R^2 value of 0.9986.

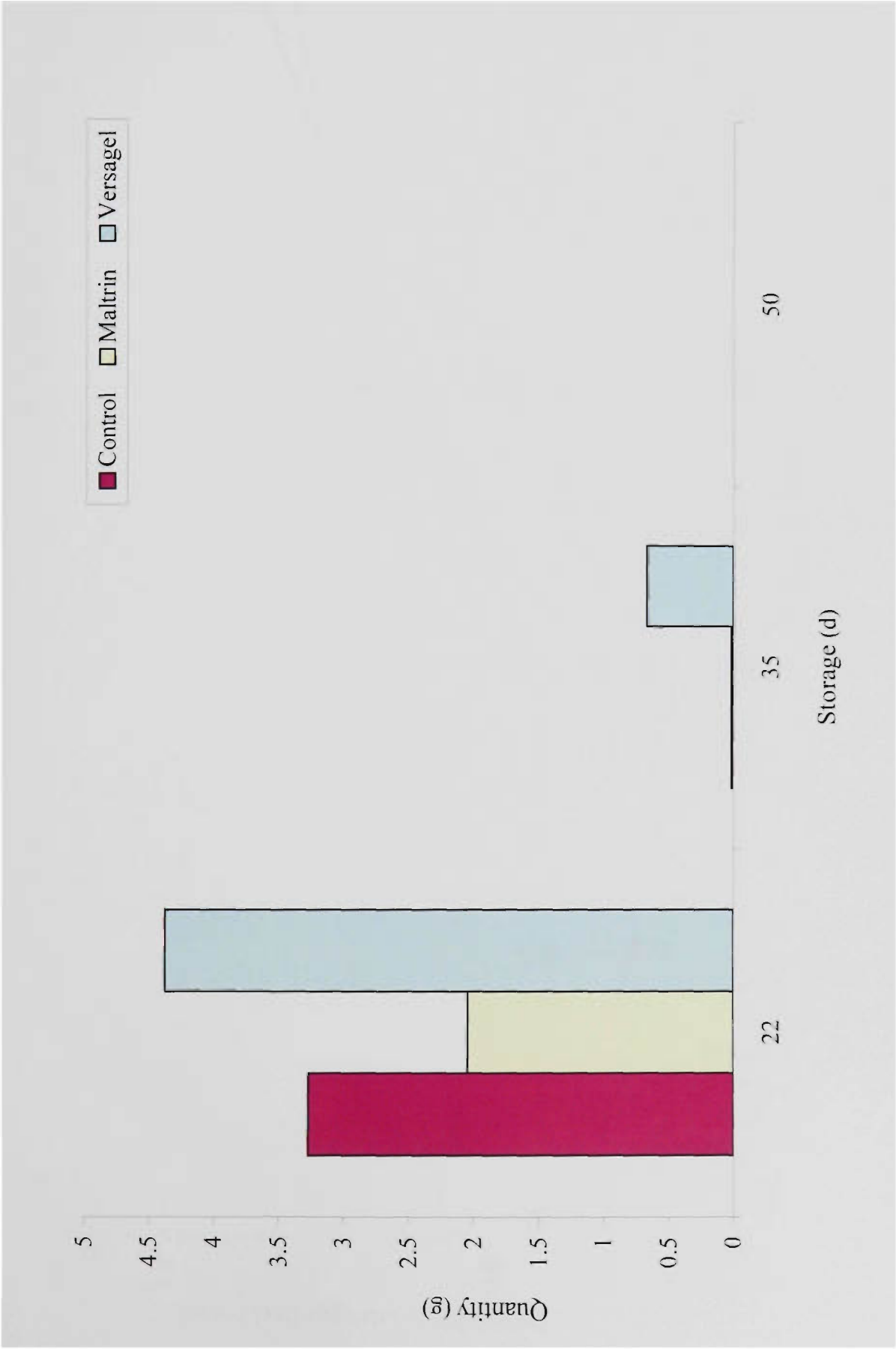


Figure 10.4.2 The expressed serum measured for control, Maltrin based and Versagel based cheeses at 22, 35 and 50 d of storage.

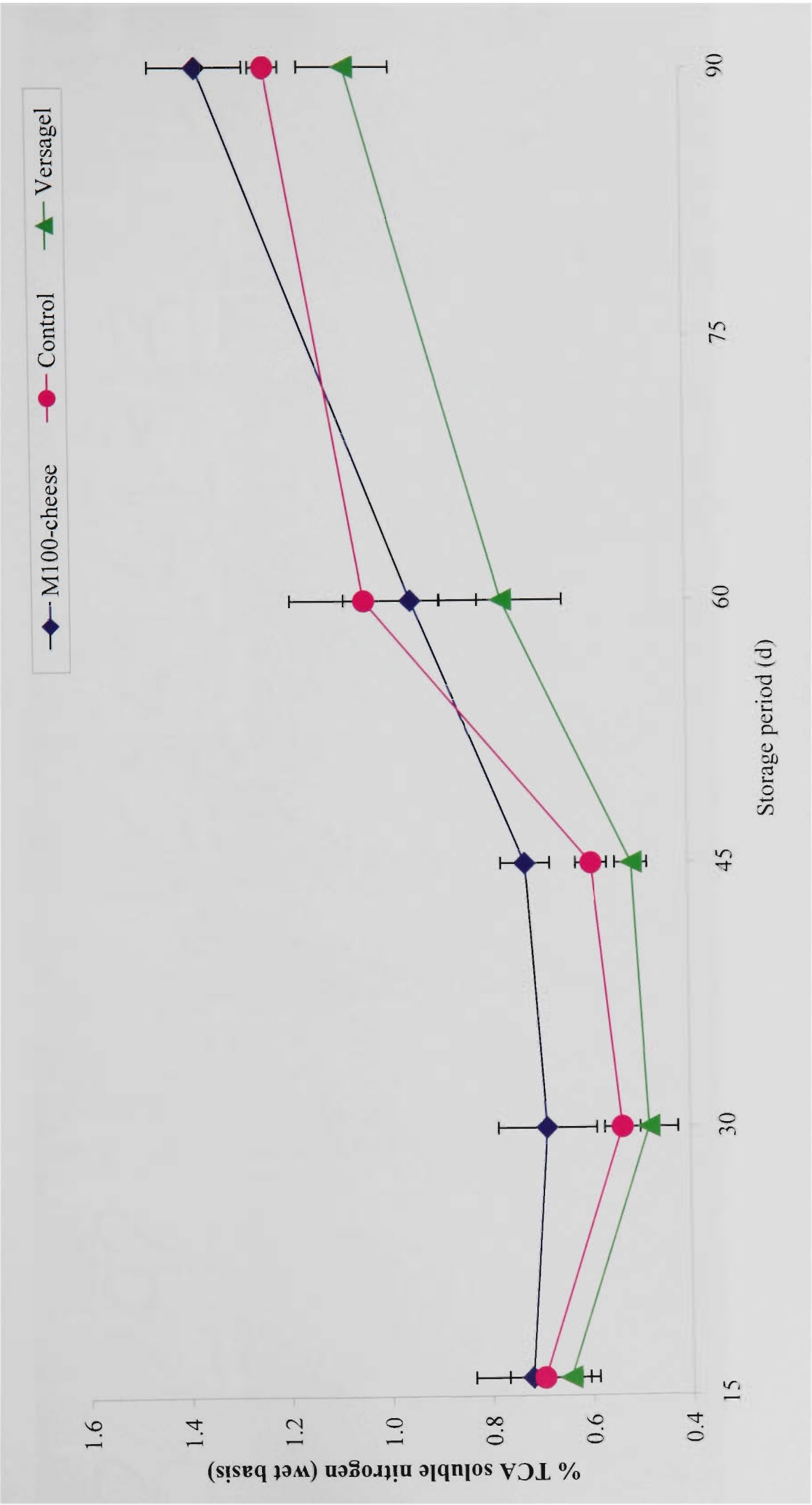


Figure 10.4.3 Effect of storage on the TCA soluble nitrogen content of Maltrin based, control and Versagel based cheeses.



Figure10.4.4 A typical melt test analyses for Maltrin based cheeses performed after storing for 29 d.



Figure10.4.5 A typical melt test analyses for control cheeses performed after storing for 29 d.

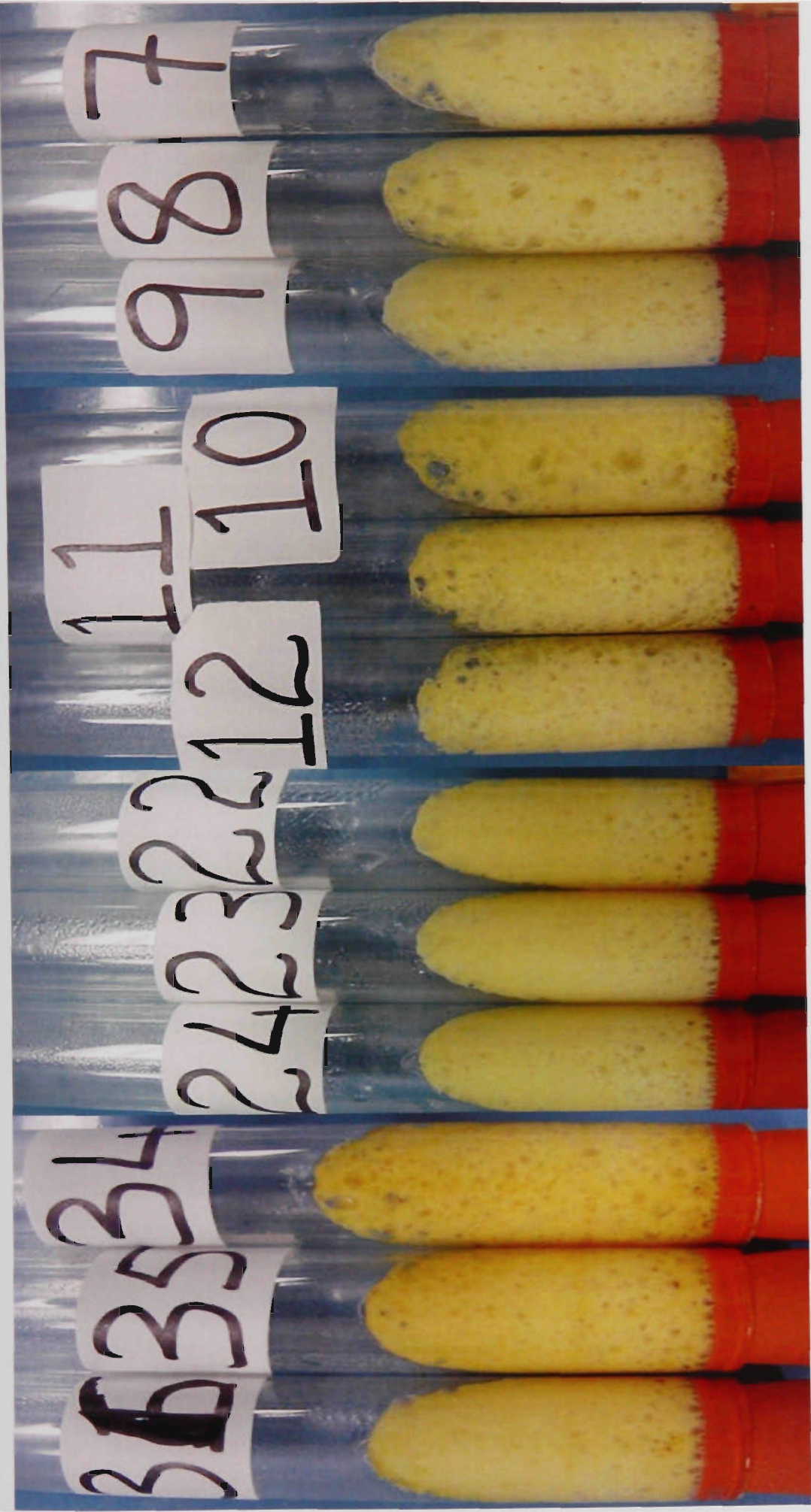


Figure 10.4.6 A typical melt test analyses for Versagel based cheeses performed after storing for 29 d.

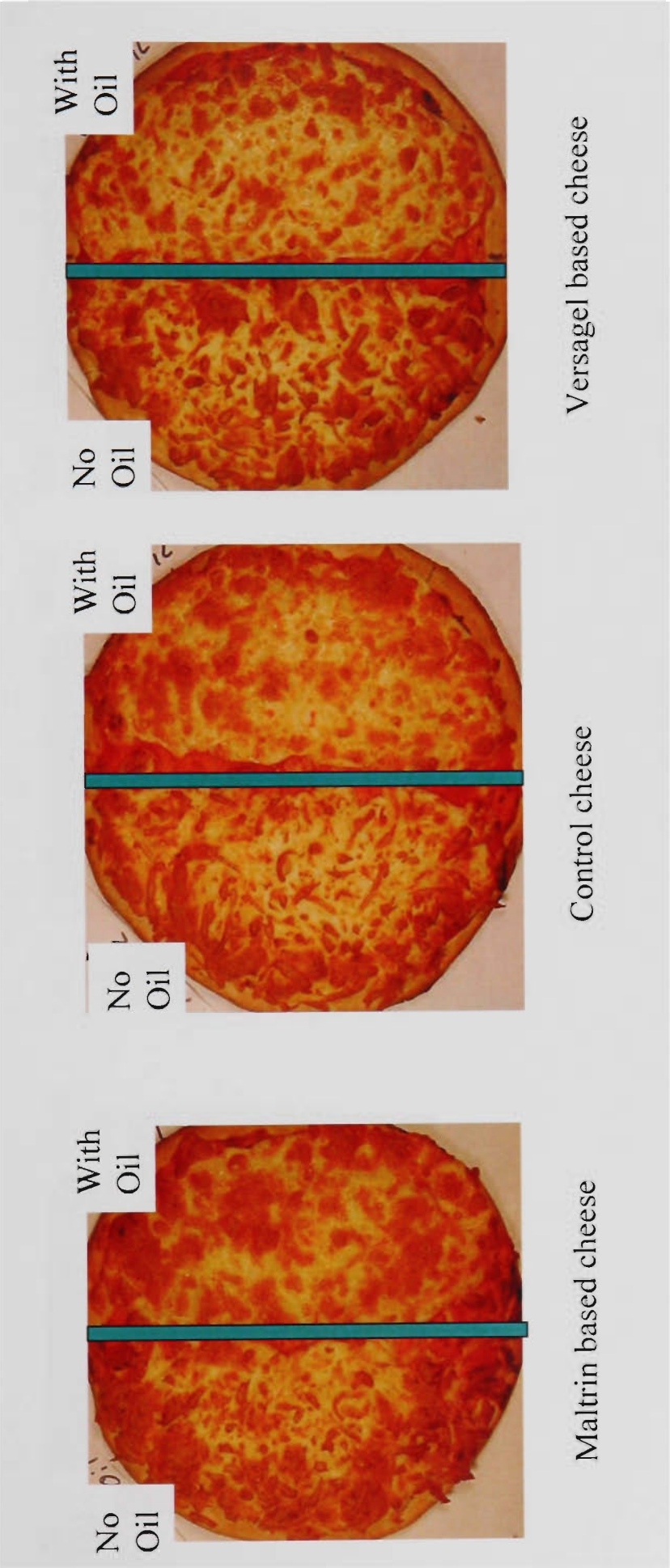


Figure 10.4.7 The effect of addition of fat replacers and application of oil on the pizza bake characteristics of Maltrin based, control and Versagel based cheeses stored for 29 d.

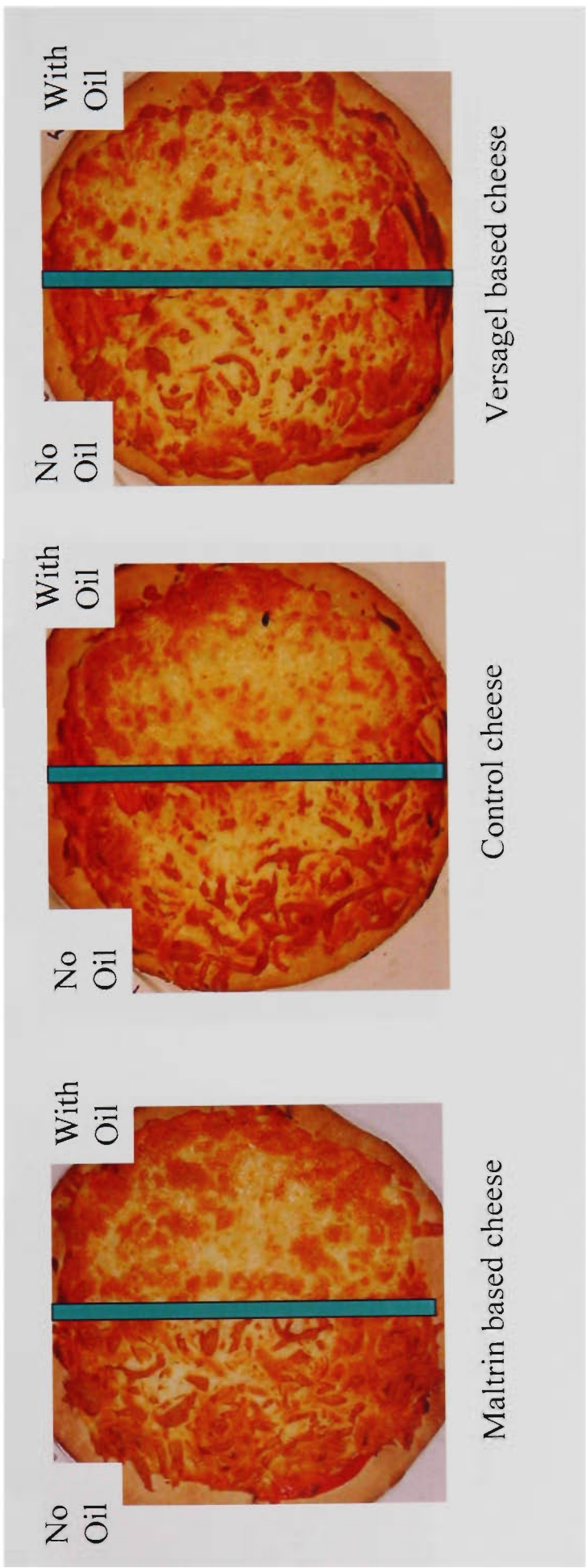


Figure 10.4.8 The effect of addition of fat replacers and application of oil on the pizza bake characteristics of Maltrin based, control and Versagel based cheeses stored for 44 d.

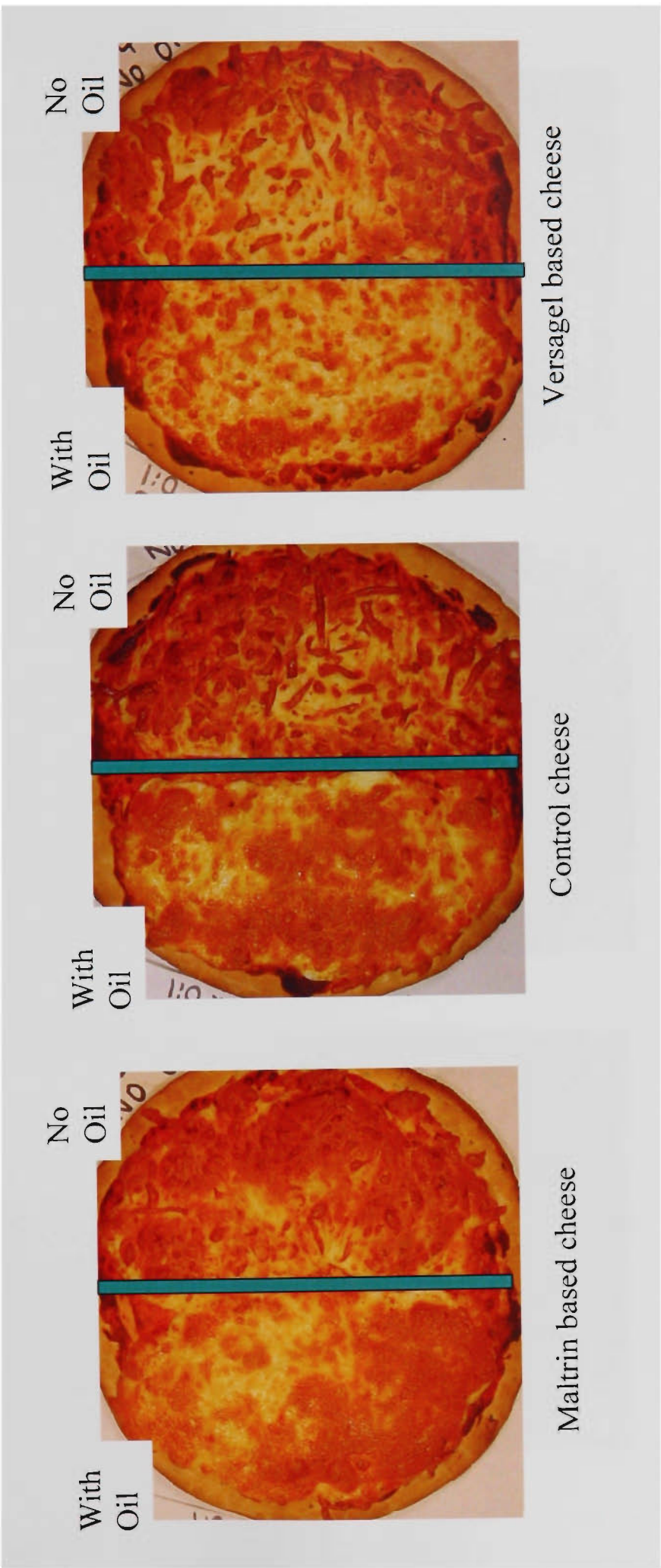


Figure 10.4.9 The effect of addition of fat replacers and application of oil on the pizza bake characteristics of Maltrin based, control and Versagel based cheeses stored for 56 d.

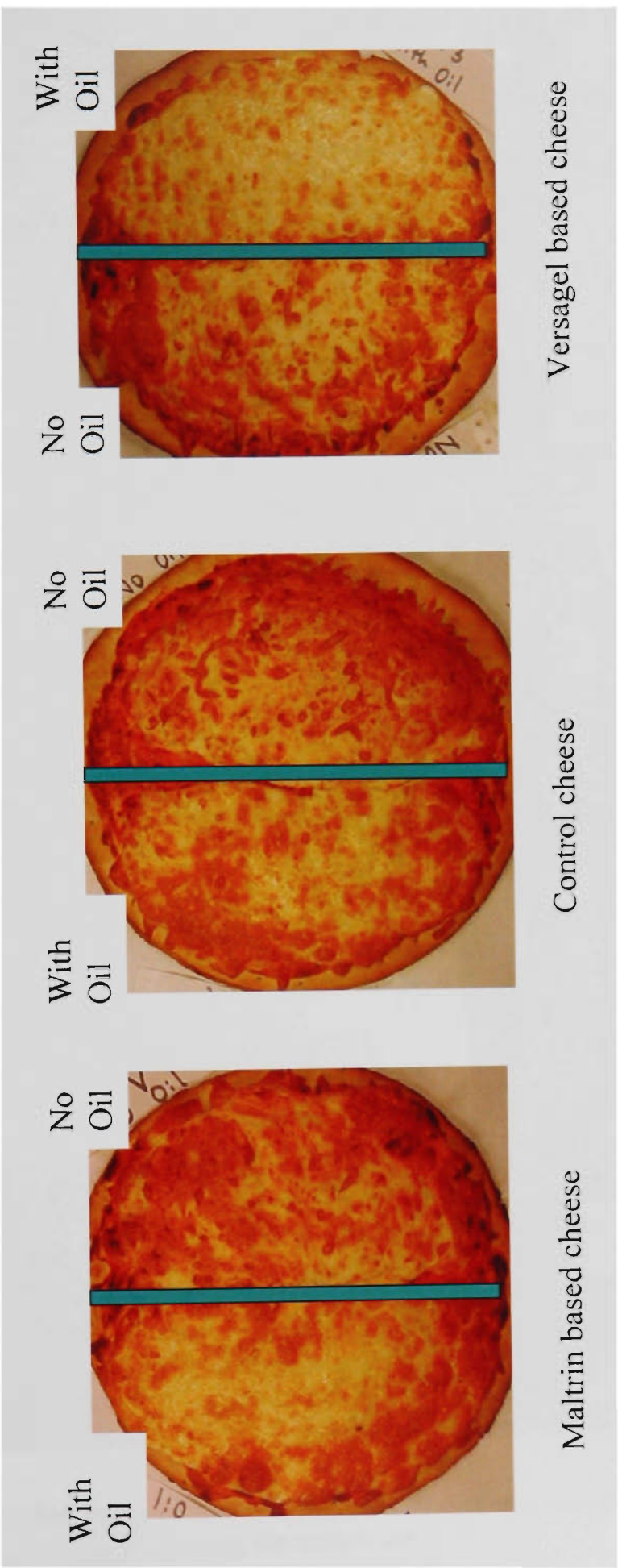


Figure 10.4.10 The effect of addition of fat replacers and application of oil on the pizza bake characteristics of Maltrin based, control and Versagel based cheeses stored for 80 d.

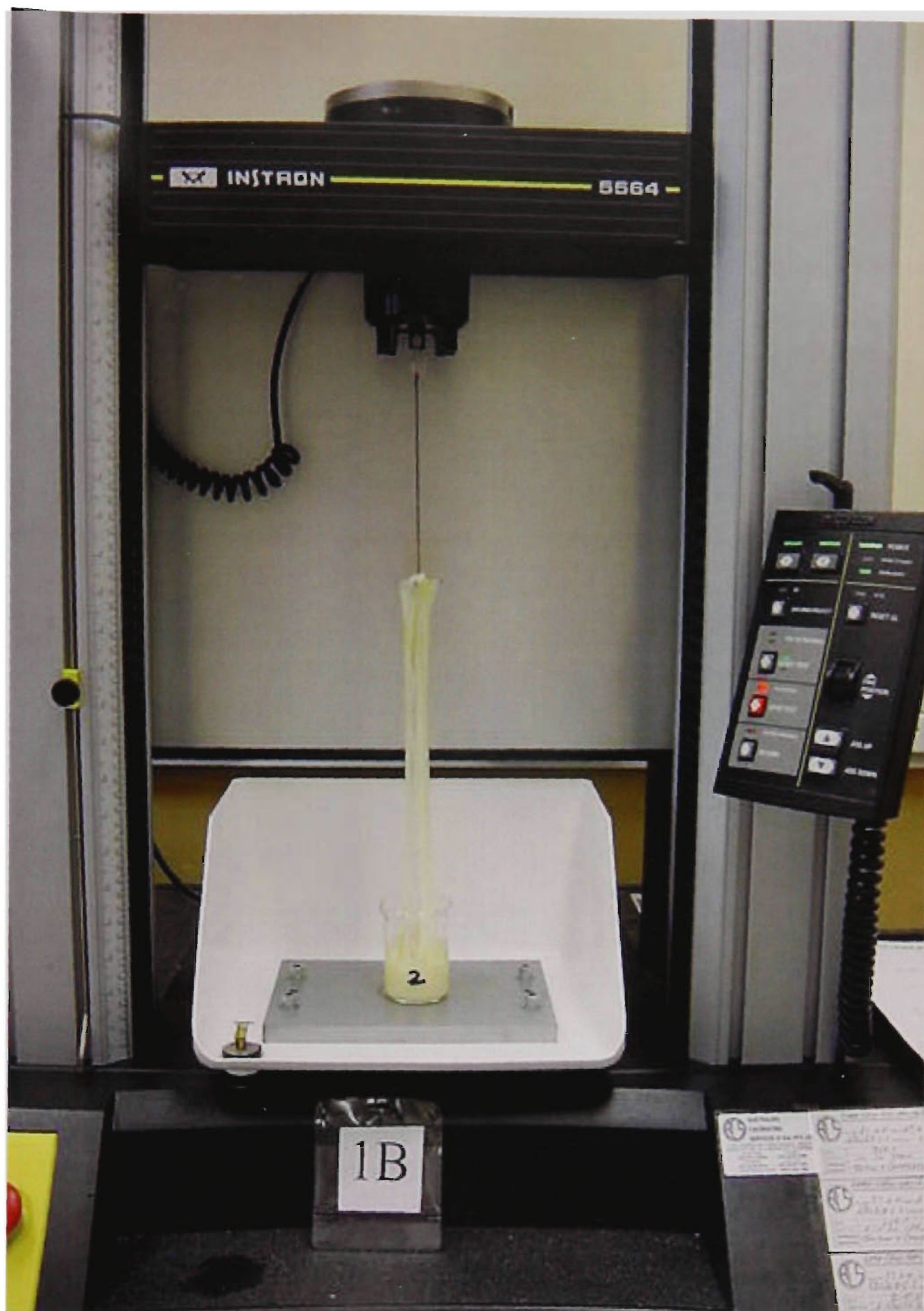


Figure 10.4.11 A typical image of stretched Maltrin based cheese stored for 29 d analysed using the stretch test.

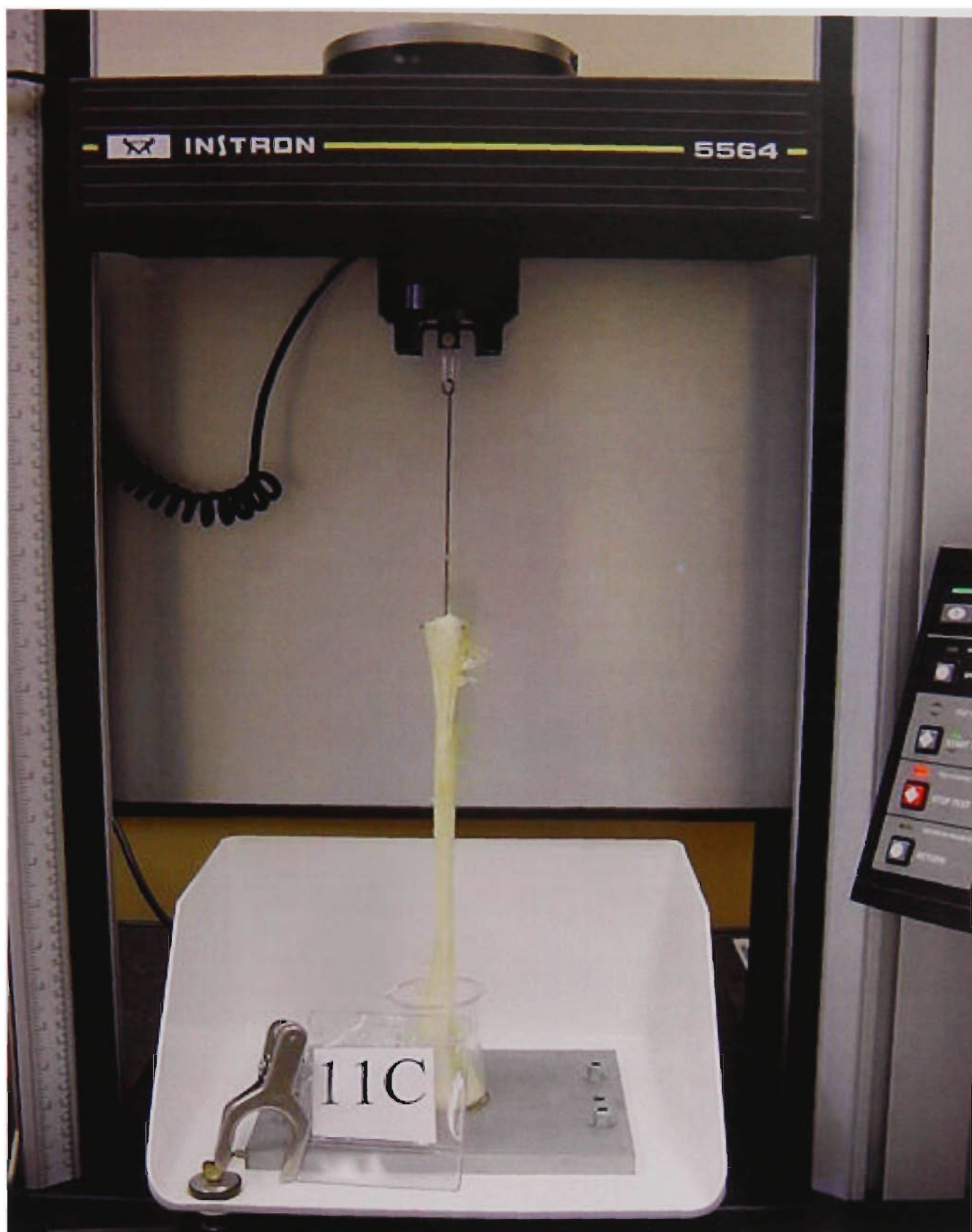


Figure 10.4.12 A typical image of stretched control cheese stored for 29 d analysed using the stretch test.



Figure 10.4.13 A typical image of stretched Versagel based cheese stored for 40 d analysed using the stretch test.

11.0 EFFECT OF RATE OF FAT REPLACER ADDITION TO PRE-ACIDIFIED MILK ON COMPOSITION, TEXTURE AND FUNCTIONALITY OF ULFM CHEESES

11.1 Introduction

Manufacture of low fat mozzarella cheeses has gained importance in recent times. Full fat mozzarella cheeses have fat content in excess of 20%. The demand for low fat cheeses is due to their beneficial effects on health and only a few consumers claim to prefer them for their organoleptic characteristics (Sandrou and Arvanitoyannis, 2000).

Low fat mozzarella cheeses show poor melt and stretch characteristics compared to a full fat mozzarella cheese (Mistry and Anderson, 1993). Reduction in the fat content of cheeses decreases the amount of moisture retained. Increasing the moisture content in low fat mozzarella cheeses improves their melt and flow characteristics. However, higher moisture content in low fat mozzarella cheeses leads to watering-off defect.

In mozzarella cheeses, stretching causes orientation of protein into bundles of stretched strands and molten fat along with the serum phase is trapped between these strands (McMahon *et al.*, 1999). When the fat is removed, these serum and molten fat channels reduce in diameter. Molten fat in low fat mozzarella cheese forms fewer fat globules after cooling and there is little resistance to the contracting protein strands. The serum is expelled from between the protein strands and the low fat mozzarella cheese retains less moisture.

Several methods have been tried to aid moisture retention in low fat mozzarella cheeses including lower manufacturing temperatures, removal of increased amounts of calcium using

direct acidification and pre-acidification techniques, use of exo-polysaccharide producing starter cultures and addition of fat replacers. Lowering of the temperature of cooking and cheddaring decreases syneresis of whey. But lower temperatures have also been attributed to cause decreased starter culture activity. Pre-acidification using acetic, lactic or citric acid to a lower pH, decreases the total calcium content of the cheeses and has been shown to improve the functional characteristics of low fat mozzarella cheeses. Exo-polysaccharide producing starter cultures have shown increased moisture retention in cheeses. But use of ropy strains causes slime formation on the surface of cheese and problems of increase in membrane fouling and back pressure on membrane during membrane filtration of whey. Capsule formers are a better option over ropy strains of exo-polysaccharide producing starter cultures because in case of former organisms the exo-polysaccharide is bound to the bacterial cell. Exo-polysaccharide producing starter cultures are currently being investigated to study their genetic mechanisms and use of such bacterial cultures could still prove to be advantageous for manufacturing low fat high moisture mozzarella cheeses (Bhaskaracharya and Shah, 2000b, 2001a).

Fat replacers such as Novagel, Simplesse, starches and gums have been shown to help in retaining moisture, increasing cheese yield and improving the functional characteristics of low fat mozzarella cheeses. Starches such as maltodextrins, potato starch, corn starch and rice starches can be used either in their native state or as modified starches. All major agricultural crops such as wheat, maize, rice and cassava contain starch. Starch is a versatile raw material, which has shown applicability in many food preparations. The performance of starch depends on the physical properties of the starch granule such as size and surface area and chemical properties such as amount of amylose and amylopectin contents in the starch granule (FAO, 1997).

Enzymatic methods are preferable when a single carbohydrate is to be analysed. e.g. glucose. as the end point of starch analysis. When several different monosaccharides are to be determined simultaneously, HPLC or GLC methods are preferable. Oligosaccharides can also be determined by GLC or HPLC methods. These methods require purified preparations of the product to be analysed, but in complex foods such as cheeses, enzymatic hydrolysis and determination of liberated monosaccharides is an alternative for specific determination.

Polysaccharides have 10 or more monomeric units and oligosaccharides have less than 10 monomers. Analytical separation of carbohydrates is based on their solubility in aqueous ethanol. The alcohol solubility of carbohydrates, however, is dependent upon the degree of polymerization (DP) and their molecular structure. Highly branched carbohydrates may be soluble in 80% ethanol in spite of a DP considerably higher than 10. In practice, therefore, the separation of oligosaccharides from polysaccharides is empirical and does not provide an exact division based on DP (Morgan and Zabik, 1981).

Quantitative analysis of starch in foods by most current methods is based on enzymatic degradation and specific determination of liberated glucose. Nutritionally, starch can be divided into glucogenic and resistant starch. The latter is not absorbed in the small intestine. Resistant starch is poorly soluble in water and methods aiming at a total starch analysis employ an initial 2 M potassium hydroxide (KOH) or dimethyl sulfoxide solvent (DMSO) treatment to disperse crystalline starch fractions that would otherwise remain unhydrolyzed. The methods presently available for estimation of resistant starch are based on simulating normal starch digestion in the small intestine.

The determination of non-starch polysaccharides (NSP) is based on the following steps: (a) degradation of starch by enzymatic hydrolysis after solubilization, (b) removal of low

molecular weight carbohydrates, including starch hydrolysis products, (c) hydrolysis of the NSP to their constituent monomers, and (d) quantitative determination of those monomers. The acid hydrolysis step is a critical one, and it has to be designed as an optimal balance between complete hydrolysis and destruction of the liberated monomers (Stephen, 1995; Wursch, 1989). HPLC detection is a popular method for specific determination of the liberated monomers.

During acidification of milk the stability of casein micelles decreases and the micelles dissociate with decrease in pH. The colloidal calcium phosphate is expelled from the micelles at lower pH. The dissociation of casein micelles has been attributed to the increase in zeta potential towards zero with the decrease in pH to 5.2 (Swaigood, 1992; Kosikowski, 1977). Small changes in the pH at different stages during manufacture of cheese can affect the network formation of caseins, which alters the structure of the finished cheese. Kiely *et al.* (1992 a) showed that cheddared curd at pH 5.2 had 17% lower calcium content if the curd was drained at pH 5.9 instead of at a higher pH of 6.4. The free oil formation was reported to be increased for mozzarella cheese containing lower calcium content or lower moisture content (Kindstedt *et al.*, 1992).

Serum extracted from low fat or part skim mozzarella cheeses when heated from 7 to 60°C showed an increase in whiteness (measured as L value) and a decrease when cooled (Metzger *et al.*, 2000). This was suggested to be due to formation of a gel, which was mainly composed of intact caseins and a few breakdown products of proteolysis, in the serum phase of the cheese during heating and this phenomenon was found to be reversible.

Kindstedt proposed a model to explain the emulsifying ability of caseins for fat in the presence of salt (sodium ions). Sodium was suggested to replace some of the casein bound calcium and

reduce leakage of fat from the micellar network. Thus salting reduced the free oil formation and decreased the tendency of mozzarella cheese to flow upon melting.

A typical waxy corn starch during initial heating phase shows an increase in viscosity as the granules swell. The swelling of granules is dependent upon the granule size, extent of heat applied, amount of moisture available, nature of starch, i.e. native starch or modified starch even though from the same source. Thus all granules do not swell at the same rate or to the same extent. The swelling is usually accompanied by loss in semi-crystalline structure of the starch granule and a loss in birefringence. The temperature at which the starch begins to undergo these changes is termed as gelatinisation temperature and because different granules do not gelatinise at the exact same temperature, the gelatinisation temperature is usually expressed as a narrow temperature range rather than one specific temperature.

During gelatinisation the amylose molecules with low molecular weights start to leach from the granules and the solution viscosity reaches a maximum during further heating close to pasting temperatures (eg $<95^{\circ}\text{C}$). At this stage most of the starch granules are completely hydrated and swollen but the granules are intact and no molecular arrangements have yet occurred. When the starch granules are heated to $>95^{\circ}\text{C}$ and held at that temperature, the granules breakdown and polymers are found to leach out. These polymers re-align and a decrease in viscosity reflects phenomenon of pasting. The starch slurries when cooled show the re-association of solubilised amylose and amylopectin with an increase in viscosity. Higher amylose containing starches show greater increase in viscosity during cooling.

Mozzarella cheese is popularly used as topping on pizzas because of its stretch and melt characteristics. However stretch and melt tests are subjective and for commercial and research purposes there is a need for a simple objective test to measure these characteristics. Several

tests for measuring stretch have been reported (Apostolopoulos, 1994; Cavella *et al.*, 1992). however, these methods require complex procedures for sample analysis.

11.2 Aims

The aims of this study were to manufacture ULFM cheeses with and without fat replacers and analyse the cheeses for pizza bake characteristics; to examine the characteristics of fat replacers such as hydration behaviour of various fat replacers using light microscopy, rheological measurements and physical characteristics such as gel formation; to utilise the fat replacer in developing an ULFM cheese with characteristics of a full fat mozzarella; to study the partitioning of starch in whey, stretch water and mozzarella cheese using HPLC techniques; and to investigate the repeatability of the new stretch test developed for analysing stretchability of mozzarella cheeses.

The hydration behaviour of the fat replacers are dependent upon the heat treatment and holding time provided to the slurry prepared from fat replacers before adding to cheese-milk. In this study the rate of addition and the type of fat replacer used were additional experimental variables to obtain desirable characteristics in ULFM cheeses similar to full fat mozzarella cheese.

11.3 Materials and methods

11.3.1 Cheese making

ULFM cheeses containing 6.37% fat were manufactured using *Streptococcus thermophilus* and *Lactobacillus helveticus* starter cultures. Three batches (20 kg each) of cheeses were made as described in sections 3.2.6.1, 3.2.6.2, 3.2.6.3 and 3.2.6.4 and the cheeses were stored at 4°C. ULFM cheeses were made with addition of 0.1%, 0.25% or 0.5% of fat replacer either Maltrin

(M100) or Versagel. During cheese making samples of milk, whey, stretch water and cheese were collected and the amount of fat replacer Maltrin (M100) retained in the cheese and its by-products was ascertained using HPLC analysis. Four commercial shredded mozzarella cheeses (A, B, C, D) were obtained from a local supermarket. The cheeses were chosen to have a use-by date as close as possible and to contain different fat contents. The composition of these cheeses was analysed and the cheeses were subjected to the stretch test developed.

11.3.2 Analysis of cheeses

During cheese manufacture samples of milk, whey, curd at whey drain, milled curd, stretched curd and brine solution used for stretching curd were collected for analysis of moisture, protein, minerals including sodium, calcium, potassium and magnesium contents. Also the samples were analysed for the distribution of Maltrin (M100) in curd and its losses into whey and brine solution used for stretching using HPLC technique. The cheese samples were prepared as described in section 3.2.9 for the measurement of composition and pizza bake characteristics (section 3.2.7 and 3.2.24). The moisture/total solids was estimated using the method described in section 3.2.20, fat content as in section 3.2.22, and protein content as in section 3.2.25. Pizza-bake test was carried out (section 3.2.24) at 30, 45 and 75 d of storage and the colour, size of blisters and their distribution upon baking were assessed.

The mineral content distribution was also examined. Fat replacers were dissolved in 'Lite' milk containing 1.5% fat and heated to 50, 75 and 90°C followed by cooling to 22°C. These heat-treated milks were observed for physical characteristics of gel formation as in section 3.2.19.2, light microscopy as in section 3.2.19.3, refractive index and gelatinisation characteristics. Based on the physical characteristics of fat replacers a manufacture protocol was developed for ULFM cheeses with these fat replacers in order for the ULFM cheeses to have characteristics of a full fat mozzarella cheese.

The new method of measuring stretchability of cheeses was investigated for repeatability. Commercial mozzarella cheeses, which had similar expiry date were obtained from the supermarket and these cheeses were stretched using the method described in section 3.2.16.

11.4 Results and discussion

11.4.1 Composition

Mozzarella cheeses containing 6.37% fat were made using 0.5% of Maltrin or 0.5% of Versagel added to standardised cheese milk. The cheeses were made in 4 h 30 min from starter addition to milling. Samples of standardised milk before addition of fat replacers, milk mixed with 0.5% of either Maltrin or Versagel, whey collected at whey draining stage, brine solution used for stretching curd and the finished mozzarella cheeses, were collected and their moisture and protein contents were determined. Table 11.4.1 shows the moisture and protein contents of the Maltrin based samples. Addition of Maltrin decreased the moisture content and increased the total solids of the milk by 0.45%. At whey draining stage (pH 6.1) about 1% protein was lost in the whey while 0.13% was lost along with the stretch water. The Maltrin based cheese contained ~ 55% moisture and ~ 34% protein.

Table 11.4.2 shows the moisture and protein contents of Versagel based samples of standardised milk, fat replaced milk, whey, stretch water after use and mozzarella cheese. Addition of Versagel increased the total solids and protein contents of the fat replaced milk. Versagel being made up of 80% protein (β -lactoglobulin) and 20% carbohydrate (carageenan and xanthan gums) marginally increased both total solids and protein contents of fat replaced milk. The whey obtained during cheese making (pH 6.1) had 1.4% protein content, which was

higher than that observed for Maltrin based cheese. Thus some of the Versagel could have been lost in the whey. The brine solution used for stretching Versagel based curd indicated no marked change in protein content compared to that of Maltrin based curd. The Versagel based cheese had increased moisture content possibly due to better moisture retention ability of Versagel compared to Maltrin. Due to the availability of carageenan and xanthan gums (which have high water binding ability compared to starches) in Versagel along with β -lactoglobulin the cheeses made using Versagel were expected to retain more moisture compared to the maltodextrins contained in Maltrin. Due to the increased moisture content of the Versagel based cheese their protein and other solids content decreased.

Addition of 0.5% of fat replacer increases the cost of manufacturing ULFM cheeses. Thus it was decided to reduce the rate of fat replacer addition. Moreover, in order to compare the effect of addition of Maltrin or Versagel to the cheese milk, two rates namely, 0.25% and 0.1% of each fat replacer were determined for addition into the cheese milk. The cheeses were made in 4 h 30 min from starter addition to milling and contained 6.37% fat content. A comparison of the moisture contents of standardised milk (no fat replacer added), whey, curd at whey drain, milled curd, stretched curd, brine solution used for stretching the curd and mozzarella cheeses are shown in Table 11.4.3. Control indicates samples collected at the various stages of manufacture of ULFM cheese made without addition of fat replacer, M2 and M1 indicate samples obtained when Maltrin was added at 0.25% and 0.1% respectively while V2 and V1 indicate samples obtained when Versagel was added at 0.25% and 0.1% respectively to the cheese milk. The standardised milk (without addition of fat replacers) used for making control, M2, M1, V2 and V1 cheeses had similar moisture contents. Thus variations in cheese milk used for making the different varieties of cheeses were avoided.

Addition of Versagel at either 0.25% or 0.1% and Maltrin at 0.1% did not change the moisture or total solids contents of whey expelled from the curd while addition of 0.25% of Maltrin decreased the moisture content of the whey by 0.2%. Curd samples obtained immediately after draining the whey at pH 6.1 for all cheese varieties had similar moisture contents. The continuous syneresis from the curd caused variations in the estimated moisture within each variety of cheese. Such variations obscured any differences between the different varieties of cheese. Although milling was carried out for all cheeses at similar pH of 5.25 ± 0.2 , the curd with 0.25% Versagel showed the highest moisture retention while control samples had the lowest moisture content. However, a higher rate of addition was required to show a definite increase in moisture content. The stretched V2 samples had significantly higher moisture contents while V1, M2 and M1 samples had similar moisture contents. Control stretched curd samples had the lowest moisture content. Stretch water contained similar amounts of total solids for all the cheeses. Among the mozzarella cheeses, V2 samples had similar moisture contents to the control cheeses and M2 cheeses while M1 and V1 cheeses had the least moisture content. M2 cheeses also had higher moisture content than V1 cheeses. Thus 0.25% of Versagel or Maltrin when added to cheese milk increased the moisture content of the cheeses while lower rates of addition did not seem to improve moisture retained in the cheeses.

The protein contents measured for control, M2, M1, V2 and V1 samples during manufacture of ULFM cheeses are shown in Table 11.4.4. The cheese milk used for manufacture had similar protein contents. Whey samples obtained from V2 cheese making process contained higher (0.16%) protein content compared to M2 samples. Curd samples for all varieties did not show significant differences while V2 milled curd showed a lower protein content. This could be due to loss of protein along with whey or due to the increase in moisture contents as shown in Table 11.4.3. The stretched curd and stretch water samples for all cheeses had similar protein contents. The protein contents of V2 and V1 cheeses correlated with their moisture contents.

An increase in moisture content of V2 and V1 cheeses possibly caused a decrease in percentage protein contents measured for these cheeses.

11.4.2 Partitioning of Maltrin in whey, stretch water and cheese

HPLC analysis of several standards and samples of milk, whey, stretch water and cheeses showed that only DP7 was of importance in ascertaining the starch partitioning. Thus standards of DP7 were run and the standard curve obtained is shown in Figure 11.4.1. Based on the standard curve the equation for calculating concentration was obtained which is shown below:

$$y = 0.0371 x.$$

Table 11.4.5 shows the distribution in percentage of DP7 in milk measured for whey, stretch water and in cheeses. As shown in the table, about 45- 60% of the DP7 and similar oligosaccharides was lost in whey at whey drain stage during mozzarella cheese manufacture from citric acid pre-acidified milk to pH 6.1 and mixed with 0.25% Maltrin M100. About 20- 25% of DP7 was lost in stretch water during stretching of curd. The mozzarella cheese showed 6- 7.5% of DP7 and similar oligosaccharides to be retained in the cheese matrix. The variation in percentages of DP7 estimated is due to the samples, which were obtained from different batches of cheeses. The very low levels of DP7 in cheeses indicated that the oligosaccharides below DP7 (i.e. DP1 to DP7) were lost in whey or stretch water while higher than DP7 oligosaccharides might still be present in the cheese.

11.4.3 Calcium content

The calcium contents measured for samples of standardised milk, whey, curd after whey draining, milled curd, stretched curd, stretch water and mozzarella cheeses are shown in Table 11.4.6. The calibration curve obtained by measuring the absorbance values for calcium

standards is shown in Figure 11.4.2 with an R^2 value of 0.9986. The equation used to calculate the concentration (mg/kg) of calcium in the cheeses and by-products is shown below:

For calcium: $y = 0.0658 x$.

During cheese making about 600- 700 mg of calcium per kg of whey was lost for control and Maltrin based cheeses while 550- 580 mg of calcium per kg of whey was lost for Versagel based cheeses. The calcium contents of curd obtained after draining whey for all varieties of cheeses were similar. M1, V2 and V1 milled curd samples showed increased calcium retention, which could be due to increased amounts of serum/moisture present in them. Stretched curd samples also showed a similar trend as their respective milled curd samples. Losses in calcium for all the cheeses appeared to be similar. About 250- 350 mg calcium was lost per kg of stretch water. Control mozzarella cheeses had the lowest calcium concentration. Addition of fat replacers appeared to decrease the amount of calcium loss during manufacture of the cheeses, which could be due to formation of gel like network by the fat replacer and a probable decrease in the dynamic transfer of calcium from micelles into serum phase as proposed by Kindstedt and Guo (1997).

11.4.4 Functional characteristics

11.4.4.1 Pizza bake characteristics for 30 d old cheese

The Hunter L values were measured for cheeses made from milk mixed with or without fat replacer. The cheeses were stored for 30 d and tri stimulus colour measurements were taken for fresh, baked and cooled cheeses indicated by L^2 , L^3 and L^4 values, respectively (Table 11.4.7). The fresh control, M2, M1, V2 and V1 cheeses had similar L^2 values and after baking had increased whiteness (L^3 values), which were similar for all cheeses. When the baked cheeses were cooled, V2 (with oil) and V1 (with/no oil) cheeses showed significantly higher L^4 values compared to control (with/no oil) and M1 (with/no oil) cheeses. M2 (with/no oil) cheeses had

higher L^4 values compared to control (with/no oil) and M1 (with/no oil) cheeses. Also application of oil to the cheese shreds did not show any marked change in L value for any of the cheeses before and after baking or after cooling.

The control and fat replaced cheeses showed increased whiteness (L^3 values) when baked. Control and Maltrin based (M2 and M1) cheeses upon cooling were less white than their respective fresh cheeses while Versagel based (V2 and V1) cheeses continued to remain white even after cooling.

The Hunter a-values were measured for cheeses made from milk either with or without fat replacer. The a^2 , a^3 and a^4 values shown in Table 11.4.8 indicate the tristimulus colour measurements for fresh, baked and cooled cheeses respectively. The a-values show the extent of browning of cheeses with values greater than zero indicating red colour. The control cheeses or fat replaced cheeses showed similar a^2 , a^3 and a^4 values. Some differences in a^2 values could have been due to the application of tomato paste before the shredded cheeses were spread on the pizza bases.

Control (with/no oil), M2 (no oil), M1 (with/no oil), V2 (no oil) and V1 (no oil) cheeses showed significantly higher a^3 values due to baking compared to their respective a^2 values when fresh. Upon cooling, the a^4 values remained similar to their a^3 values. M2 (with oil), V2 (with oil) and V1 (with oil) cheeses did not show an increase in a^3 or a^4 values due to baking or cooling. Thus addition of fat replacers along with application of a protective coating such as of oil to the shredded cheeses could reduce the browning of ULFM cheeses. A higher rate (0.25%) addition of Maltrin was required to show a similar effect as at 0.25% or 0.1% of Versagel addition. Hence, Versagel at 0.1% concentration in cheese milk was able to reduce the browning of ULFM cheeses made from the fat replaced milk when the cheeses were baked.

The Hunter b-values were measured for control and fat replaced cheeses indicated the extent of yellow colouration of the cheese shreds. The b^2 , b^3 and b^4 values indicate the b-values measured for fresh, baked and cooled cheeses respectively, as shown in Table 11.4.9. The fresh cheeses made from control milk or fat replaced milk showed similar b^2 values. Moreover, all the cheeses had similar b^3 values. The baked V2 and V1 cheeses when cooled showed significantly higher b^4 values while control cheeses had significantly lower b^4 values. The increase in yellowness of M2 and M1 cheeses was not significantly different to V2, V1 and control cheeses. Application of oil did not change the b-values for any of the cheeses.

The control and fat replaced cheeses showed increases in b-values due to baking (b^3 values) compared to fresh cheeses. Cooled control (with/no oil), M2 (no oil) and M1 (with/no oil) cheeses had lower b^4 values compared to their respective b^3 values while M2 (with oil), V2 (with/no oil) and V1 (with/no oil) cheeses had b^4 values similar to their b^3 values.

11.4.5 Sodium, potassium and magnesium contents

The mineral content of standardised milk (S1), whey (S2), curd (S3), milled curd (S4), stretched curd (S5), stretch water (S6) and 2 d old cheese (S7) samples were measured for sodium, potassium and magnesium concentrations. The mineral concentrations measured for control samples are shown in Table 11.4.10, M2 samples in Table 11.4.11, M1 samples in Table 11.4.12, V2 samples in Table 11.4.13 and V1 samples in Table 11.4.14. The calibration curves obtained by measuring the absorbance values for sodium standards is shown in Figure 11.4.3 (R^2 value = 0.9978), for potassium standards is shown in Figure 11.4.4 (R^2 value = 0.9974) and for magnesium standards is shown in Figure 11.4.5 (R^2 value = 0.9986). The equations used to calculate the concentration (mg/kg) of each mineral are shown below:

for sodium: $y = 0.0501 x$

for potassium: $y = 0.0685 x$

and for magnesium: $y = -0.0064 x^2 + 0.2098 x + 0.0403$

The concentration of minerals was measured as a single element analysis and AAS was calibrated using standards before, in between and after sample analysis with similar R^2 values and absorbance readings. The concentration of sodium in milk was measured to be about 247-271 mg/kg while that of potassium was about 1632- 1656 mg/kg and magnesium was about 71-114 mg/kg. Most of the sodium, potassium and magnesium salts were lost in whey and reduced amounts of these minerals were retained per unit of curd weight compared to per unit weight of whey. This was expected, as both sodium and potassium are soluble. Magnesium was retained to a greater extent than was sodium or potassium in the curd. Throughout cheddaring the minerals were lost into whey leaving about 241- 268 mg/kg of sodium, 1343- 1405 mg/kg of potassium and 291- 357 mg/kg of magnesium in the unsalted milled curd samples. The samples of 0.1% Maltrin based (M1) milled curd were dry salted and the mineral composition was estimated to study the changes due to dry salting. Because the salt was added in dry form rather than in liquid form, the sodium concentration per kg of milled curd increased but the concentrations of potassium and magnesium did not show a great decrease.

The increased sodium concentration in stretched curd is due to stretching the dry salted curd in 3% brine solution. Loss of potassium and magnesium ions into the stretch water could be due to leaching of these ions into brine or caused by replacement with sodium ions in the stretched cheese. There was a transfer of 127- 160 mg of potassium per kg of stretch water and about 8-14 mg of magnesium per kg of stretch water. The control mozzarella cheese samples contained about 2950 mg sodium, while Maltrin based cheeses had about 3560- 3945 mg and Versagel based cheeses had 4095- 4118 mg per kg, which could be due to their exchange with the colloidal calcium bound within the cheese (Kindstedt and Guo, 1997). The variations in the

estimated mineral contents were mainly between the batches and could have been affected by the moisture and protein contents of the samples analysed. These variations in mineral balance could have affected some of the functional characteristics of the mozzarella cheeses as described earlier.

11.4.6 Physical properties of fat replacers as observed under a light microscope and measured for refractive index

The effect of heating on gel formation by fat replacers was observed by heating the starch solution for 20 min at 50, 75 and 90°C. Table 11.4.15 shows the fat replacers which formed a gel when heated. The modified corn starches namely B950, B965, B990 and B994 along with the potato starch SS143 formed a gel when these solutions were heated to 90°C and later cooled to 22°C. Heating to 50 or 75°C and subsequent cooling of these starches did not lead to gel formation. Although Versagel on its own did not form a gel when heated to 90°C and cooled, an addition of equal amounts (0.25% each) of Maltrin M100 and Versagel to the 'Lite' milk caused a firm gel formation. This was probably due to the increased amount of solids, which became available for network formation leading to a firm gel. At 0.25% when individually added to 'Lite' milk neither Versagel nor Maltrin M100 were able to form a gel due to the very low concentration of solids. The large distances between the particles as a result of the very dilute concentrations could have inhibited gel formation.

The evidence for increased network formation could also be seen from the measurement of refractive index at 22°C of the fat replaced milk as shown in Table 11.4.16. There was a relatively greater decrease in 90°C heated milk containing combined Maltrin M100 and Versagel than individually for either of the fat replacers. All the fat replaced milks showed a decrease in refractive index when heated to 90°C probably due to the breakdown of intact starch granules and leaching of their contents into the milk. Only the control milk, which was

not mixed with any fat replacer, showed a constant refractive index reading at 22, 50, 75 and 90°C. Heating the fat replaced milks to below 90°C did not markedly alter their refractive index nor did these solutions form a gel.

Light microscopic study was conducted to observe effect of heat-treatment to 50, 75 or 90°C on fat replacers. Figure 11.4.6 shows the changes in starch granules when modified corn starch (B950) was heated to 50, 75 or 90°C. The B950 starch showed swollen granules formed at 75°C compared to 50°C and these granules were disrupted at 90°C causing leaching of the granular components. Only a few granules were found to be intact when the starch was heated to 90°C. Figure 11.4.7 shows the modified corn starch (B965) heated to 50, 75 and 90°C. The light microscopy images showed no disruption of starch granules at 50°C, partial breakdown of granules at 75°C and increased breakdown and exudation of molecular components. Some of the swollen starch granules could also be seen at 90°C indicating that the starch was not completely pasted. Figure 11.4.8 shows the changes in starch granules when modified corn starch (B990) was heated to 50, 75 or 90°C. The B990 starch showed swollen granules formed at 75°C compared to 50°C and these granules were disrupted at 90°C causing leaching of the granular components. Only a few granules were found to be intact when the starch was heated to 90°C.

In Figure 11.4.9 the light microscopic images for B994, modified corn starch are shown when heated to 50, 75 or 90°C. The starch showed larger granules at 50°C compared to B950 or B965 corn starches. These B994 starch granules became swollen at 75°C and showed complete breakdown when heated to 90°C. Corn starch N1658 was observed for changes in granule structure due to heat treatment (Figure 11.4.10). The granules observed after heating to 50°C did not show any breakdown at 75°C but at 90°C there was a large-scale disruption observed. Fewer intact granules could be seen when the N1658 was heated to 90°C.

Figure 11.4.11 shows the effect of heating on a mixture of fat replacer solution. The solution was made up of both Maltrin M100 and Versagel fat replacers added at 0.25% each. When this mix solution was heat treated to 50°C, numerous granules were observed, which were small in size. Heating to 75°C caused the granules to swell and hydrate which upon further heating to a higher temperature of 90°C showed disruption of granules and dispersed molecular components of starch in the solution. A network formation of the fat replacer molecules could be possible after cooling when the exudate of amylose and amylopectin can reassociate and form a gel as was observed in Table 11.4.16.

The effect of heat treatment given to Maltrin M040 is shown in Figure 11.4.12. The starch granules appeared to increase in diameter when heated to 75°C with negligible collapse of the granules. At 90°C the granules lost their birefringence and appeared swollen and partially disrupted similar to that reported by Thomas and Atwell (1999). A potato starch, StaSlim SS143 shown in Figure 11.4.13 had similar characteristics to that of Maltrin M040. The SS143 granules had increased starch disruption compared to M040 at 75°C and at 90°C the pasting appeared to be complete. There were no intact granules observed for the SS143 after heating to 90°C.

In Figure 11.4.14 the changes occurring when Versagel is heated have been shown. Only a few of the granules appeared to be swollen at 75°C as was expected due to the major proportion of Versagel being constituted of β -lactoglobulin. The carageenan and xanthan gums required higher temperatures to gelatinise and this was evident at 90°C. The granules of Versagel remained largely intact even after heating to 90°C and holding for 20 min.

Figure 11.4.15 shows the maltodextrin M100 heat treated to 50, 75 and 90°C and observed under a light microscope. Initially when Maltrin M100 was heated to 50°C the granules appeared to be very small and dispersed. These granules became enlarged and swollen due to increased hydration at 75°C and the starch was completely pasted at 90°C.

11.4.7 Rheological characteristics

Rheological characteristics such as G' , G'' and $\tan(\delta)$ were measured using a rheometer (section 3.1.3.16) to provide information about the heat-induced changes in the granules of fat replacers. The storage modulus (G') indicates the strength of intramolecular bonds while loss modulus (G'') is a measure of inter-molecular bonds. The ability of a molecule to overcome the heat energy applied to it without breaking down represents storage modulus while the intermolecular bonds, which can be broken by providing energy indicates loss modulus. Fat replacers showing $\tan(\delta)$ less than 1 indicate a gel formation.

Figure 11.4.16 shows the Deflection angle, Loss modulus, Torque, Complex viscosity and Storage modulus for Versagel plotted over the temperature regime of 20°C to 90°C. The Versagel sample when heated showed increase in the loss modulus (G'') from 0.40 to 0.50 while the storage modulus (G') remained constant close to zero till the sample reached about 85°C. There was no cross over of G' and G'' indicating that there was insufficient amount of Versagel to form a network. The increased G'' at 85°C was considered to be the gelatinisation temperature for Versagel samples. Versagel samples when heated showed increase in Torque and complex viscosity with temperature. During the cooling process of Versagel sample from 90°C to 20°C the storage modulus, loss modulus, Torque and complex viscosity increased as shown in Figure 11.4.17.

Heat treatment of Maltrin M100 added to 'Lite' milk showed an increase in loss modulus while the storage modulus remained constant (Figure 11.4.18). The deflection angle, torque and complex viscosity also remained constant through out the heating process. The values were very low and appeared similar to that of Versagel except that there was no increase observed for storage modulus. A higher concentration of Maltrin M100 in 'Lite' milk could have probably increased the values of storage modulus. Cooling of Maltrin M100 solution after heat treatment to 90 °C showed an increase in storage modulus with decrease in temperature (Figure 11.4.19). The storage modulus and loss modulus values were significantly lower for Maltrin compared to those for Versagel. This could be due to the early disruption of maltodextrin granules by heat. Versagel consists of β -lactoglobulin, and carageenan and xanthan gums. The gums present in Versagel would be able to form a network with stronger inter molecular bonding compared to maltodextrins. This could be the reason for the higher values of the rheological parameters for Versagel compared to Maltrin. The results correlated with the suppliers specifications.

In order to observe the effect of a combined addition of Versagel and Maltrin M100 to 'Lite' milk on the storage modulus and loss modulus, a 0.25% of each fat replacer was added to the 'Lite' milk and rheological measurements were performed. The loss modulus, torque, complex viscosity and storage modulus remained constant unlike the Versagel only samples (Figure 11.4.20). The trend was similar to that observed when Maltrin M100 was heated. Upon cooling the storage modulus values measured for Versagel and Maltrin added 'Lite' milk was similar to that for Maltrin added 'Lite' milk as shown in Figure 11.4.21. The other parameters were also similar to that of maltodextrin added milk.

11.4.8 ULFM cheeses made using N1658 and B965 fat replacers

The study of the effects of heating and cooling on the hydration and gelatinisation characteristics of the fat replacers prompted us to modify the cheese manufacturing method. Thus based on all the above results it was felt that an optimum hydration of the fat replacer was necessary and the type of fat replacer was an important consideration for successful manufacturing of an ULFM cheese with improved functionality. Details of manufacturing conditions and optimisation of the rate of fat replacer added to cheese milk are not being provided due to confidentiality reasons.

The results from cheese making and later pizza bake characteristics indicated an increase in yield (wet basis) by 10- 12% using N1658 fat replacer. The pizza bake characteristics of freshly baked cheeses containing N1658 and B965 fat replacers and stored for 30 d are shown in Figure 11.4.22, while these cheeses when cooled are shown in Figure 11.4.23. The cheeses were again analysed for pizza bake characteristics after 45 d and the freshly baked pizzas obtained are shown in Figure 11.4.24 while the 45 d old baked and cooled cheeses are shown in Figure 11.4.25. The N1658 based cheeses showed reduced browning, had an appealing whiteness and gloss especially when these cheeses were applied with oil. The N1658 (with oil) cheeses had similar functionality to that of a full fat mozzarella cheese after 30 d of storage. Blister formation was also reduced and the shredded cheeses melted, fused and showed excellent flow characteristics on the pizza. Due to very low fat content in the cheeses (<6.0% milk fat on wet basis) no free oil formation was observed. At 30 d and 45 d the N1658 based cheeses showed good pizza bake characteristics without burning and remained the same after the pizzas were cooled. The B965 based cheeses showed browning although the cheeses were not burnt. B965 based cheeses did not perform similar to a full fat mozzarella cheeses after 30 d or 45 d whether oil was applied or not.

Thus although the starches were from similar origin (corn starches), N1658 and B965 were found to have different functionality within the cheeses which affected the ULFM cheese characteristics when baked.

11.4.9 Stretchability testing of commercial mozzarella cheeses

The melted commercial cheeses described in section 11.3.1, were stretched using a cross bar spindle. The force exerted by the cheese on the spindle was recorded along with the instantaneous distance of load cell movement. Data such as extension at which the strands broke, peak load before breaking and break point were collected during the stretch tests using a Merlin Software as shown in Table 11.4.17. The weight of 100 mm cheese strand cut from the middle of the stretched strand was measured using an analytical balance, converted to a standard unit (tex value) and compared among the four cheeses. Table 11.4.17 shows the composition of the cheeses. The protein content of the cheeses A, B and C was similar (26.5%) while cheese D had 31.7% protein. The fat contents ranged from 14.5% (low-fat) to 23.5% (full-fat). Cheeses A, B, C and D showed calcium contents of 865, 765, 800 and 950 mg per 100 g of samples, respectively.

Figure 11.4.26 shows an image of a cheese stretched using the developed stretch test. A typical texture profile curve was obtained as shown in Figure 11.4.27. The curve is shown only up to 60 mm whereas the cheeses were actually stretched to 300 mm. The stretch-distance appeared to be a good measure of the stretchability of cheeses and that below 300 mm was considered of inferior quality. The force required to stretch the cheeses increased sharply as the spindle exited from the sample and decreased as the strands of the cheese were further pulled as shown in the typical stretch-test curve (Figure 11.4.27). The tex value indicates the linear density and is calculated as weight in g of 1000 m of yarn (stretched cheese). An alternative unit for tex is denier which is given by weight in g of 9000 m of yarn. Another measure termed as tenacity

(or toughness) was also considered to estimate the differences in stretchability of the cheeses. Tenacity is measured as the tensile stress at breakpoint. It is expressed as force per unit linear density of unstrained specimen. The cheese strands were considered similar to cotton or nylon fibres (in general yarn) as explained by Othmer (1999). For the same brand (Cheese C and D) the peak load before breaking and tenacity seem to increase with decreased fat content and increased protein content (Table 11.4.17). Definite correlations between the composition, and stretch measures such as extension, strand weight, tex, peak load before breaking and tenacity, could not be established due to differences in composition, and also possibly due to manufacturing parameters, cheese pH, calcium and salt contents and degree of proteolysis in cheeses, which would have an effect on their stretchability. The results show however, that this method of testing stretchability of cheeses can be objectively carried out with great reliability. The data were reproducible. The test was easy to perform and was less time-consuming than the subjective test of using a fork. The tex and tenacity values can be directly computed using the Merlin software.

11.5 Conclusions

Maltrin based cheeses retained reduced amounts of moisture and about 1.0% protein was lost in whey. Addition of Maltrin M100 increased the total solids of the cheeses but did not increase their moisture or protein contents. Versagel based cheeses showed increased loss of protein in whey during manufacture. Stretch water used for stretching the Maltrin based and Versagel based cheeses had similar total solids and proteins contents. Negligible amounts of proteins were lost in stretch water for both cheeses. Versagel based cheeses had increased moisture content. Addition of 0.25% or 0.1% of Maltrin or Versagel did not alter the composition of whey. The curd samples for all the fat replaced cheeses showed similar moisture content. During cheddaring some of the Versagel could have been hydrated and thus helped the cheddared curd retain more moisture. Cheeses made from 0.25% Versagel showed

higher moisture content than Maltrin and control cheeses. Control and Maltrin based cheeses had similar moisture content. The increase in moisture content of V2 and V1 cheeses probably caused a decrease in their apparent protein content. Calcium content of curd and cheese samples obtained from fat replaced milk was higher than for control cheeses. Versagel based cheeses retained more calcium than Maltrin based or control cheeses.

The pizza bake colour measurements showed increased whiteness for Versagel based cheeses compared to Maltrin based or control cheeses. Application of oil to the fat replaced cheeses seemed to reduce the browning. Cheeses made from 0.1% Versagel added to cheese milk showed pizza bake characteristics similar to the 0.25% Maltrin based cheeses. Thus reduced rates of Versagel addition to cheese milk had similar effect as the higher rates of addition of Maltrin. Increased loss in sodium, potassium and magnesium into whey reduced their concentration in the curd. Magnesium was retained in the curd to a greater extent than was sodium or potassium. Stretching the curd in brine caused increased loss of magnesium and potassium ions from the curd. Size of starch granules, the extent of the starch modifications and its origin caused several differences in their hydration characteristics. The starches showing increased hydration also showed greater gelatinisation. Heating the starches to 90°C caused a reduction in their refractive index possibly due to exudation of the granular constituents. Lite milk mixed with Maltrin showed reduced values of storage modulus and loss modulus compared to milk mixed with Versagel. The rate of addition (0.25%) was too low for both Maltrin and Versagel to form gels but together they were able to form a firm gel. Finally, cheeses made using N1658, corn starch showed excellent melt, shred fusion and flow characteristics when baked on pizza. These cheeses had the desired characteristics similar to that of a full fat mozzarella cheese. Stretchability of cheeses could be objectively measured using Instron with great reliability. The data were reproducible and the test was easy to perform.

Table 11.4.1 The effect of addition of 0.5% Maltrin to cheese milk on the mean \pm SE¹ of moisture and protein contents (n = 3) of milk, whey and mozzarella cheese.

Product	Moisture (%)	Protein (%)
Standardised milk	89.13 \pm 0.01	4.08 \pm 0.01
Milk mixed with 0.5% M100	88.68 \pm 0.01	4.06 \pm 0.02
Whey	91.83 \pm 0.01	1.11 \pm 0.00
Stretch water after use	96.94 \pm 0.01	0.13 \pm 0.00
Mozzarella cheese	55.25 \pm 0.10	33.83 \pm 0.08

¹SE = Standard error; n = Number of samples.

Table 11.4.2 The effect of addition of 0.5% Versagel to cheese milk on the mean \pm SE¹ of moisture and protein contents (n = 3) of milk, whey and mozzarella cheese.

Product	Moisture (%)	Protein (%)
Standardised milk	89.13 \pm 0.01	4.08 \pm 0.01
Milk mixed with 0.5% Versagel	88.66 \pm 0.01	4.44 \pm 0.00
Whey	91.96 \pm 0.01	1.44 \pm 0.01
Stretch water after use	96.94 \pm 0.01	0.13 \pm 0.00
Mozzarella cheese	55.95 \pm 0.00	33.12 \pm 0.17

¹SE = Standard error; n = Number of samples.

Table 11.4.3 The mean \pm SE¹ of moisture contents (n = 6) of milk, whey, curd, milled curd, stretched curd and mozzarella cheeses made with or without the addition of fat replacer.

Product	Standardised milk	Whey	Curd at whey drain	Milled curd	Stretched curd	Stretch water after use	Mozzarella cheese
Control	90.47 \pm 0.01 ^a	93.38 \pm 0.01 ^a	59.99 \pm 1.43 ^a	51.44 \pm 0.44 ^d	50.27 \pm 0.41 ^d	96.46 \pm 0.09 ^a	54.89 \pm 0.04 ^{ab}
M2	90.43 \pm 0.02 ^a	93.18 \pm 0.01 ^b	62.78 \pm 1.93 ^a	52.83 \pm 0.56 ^{bcd}	52.44 \pm 0.39 ^c	96.53 \pm 0.04 ^a	54.67 \pm 0.49 ^{ab}
M1	90.64 \pm 0.03 ^a	93.43 \pm 0.02 ^a	64.80 \pm 0.39 ^a	53.54 \pm 0.40 ^{abcd}	53.16 \pm 0.44 ^{bc}	96.49 \pm 0.00 ^a	54.28 \pm 0.09 ^{bc}
V2	90.45 \pm 0.10 ^a	93.34 \pm 0.03 ^a	64.56 \pm 0.58 ^a	55.51 \pm 0.52 ^a	55.00 \pm 0.34 ^a	96.51 \pm 0.04 ^a	55.62 \pm 0.15 ^a
V1	90.54 \pm 0.08 ^a	93.47 \pm 0.06 ^a	59.94 \pm 1.38 ^a	52.27 \pm 0.26 ^{cd}	53.51 \pm 0.10 ^{abc}	96.54 \pm 0.02 ^a	53.21 \pm 0.08 ^c
p-value ²	0.1322	0.0000 [*]	0.2300	0.0000 [*]	0.0000 [*]	0.7314	0.0000 [*]

¹SE = Standard error; ²ANOVA of means arranged within the same column; ^{a, b, c, d} Means within same column not sharing common superscripts differ (p<0.05); ^{*} Significant (p<0.05); n = Number of samples; Control = Samples collected when making cheeses containing no fat replacer; M2 = Samples collected when making cheeses from milk mixed with 0.25% Maltrin; M1 = Samples collected when making cheeses from milk mixed with 0.1% Maltrin; V2 = Samples collected when making cheeses from milk mixed with 0.25% Versagel; V1 = Samples collected when making cheeses from milk mixed with 0.1% Versagel.

Table 11.4.4 The mean \pm SE¹ of protein contents (n = 6) of milk, whey, curd, milled curd, stretched curd and mozzarella cheeses made with or without the addition of fat replacer.

Product	Standardised milk	Whey	Curd at whey drain	Milled curd	Stretched curd	Stretch water after use	Mozzarella cheese
Control	3.69 \pm 0.88 ^a	1.11 \pm 0.02 ^{ab}	31.42 \pm 0.44 ^a	35.73 \pm 0.18 ^a	34.99 \pm 0.43 ^a	0.09 \pm 0.00 ^a	35.13 \pm 0.12 ^{ab}
M2	3.57 \pm 0.04 ^a	1.04 \pm 0.02 ^b	28.81 \pm 2.82 ^a	35.55 \pm 0.39 ^a	34.58 \pm 0.57 ^a	0.13 \pm 0.03 ^a	35.40 \pm 0.74 ^{ab}
M1	3.61 \pm 0.02 ^a	1.08 \pm 0.03 ^{ab}	27.63 \pm 0.47 ^a	35.47 \pm 0.31 ^{ab}	35.23 \pm 0.53 ^a	0.11 \pm 0.00 ^a	35.39 \pm 0.14 ^{ab}
V2	3.49 \pm 0.16 ^a	1.20 \pm 0.03 ^a	27.22 \pm 0.44 ^a	33.52 \pm 0.70 ^b	34.63 \pm 0.14 ^a	0.11 \pm 0.01 ^a	33.84 \pm 0.25 ^b
V1	3.59 \pm 0.08 ^a	1.16 \pm 0.03 ^{ab}	30.45 \pm 1.34 ^a	36.21 \pm 0.21 ^a	35.70 \pm 0.26 ^a	0.07 \pm 0.00 ^a	36.34 \pm 0.33 ^a
p-value ²	0.6351	0.0100 [*]	0.2342	0.0028 [*]	0.3491	0.1264	0.0062 [*]

¹SE = Standard error; ²ANOVA of means arranged within the same column; ^{a, b, c, d} Means within same column not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05); n = Number of samples; Control = Samples collected when making cheeses containing no fat replacer; M2 = Samples collected when making cheeses from milk mixed with 0.25% Maltrin; M1 = Samples collected when making cheeses from milk mixed with 0.1% Maltrin; V2 = Samples collected when making cheeses from milk mixed with 0.25% Versagel; V1 = Samples collected when making cheeses from milk mixed with 0.1% Versagel.

Table 11.4.5 Percentage of DP7 (oligosaccharide from starch) in whey, stretch water and cheese expressed as percentage of DP7 in milk.

Product	Percentage of DP7 in milk
Whey at draining	45.21 – 58.04
Stretch water after kneading and stretching curd	20.00 – 24.92
2 d old cheese	5.99 – 7.40

Table 11.4.6 The calcium contents (n = 12) measured in mg/Kg of milk, whey, curd, milled curd, stretched curd and mozzarella cheeses made with or without the addition of fat replacer.

Product	Standardised milk	Whey	Curd at whey drain	Milled curd	Stretched curd	Stretch water after use	Mozzarella cheese
Control	1477.6	699.8	7366.1	9410.8	8768.8	341.7	8750.8
M2	1537.5	716.2	8342.9	9399	9561.4	287.3	9612.1
M1	1421.0	616.8	7810.1	10209.1	10100.8	353.0	9759.4
V2	1375.2	571.0	7958.8	9754.7	9838.4	310.7	9641.7
V1	1370.3	566.3	8244.2	10909.7	10611.9	231.3	10460.4

n = Number of samples; Control = Samples containing no fat replacer; M2 = Samples collected when making cheeses from milk mixed with 0.25% Maltrin; M1 = Samples collected when making cheeses from milk mixed with 0.1% Maltrin; V2 = Samples collected when making checcses from milk mixed with 0.25% Versagel; V1 = Samples collected when making cheeses from milk mixed with 0.1% Versagel.

Table 11.4.7 The mean \pm SE¹ of Hunter L values (n = 6) for control and fat replaced cheeses measured during pizza bake analysis.

Type of cheese	Oil application	L ²	L ³	L ⁴	p-value ⁵
Control	No Oil	58.08 \pm 1.27 ^{a, B}	69.49 \pm 0.57 ^{a, A}	46.73 \pm 1.06 ^{e, C}	0.0000 [*]
	With oil	59.71 \pm 1.47 ^{a, B}	70.30 \pm 0.62 ^{a, A}	50.15 \pm 0.85 ^{de, C}	0.0000 [*]
M2	No Oil	60.44 \pm 0.82 ^{a, BC}	67.77 \pm 1.58 ^{a, A}	56.34 \pm 2.50 ^{abc, C}	0.0013 [*]
	With oil	56.36 \pm 0.57 ^{a, C}	70.21 \pm 1.68 ^{a, A}	59.49 \pm 2.76 ^{abc, BC}	0.0003 [*]
M1	No Oil	58.87 \pm 1.69 ^{a, B}	70.47 \pm 1.16 ^{a, A}	51.76 \pm 0.97 ^{cde, C}	0.0000 [*]
	With oil	59.98 \pm 0.63 ^{a, B}	70.92 \pm 0.27 ^{a, A}	53.59 \pm 0.74 ^{bcd, C}	0.0000 [*]
V2	No Oil	60.63 \pm 1.05 ^{a, C}	72.71 \pm 0.98 ^{a, A}	62.15 \pm 1.63 ^{ab, BC}	0.0000 [*]
	With oil	59.16 \pm 0.52 ^{a, C}	71.97 \pm 0.42 ^{a, A}	65.25 \pm 0.84 ^{a, B}	0.0000 [*]
V1	No Oil	59.44 \pm 1.19 ^{a, C}	69.15 \pm 0.83 ^{a, A}	64.89 \pm 1.07 ^{a, B}	0.0000 [*]
	With oil	57.10 \pm 0.90 ^{a, B}	67.69 \pm 1.30 ^{a, A}	63.85 \pm 0.79 ^{a, A}	0.0000 [*]
p-value ⁶		0.1124	0.2410	0.0000 [*]	

¹SE = Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); ^{*}Significant (p<0.05); Control = Cheeses containing no fat replacer; M2 = Cheeses made from milk mixed with 0.25% Maltrin; M1 = Cheeses made from milk mixed with 0.1% Maltrin; V2 = Cheeses made from milk mixed with 0.25% Versagel; V1 = Cheeses made from milk mixed with 0.1% Versagel.

Table 11.4.8 The mean \pm SE¹ of Hunter a values (n = 6) for control and fat replaced cheeses measured during pizza bake analysis.

Type of cheese	Oil application	a ²	a ³	a ⁴	p-value ⁵
Control	No Oil	-1.01 \pm 0.21 ^{ab, B}	1.99 \pm 0.89 ^{a, A}	2.91 \pm 0.98 ^{a, A}	0.0069
	With oil	-1.41 \pm 0.13 ^{b, B}	0.63 \pm 0.70 ^{a, AB}	2.14 \pm 1.05 ^{a, A}	0.0131*
M2	No Oil	-1.07 \pm 0.16 ^{ab, B}	4.73 \pm 1.65 ^{a, A}	3.10 \pm 0.66 ^{a, A}	0.0035*
	With oil	-0.76 \pm 0.13 ^{ab, A}	1.60 \pm 1.47 ^{a, A}	0.96 \pm 0.61 ^{a, A}	0.2064
M1	No Oil	-0.22 \pm 0.26 ^{a, B}	1.24 \pm 0.39 ^{a, A}	2.08 \pm 0.25 ^{a, A}	0.0003*
	With oil	-0.77 \pm 0.15 ^{ab, C}	-0.69 \pm 0.17 ^{a, BC}	0.44 \pm 0.17 ^{a, A}	0.0001*
V2	No Oil	-0.63 \pm 0.14 ^{ab, B}	0.93 \pm 0.66 ^{a, AB}	3.23 \pm 0.95 ^{a, A}	0.0037*
	With oil	-0.56 \pm 0.15 ^{ab, A}	-0.52 \pm 0.27 ^{a, A}	0.17 \pm 0.50 ^{a, A}	0.2665
V1	No Oil	-0.25 \pm 0.22 ^{a, B}	1.96 \pm 0.41 ^{a, AB}	3.98 \pm 1.24 ^{a, A}	0.0051*
	With oil	-0.23 \pm 0.17 ^{a, A}	2.15 \pm 1.34 ^{a, A}	2.71 \pm 0.86 ^{a, A}	0.0880
p-value ⁶		0.0001*	0.1280	0.1770	

¹SE = Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; *Significant (p<0.05); ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); Control = Cheeses containing no fat replacer; M2 = Cheeses made from milk mixed with 0.25% Maltrin; M1 = Cheeses made from milk mixed with 0.1% Maltrin; V2 = Cheeses made from milk mixed with 0.25% Versagel; V1 = Cheeses made from milk mixed with 0.1% Versagel.

Table 11.4.9 The mean \pm SE¹ of Hunter b values (n = 6) for control and fat replaced cheeses measured during pizza bake analysis.

Type of cheese	Oil application	b ²	b ³	b ⁴	p-value ⁵
Control	No Oil	17.89 \pm 0.73 ^{a, BC}	29.82 \pm 0.69 ^{a, A}	17.79 \pm 0.25 ^{f, C}	0.0000 [*]
	With oil	17.97 \pm 0.35 ^{a, C}	29.51 \pm 0.61 ^{a, A}	18.38 \pm 0.50 ^{ef, BC}	0.0000 [*]
M2	No Oil	18.48 \pm 0.78 ^{a, C}	29.62 \pm 1.14 ^{a, A}	23.60 \pm 1.44 ^{abcdef, B}	0.0000 [*]
	With oil	16.76 \pm 0.35 ^{a, B}	29.03 \pm 1.41 ^{a, A}	23.02 \pm 2.40 ^{bcef, A}	0.0003 [*]
M1	No Oil	18.14 \pm 0.56 ^{a, C}	27.48 \pm 0.47 ^{a, A}	20.69 \pm 0.62 ^{cef, B}	0.0000 [*]
	With oil	18.07 \pm 0.34 ^{a, C}	27.07 \pm 0.40 ^{a, A}	18.72 \pm 0.77 ^{def, BC}	0.0000 [*]
V2	No Oil	17.80 \pm 0.32 ^{a, B}	27.01 \pm 0.77 ^{a, A}	27.49 \pm 0.60 ^{ab, A}	0.0000 [*]
	With oil	17.31 \pm 0.43 ^{a, B}	26.78 \pm 0.41 ^{a, A}	26.39 \pm 0.79 ^{abc, A}	0.0000 [*]
V1	No Oil	18.23 \pm 0.48 ^{a, B}	27.45 \pm 1.44 ^{a, A}	30.07 \pm 0.77 ^{a, A}	0.0000 [*]
	With oil	17.39 \pm 0.46 ^{a, B}	30.12 \pm 1.68 ^{a, A}	29.91 \pm 0.80 ^{a, A}	0.0000 [*]
p-value ⁶		0.4326	0.0977	0.0000 [*]	

¹SE = Standard error; ²Fresh cheeses; ³Baked cheeses; ⁴Cooled cheeses; ⁵ANOVA of means arranged within the same row; ⁶ANOVA of means arranged within the same column; ^{*}Significant (p<0.05); ^{a, b, c, d, e} Means within same column not sharing common superscripts differ (p<0.05); ^{A, B, C} Means within same row not sharing common superscripts differ (p<0.05); Control = Cheeses containing no fat replacer; M2 = Cheeses made from milk mixed with 0.25% Maltrin; M1 = Cheeses made from milk mixed with 0.1% Maltrin; V2 = Cheeses made from milk mixed with 0.25% Versagel; V1 = Cheeses made from milk mixed with 0.1% Versagel.

Table 11.4.10 The mean \pm SE¹ of minerals estimated (n = 12) at several stages during manufacture of control ULFM cheeses (no fat replacers were added).

Sample No.	Sample description	Na (mg/kg sample)	K (mg/kg sample)	Mg (mg/kg sample)
S1	Std. Milk sample	247.17 \pm 12.36	1644.54 \pm 19.49	114.51 \pm 3.59
S2	Whey at drain	380.99 \pm 5.22	1817.18 \pm 8.17	88.16 \pm 0.70
S3	Curd at whey drain	295.95 \pm 2.44	1513.57 \pm 34.96	305.02 \pm 12.56
S4	Milled curd before salting	237.44 \pm 0.22	1343.00 \pm 0.30	356.67 \pm 1.08
S5	Stretched curd	3577.98 \pm 54.29	956.13 \pm 94.93	314.14 \pm 2.16
S6	Stretch water after use	Not Determined	135.58 \pm 10.37	14.48 \pm 1.13
S7	Stored cheese sample (2 d)	2949.38 \pm 3.31	1068.37 \pm 23.93	297.81 \pm 2.48

¹SE = Standard error.

Table 11.4.11 The mean \pm SE¹ of minerals (n = 12) estimated at several stages during manufacture of 0.25% Maltrin based ULFM cheeses.

Sample No.	Sample description	Na (mg/kg sample)	K (mg/kg sample)	Mg (mg/kg sample)
S1	Std. Milk sample	266.69 \pm 4.02	1656.41 \pm 3.50	100.10 \pm 1.09
S2	Whey at drain	390.09 \pm 2.47	1796.37 \pm 6.99	83.41 \pm 2.42
S3	Curd at whey drain	308.25 \pm 6.84	1548.98 \pm 21.88	325.74 \pm 4.56
S4	Milled curd before salting	267.95 \pm 11.94	1405.21 \pm 44.12	345.93 \pm 37.06
S5	Stretched curd	4036.33 \pm 18.15	1104.59 \pm 13.45	326.61 \pm 28.64
S6	Stretch water after use	Not determined	159.90 \pm 5.94	12.04 \pm 0.05
S7	Stored cheese sample (2 d)	3945.48 \pm 68.12	1096.44 \pm 17.63	317.52 \pm 29.57

¹SE = Standard error.

Table 11.4.12 The mean \pm SE¹ of minerals (n = 12) estimated at several stages during manufacture of 0.1% Maltrin based ULFM cheeses.

Sample No.	Sample description	Na (mg/kg sample)	K (mg/kg sample)	Mg (mg/kg sample)
S1	Std. Milk sample	268.73 \pm 17.94	1632.74 \pm 9.96	80.03 \pm 8.94
S2	Whey at drain	389.45 \pm 4.86	1790.55 \pm 2.46	69.35 \pm 5.25
S3	Curd at whey drain	312.03 \pm 1.29	1532.51 \pm 42.35	244.69 \pm 31.06
S4	Milled curd after salting	1746.30 \pm 15.52	1311.44 \pm 27.26	266.50 \pm 34.27
S5	Stretched curd	3529.71 \pm 48.24	1125.97 \pm 6.29	278.48 \pm 31.44
S6	Stretch water after use	Not determined	151.95 \pm 0.75	7.80 \pm 0.90
S7	Stored cheese sample (2 d)	3559.84 \pm 16.79	1095.52 \pm 1.94	273.90 \pm 35.77

¹SE = Standard error.

Table 11.4.13 The mean \pm SE¹ of minerals estimated (n = 12) at several stages during manufacture of 0.25% Versagel based ULFM cheeses.

Sample No.	Sample description	Na (mg/kg sample)	K (mg/kg sample)	Mg (mg/kg sample)
S1	Std. Milk sample	271.39 \pm 0.30	1634.06 \pm 13.18	71.19 \pm 3.11
S2	Whey at drain	425.35 \pm 0.69	1754.29 \pm 28.54	73.68 \pm 13.09
S3	Curd at whey drain	322.71 \pm 0.35	1453.60 \pm 35.27	224.18 \pm 3.54
S4	Milled curd before salting	278.80 \pm 1.48	1312.21 \pm 15.74	290.87 \pm 1.91
S5	Stretched curd	3847.18 \pm 9.82	1095.14 \pm 16.39	259.14 \pm 11.66
S6	Stretch water after use	Not determined	163.83 \pm 6.68	12.35 \pm 2.67
S7	Stored cheese sample (2 d)	4095.16 \pm 12.58	1105.05 \pm 15.08	216.43 \pm 13.32

¹SE = Standard error.

Table 11.4.14 The mean \pm SE¹ of minerals estimated (n = 12) at several stages during manufacture of 0.1% Versagel based ULFM cheeses.

Sample No.	Sample description	Na (mg/kg sample)	K (mg/kg sample)	Mg (mg/kg sample)
S1	Std. Milk sample	257.85 \pm 1.18	1583.63 \pm 4.02	105.54 \pm 1.72
S2	Whey at drain	401.63 \pm 0.77	1726.80 \pm 1.77	71.47 \pm 4.89
S3	Curd at whey drain	273.63 \pm 7.28	1348.11 \pm 38.15	200.90 \pm 5.41
S4	Milled curd before salting	241.01 \pm 3.54	1217.36 \pm 20.02	242.12 \pm 5.42
S5	Stretched curd	4017.25 \pm 25.96	1042.50 \pm 26.21	217.19 \pm 12.08
S6	Stretch water after use	Not determined	127.39 \pm 1.27	9.66 \pm 0.90
S7	Stored cheese sample (2 d)	4117.74 \pm 36.61	1020.63 \pm 32.76	181.41 \pm 7.72

¹SE = Standard error.

Table 11.4.15 Effect of heating on fat replacers dissolved in ‘Lite’ milk to 50, 75 or 90°C followed by cooling to 22°C.

Sample ID	22°C	50°C	75°C	90°C
Control milk	No gel	No gel	No gel	No gel
M100	No gel	No gel	No gel	No gel
Versagel	No gel	No gel	No gel	No gel
M100 + Versagel	No gel	No gel	No gel	Firm gel
Corn Starch (N1658)	No gel	No gel	No gel	No gel
M040	No gel	No gel	No gel	No gel
SS143	No gel	No gel	No gel	Firm gel
B950	No gel	No gel	No gel	Firm gel
B965	No gel	No gel	No gel	Firm gel
B990	No gel	No gel	No gel	Firm gel
B994	No gel	No gel	No gel	Firm gel

Table 11.4.16 Refractive index measured for fat replaced ‘Lite’ milk after heating to 50, 75 and 90 °C and cooling to 22 °C.

Sample ID	22°C	50°C	75°C	90°C
Control milk	13	13	13	13
M100	13.2	13.4	13.4	12
Versagel	13.4	13.4	13.6	13
M100 + Versagel	13.6	13.4	13.4	9
Corn Starch (N1658)	13	13	13	12.6
M040	11	11	11	10.6
SS143	11	11	10.6	10.4
B950	11	11	10.8	10.6
B965	10.8	10.8	10.6	10.6
B990	11	11	10.8	10.6
B994	11	11	10.6	10.2

Table 11.4.17 Composition and stretch test observations for commercial shredded mozzarella cheeses.

Cheese	Protein (%)	Fat (%)	Calcium (mg/100 g)	Extension (mm)	Strand wt. (g/100 mm)	Tex ² (g/1000 m)	Peak load before breaking (gf)	Break point ³ (mm)	Tenacity ⁴ (gf/tex)
Cheese A (DOE ⁵ 28/7/02)	26.5	21.5	865	254	0.15 ± 0.03	1548.67	5.21 ± 1.88	237	0.0034
Cheese B (DOE ⁵ 13/07/02)	26.0	23.5	765	300 +	3.89 ± 0.30	38897.67	62.90 ± 1.14	300	0.0016
Cheese C (DOE ⁵ 28/07/02)	26.5	21.5	800	300 +	1.41 ± 0.20	14061.33	15.16 ± 1.23	300	0.0011
Cheese D (DOE ⁵ 28/08/02)	31.7	14.5	950	300 +	2.17 ± 0.72	21730.67	41.70 ± 3.05	300	0.0019

¹Extension = Distance that the cheese stretched; ²Tex = Measured as g of sample per 1000 m of stretched strand; ³Tbreak point = Length of cheese strand at peak load before breaking; ⁴Tenacity = Peak load divided by Tex; ⁵DOE = Date of expiry.

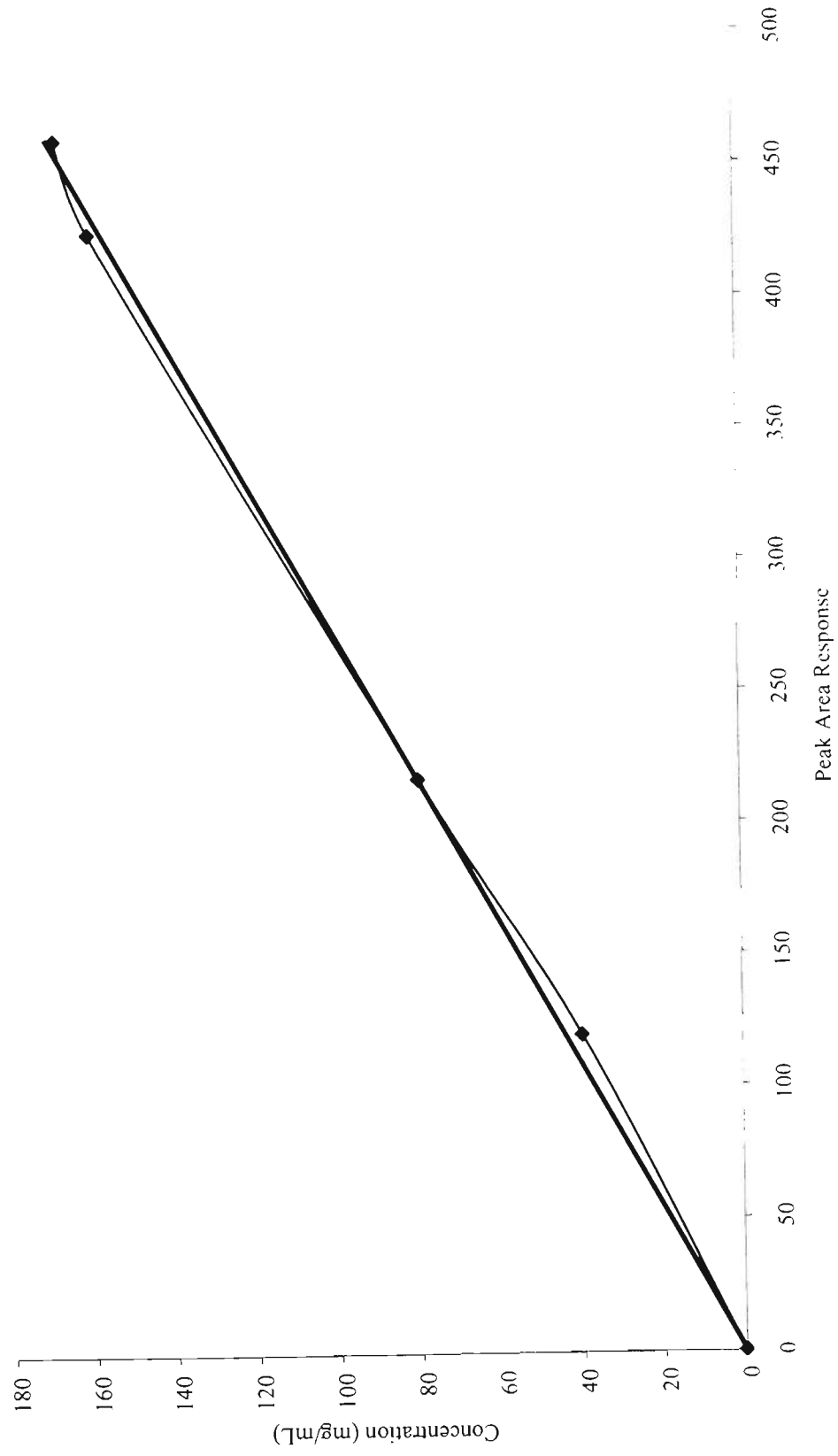


Figure 11.4.1 Peak area response for DP7 standards with elution time of 7.1 min and showing $y = 0.3708x$ and R^2 value of 0.9986.

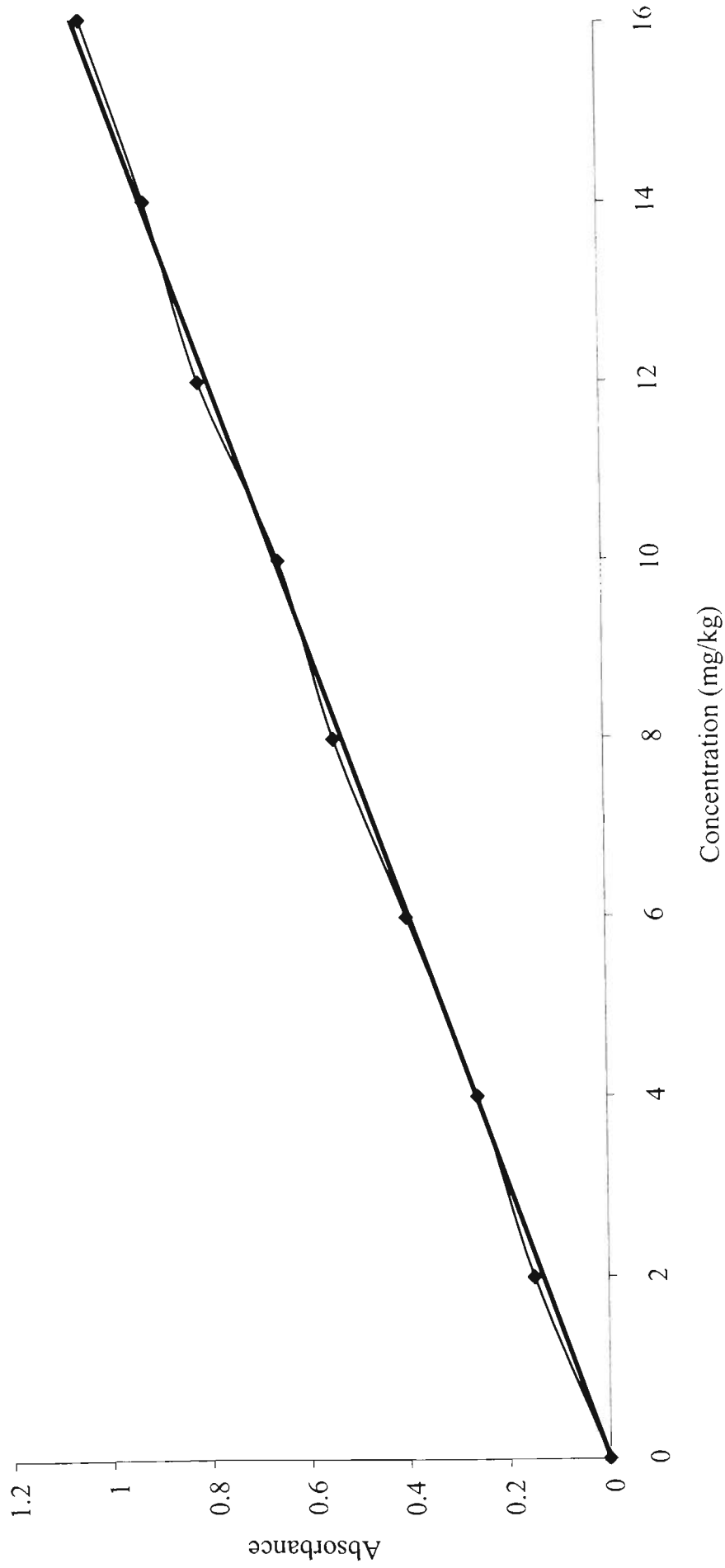


Figure 11.4.2 Absorbance values plotted against concentration (mg/kg) for calcium standards showing an R^2 value of 0.9986 and $y = 0.0658x$.

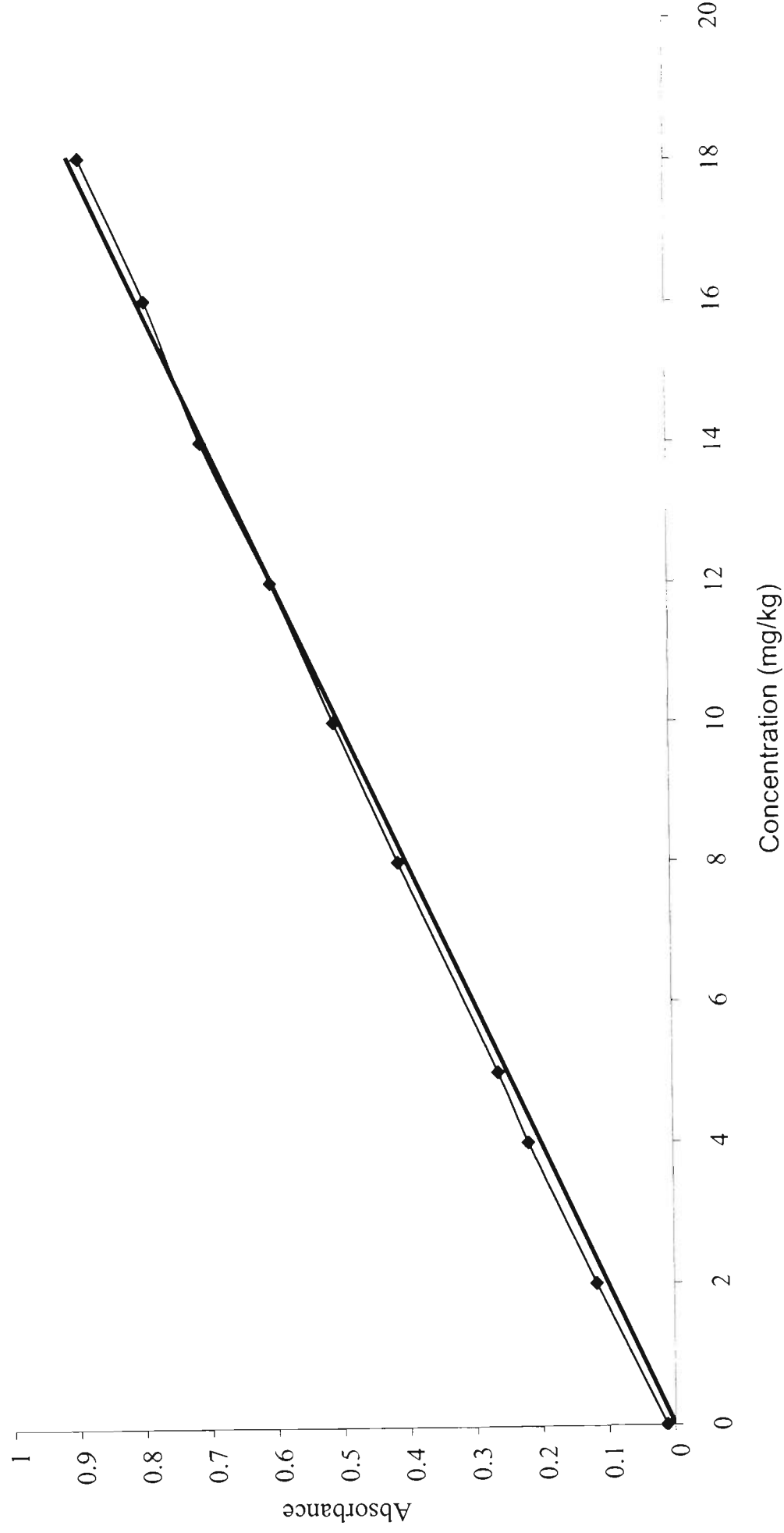


Figure 11.4.3 Absorbance values plotted against concentration (mg/kg) for sodium standards showing an R^2 value of 0.9978 and $y = 0.0501x$.

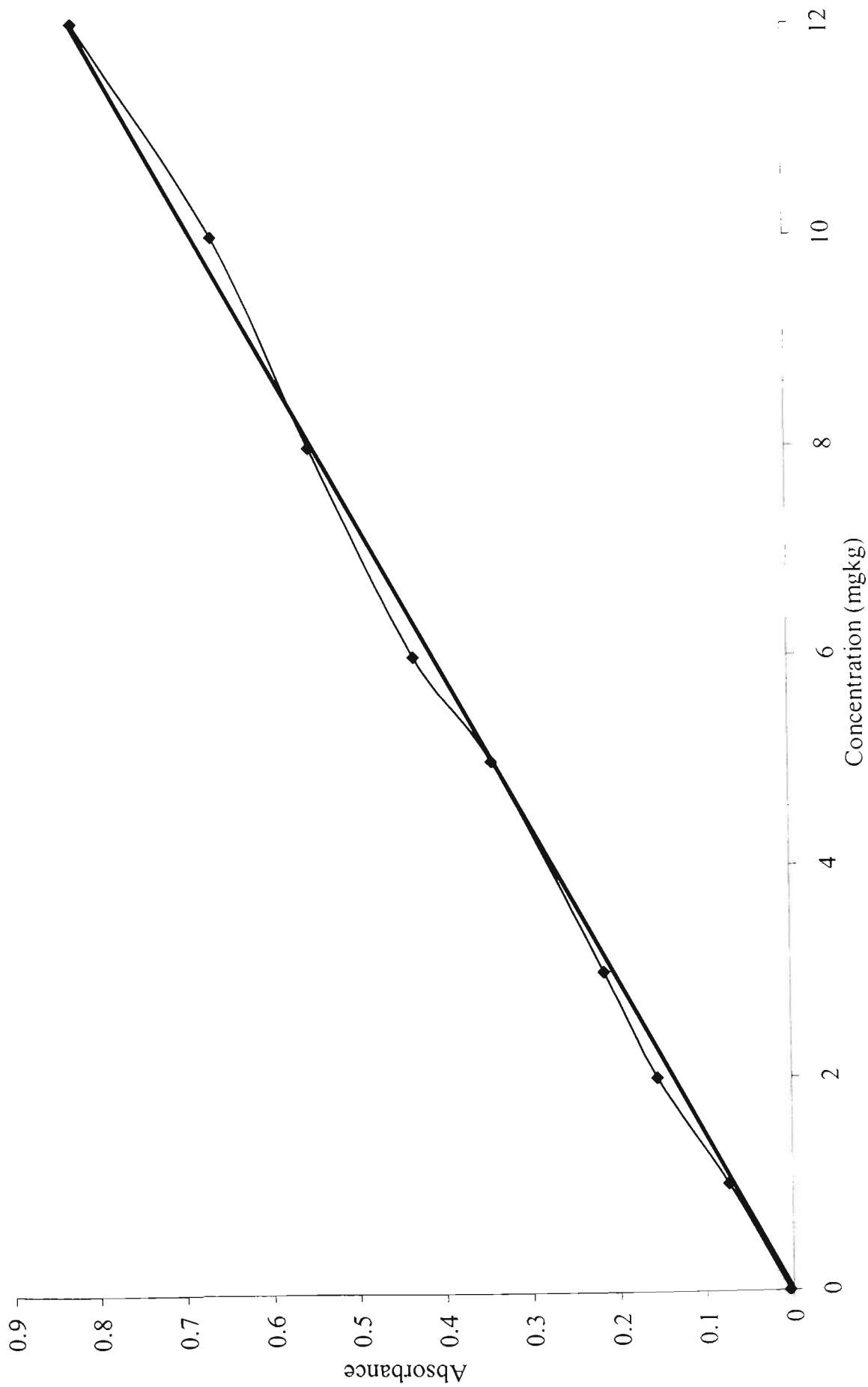


Figure 11.4.4 Absorbance values plotted against concentration (mg/kg) for potassium standards showing an R^2 value of 0.9974 and $y = 0.0685x$.

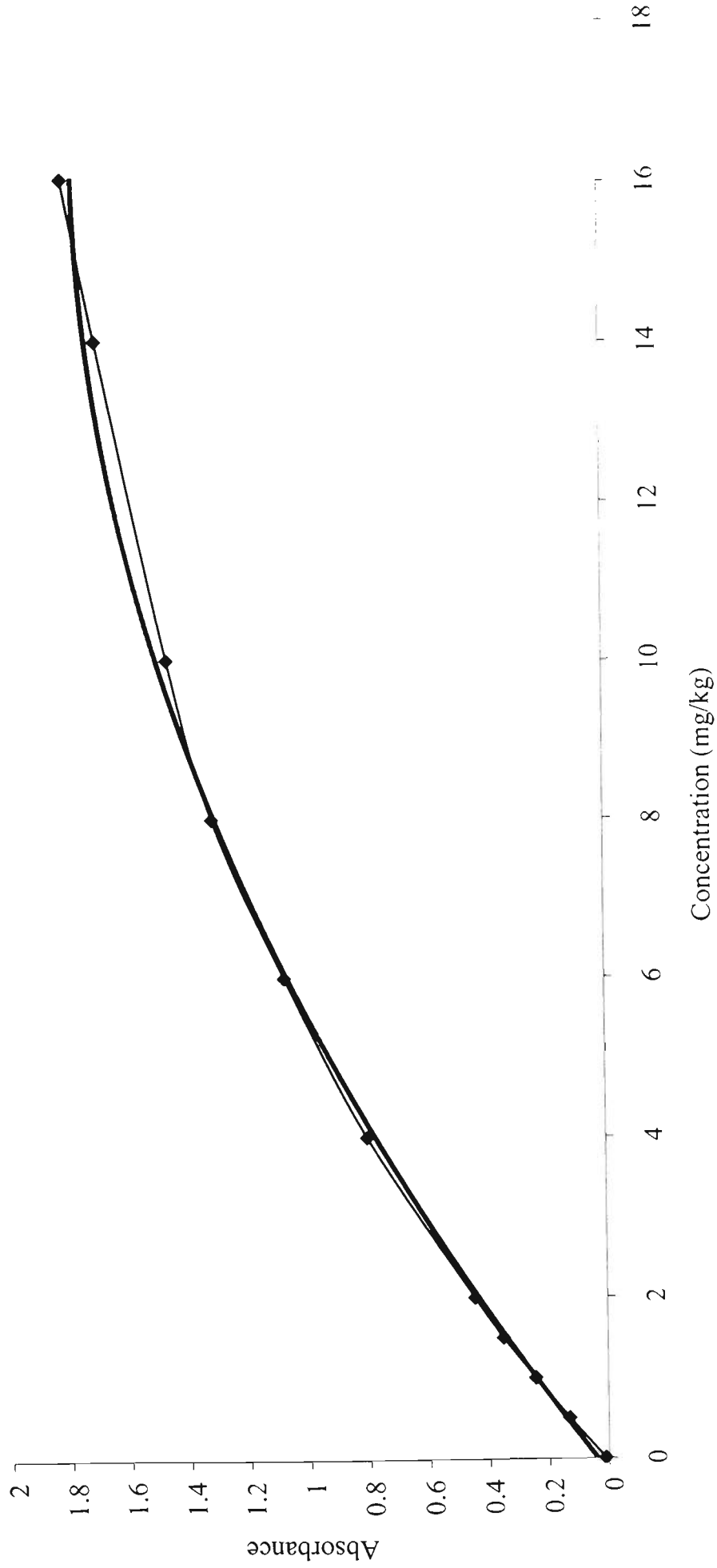


Figure 11.4.5 Absorbance values plotted against concentration (mg/kg) for magnesium standards with $y = -0.00064x^2 + 0.2098x + 0.0403$ and showing an R^2 value of 0.9986.

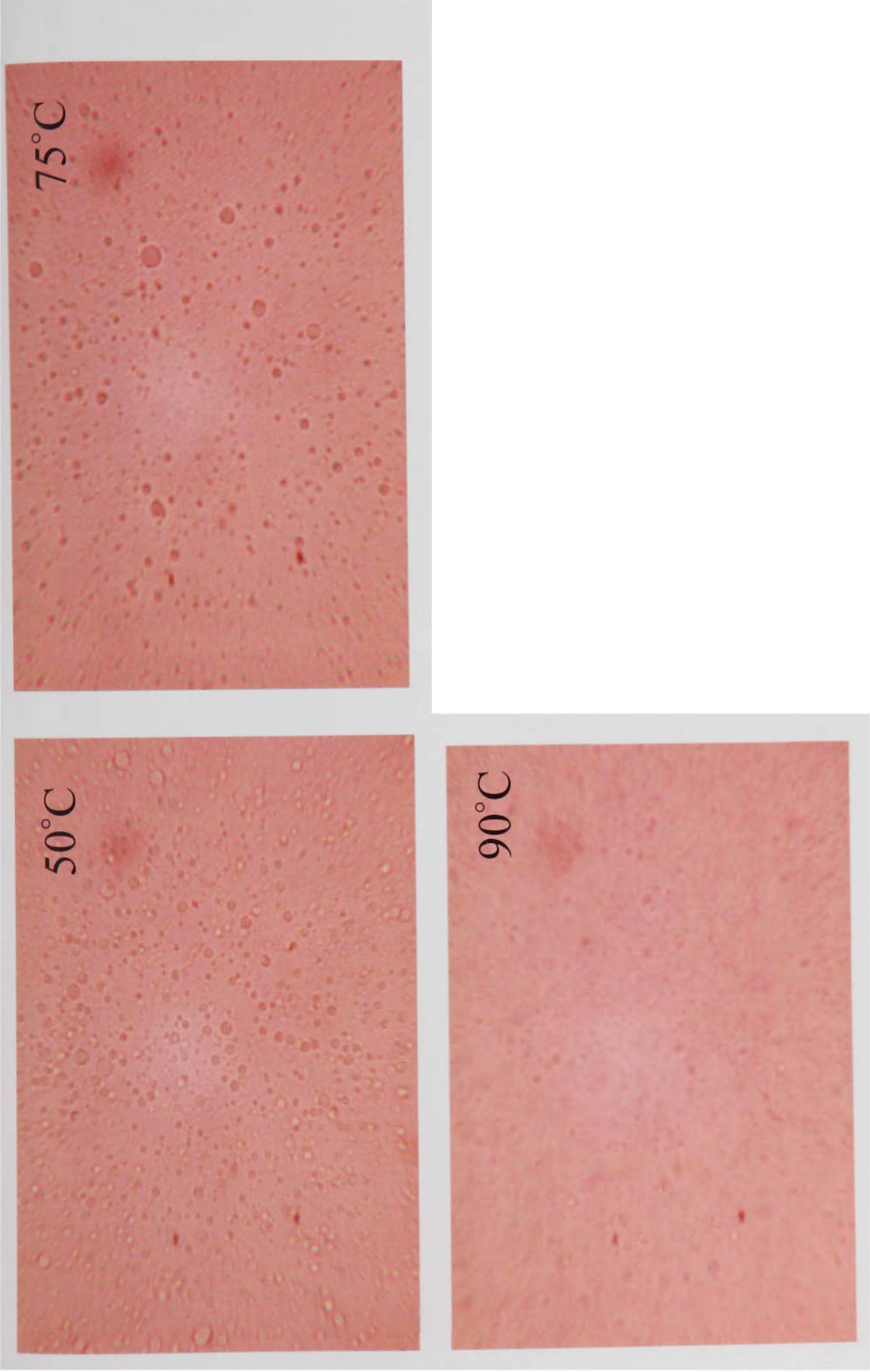


Figure 11.4.6 The effect of heating temperature on B950 corn starch granules as observed under a light microscope.

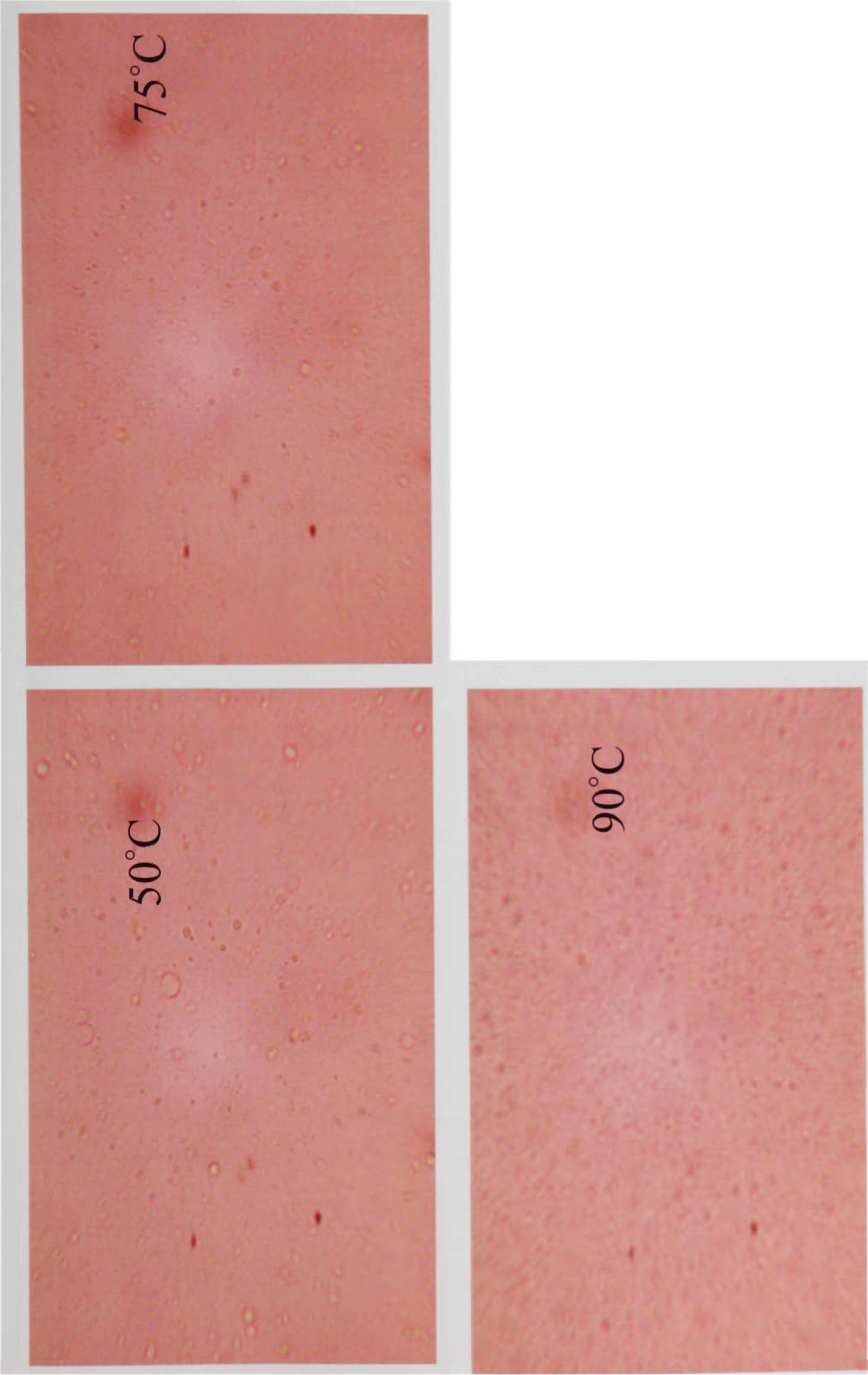


Figure 11.4.7 The effect of heating temperature on B965 corn starch granules as observed under a light microscope.

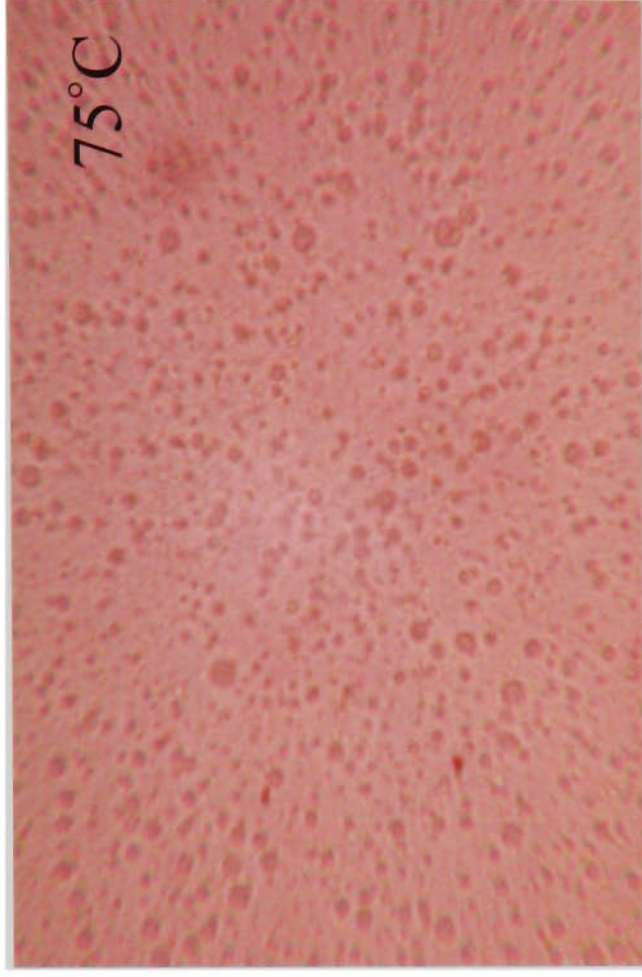
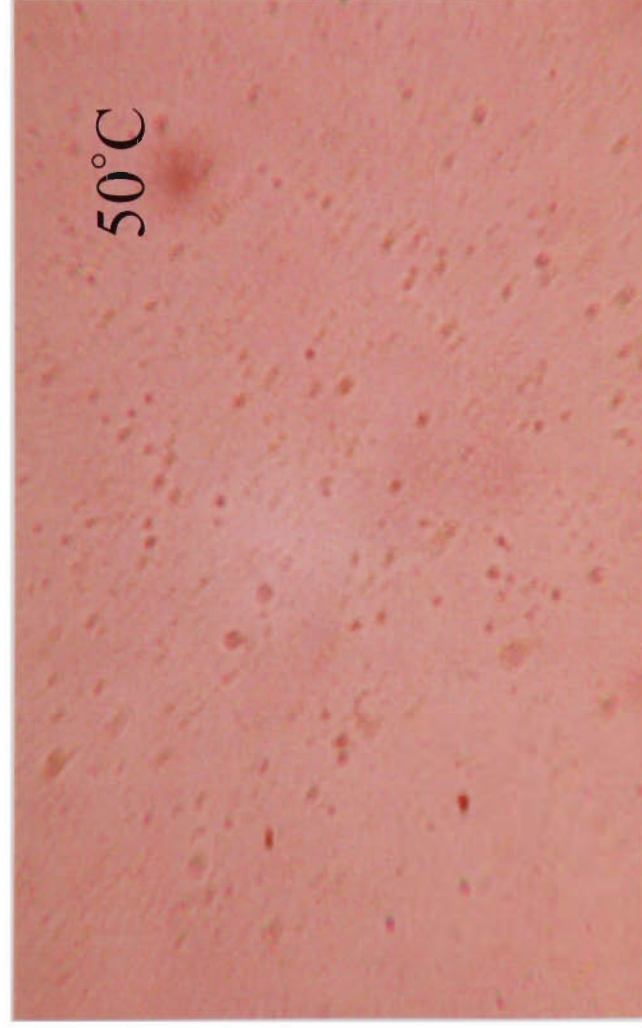


Figure 11.4.8 The effect of heating temperature on B990 corn starch granules as observed under a light microscope.

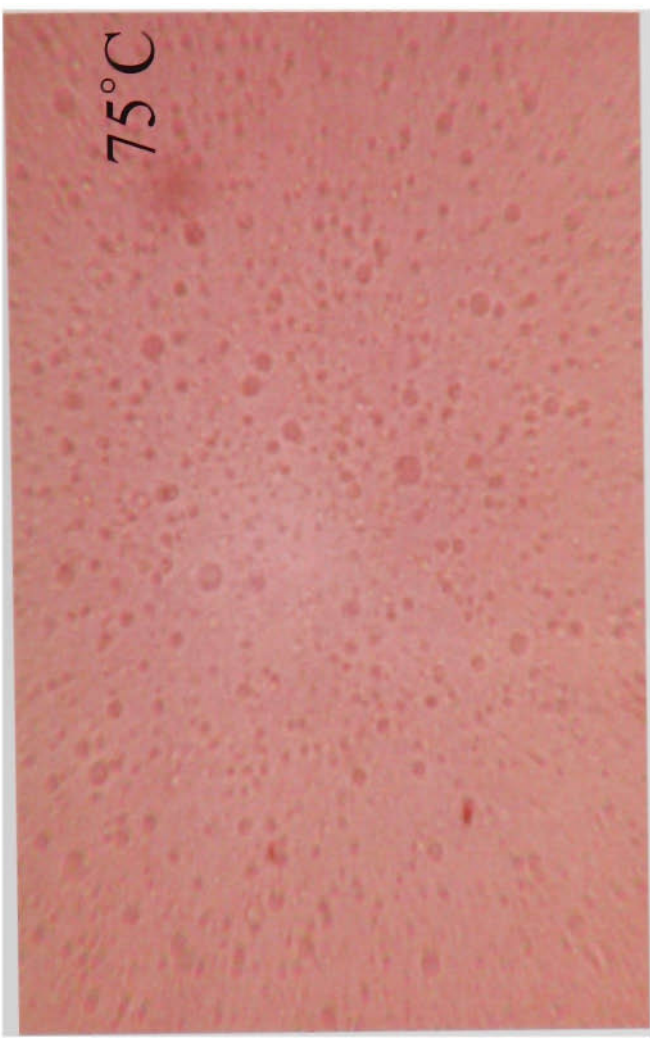
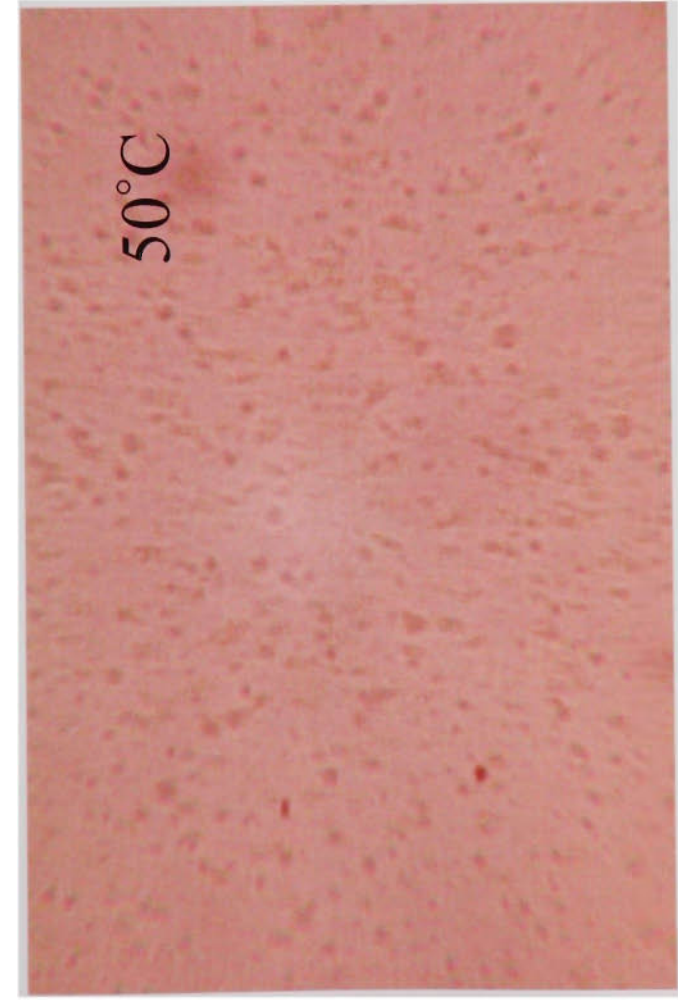


Figure 11.4.9 The effect of heating temperature on B994 corn starch granules as observed under a light microscope.

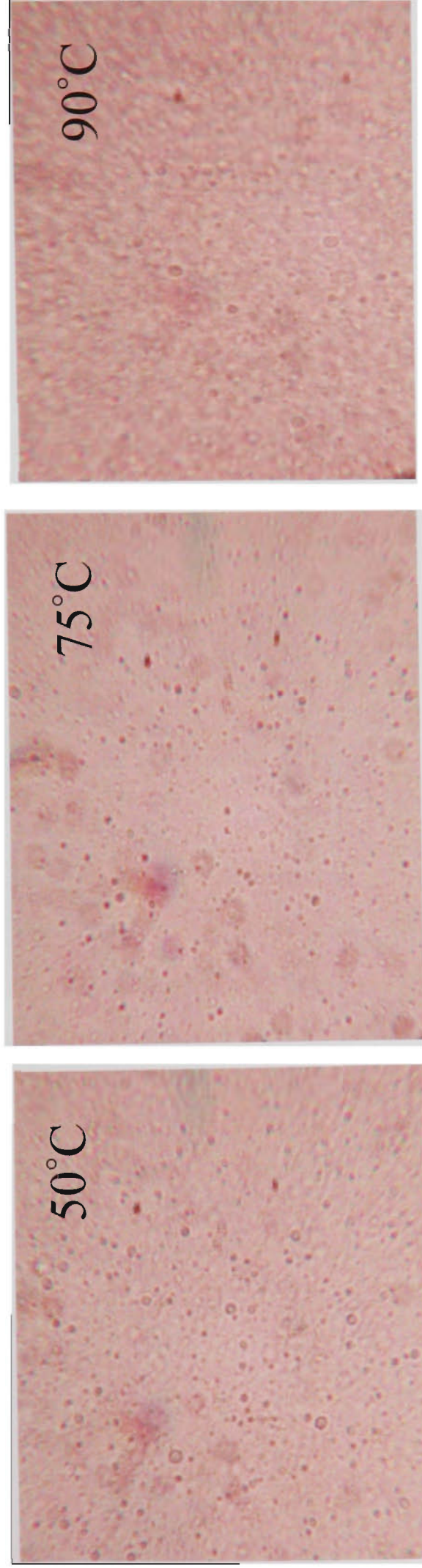


Figure 11.4.10 The effect of heating temperature on N1658 corn starch granules as observed under a light microscope.

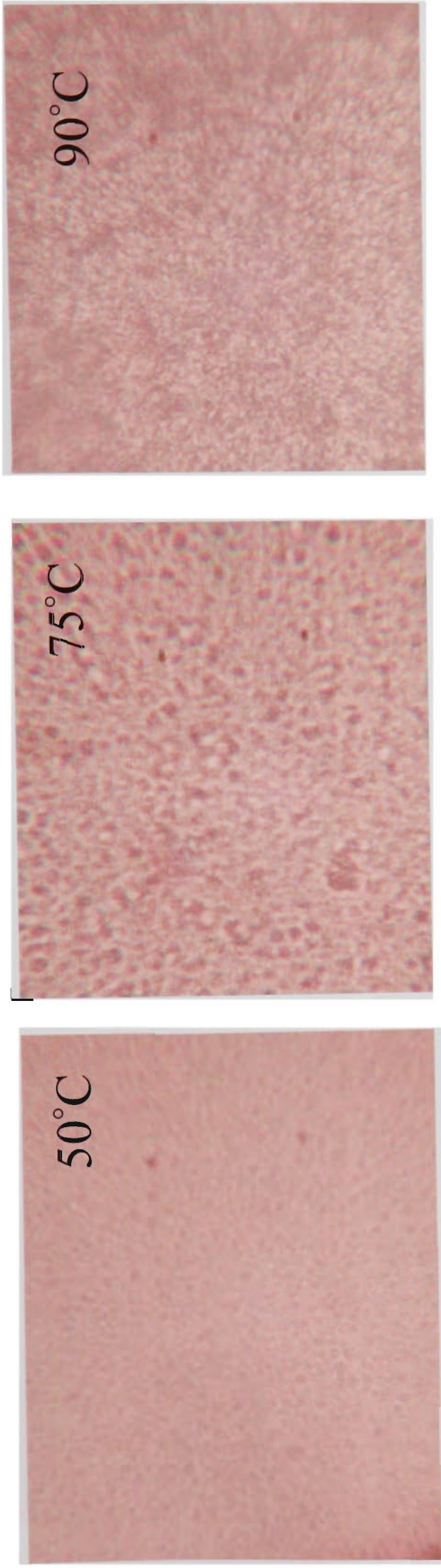


Figure 11.4.1.1 The effect of heating temperature on Maltrin M100 and Versagel added together to 'Lite' milk as observed under a light microscope.

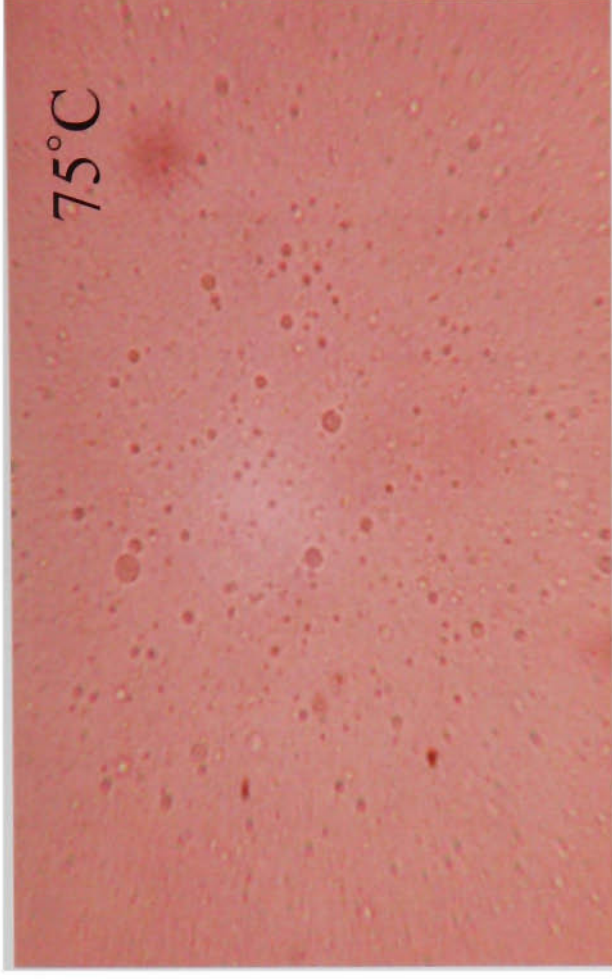
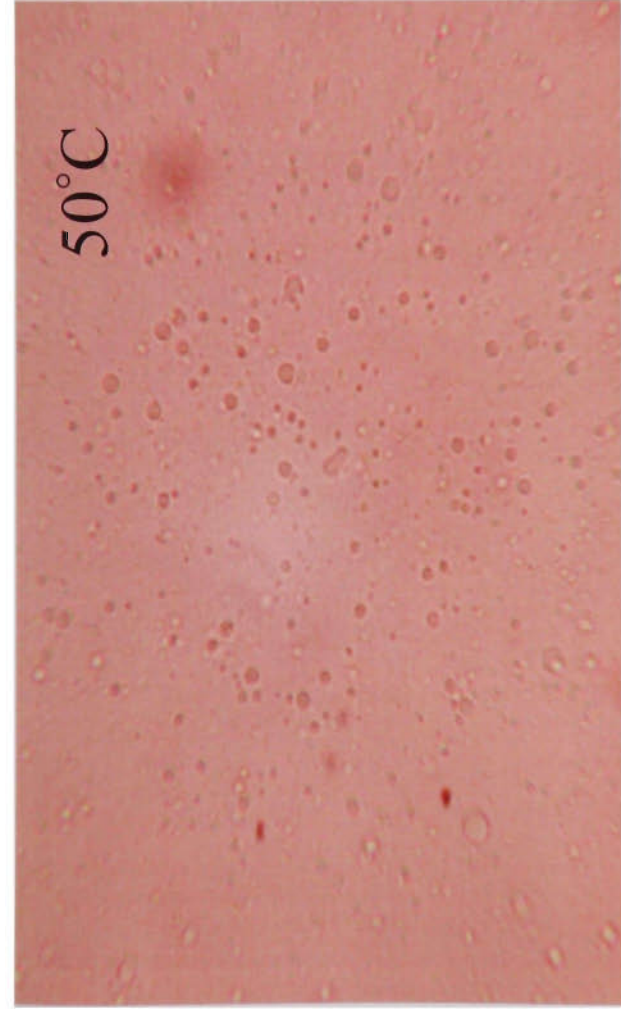


Figure 11.4.12 The effect of heating temperature on maltodextrin Maltrin M040 starch granules as observed under a light microscope.

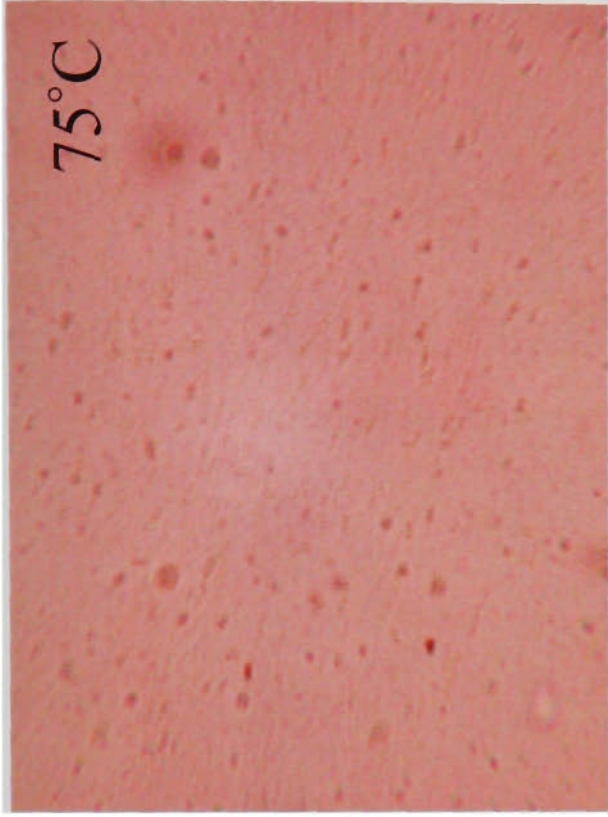
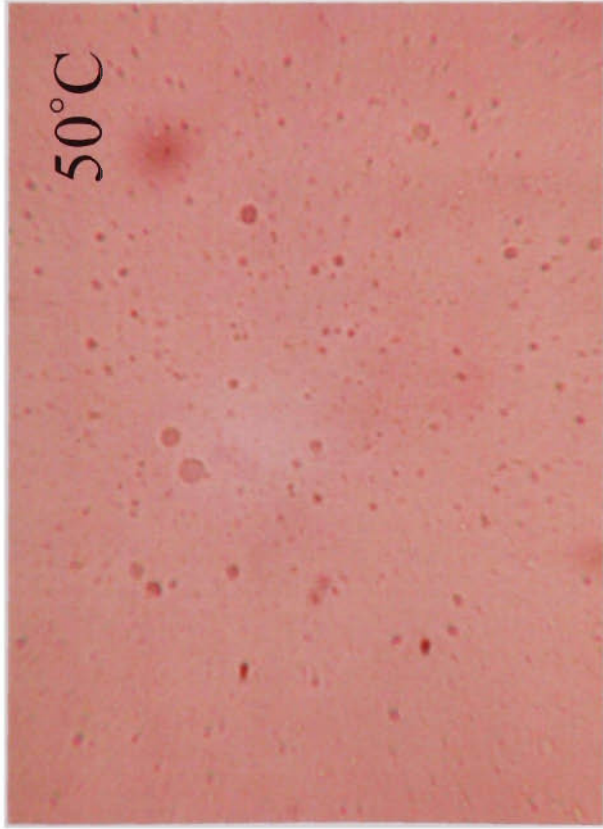


Figure 11.4.13 The effect of heating temperature on potato starch StaSlim SS143 granules as observed under a light microscope.



Figure 11.4.14 The effect of heating temperature on the constituents of Versagel, which include β -lactoglobulin, carageenan gum and xanthan gum, as observed under a light microscope.

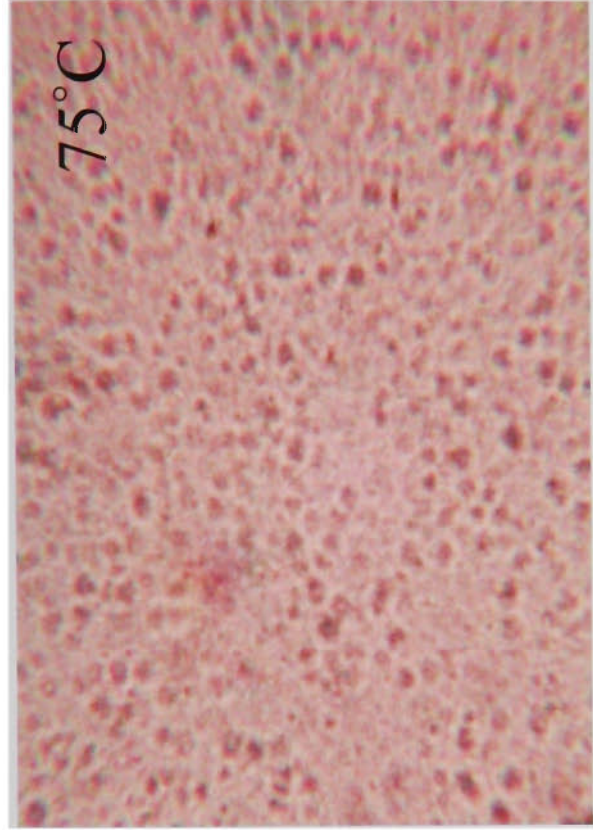


Figure 11.4.15 The effect of heating temperature on maltodextrin Maltrin M100 starch granules as observed under a light microscope.

\square — G' \triangle — G'' \times —Visc. $*$ —Def. angle \bullet —Torq.

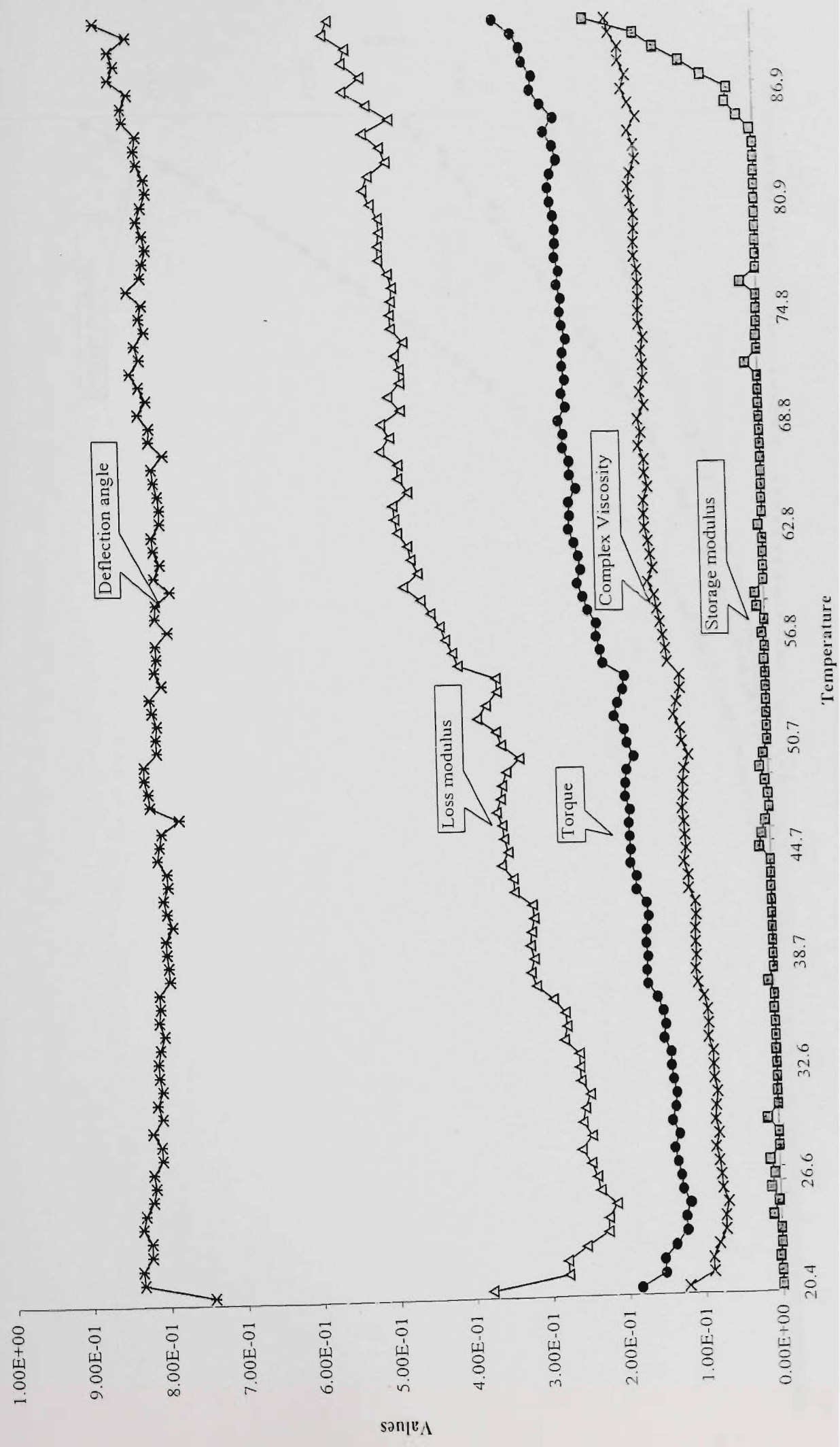


Figure 11.4.16 Deflection angle, loss modulus, torque, complex viscosity and storage modulus over 20 ° - 90°C for Versagel fat replacer at 0.25%

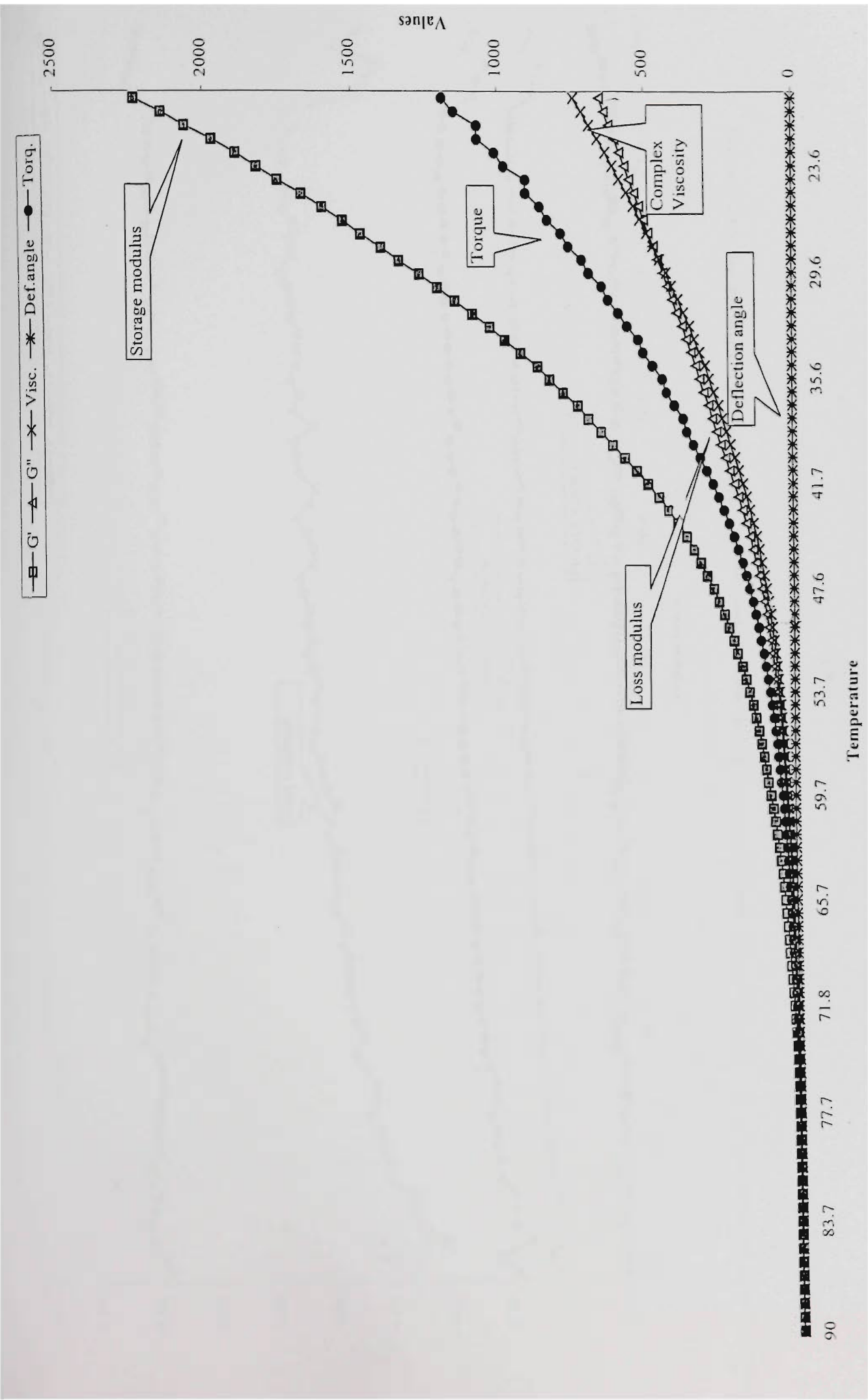


Figure 11.4.17 Deflection angle, loss modulus, torque, complex viscosity and storage modulus over 90° - 20 °C for Versagel fat replacer at 0.25%

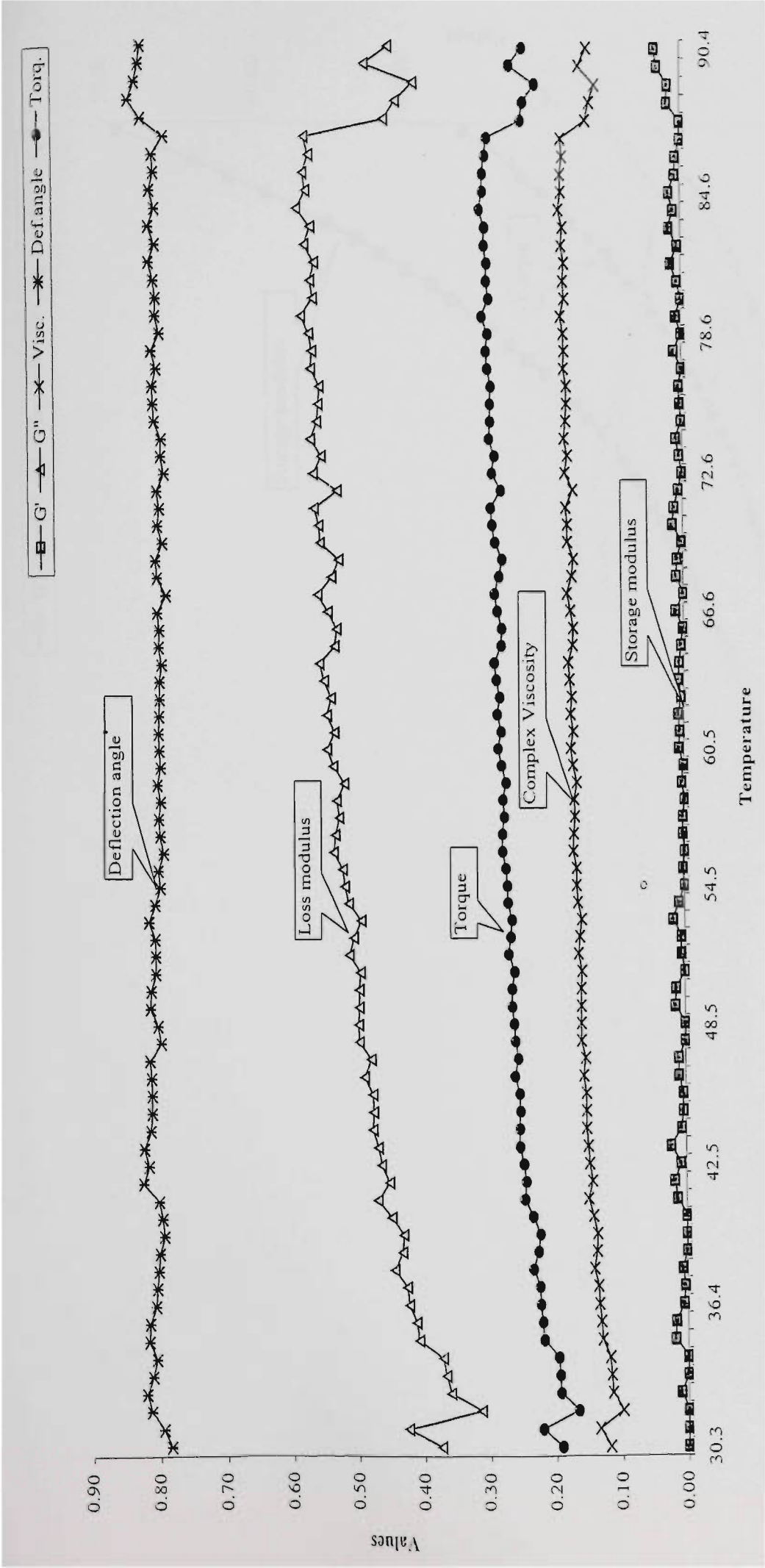


Figure 11.4.18 Deflection angle, loss modulus, torque, complex viscosity and storage modulus over 20 °- 90°C for Maltrin M100 fat replacer at 0.25%

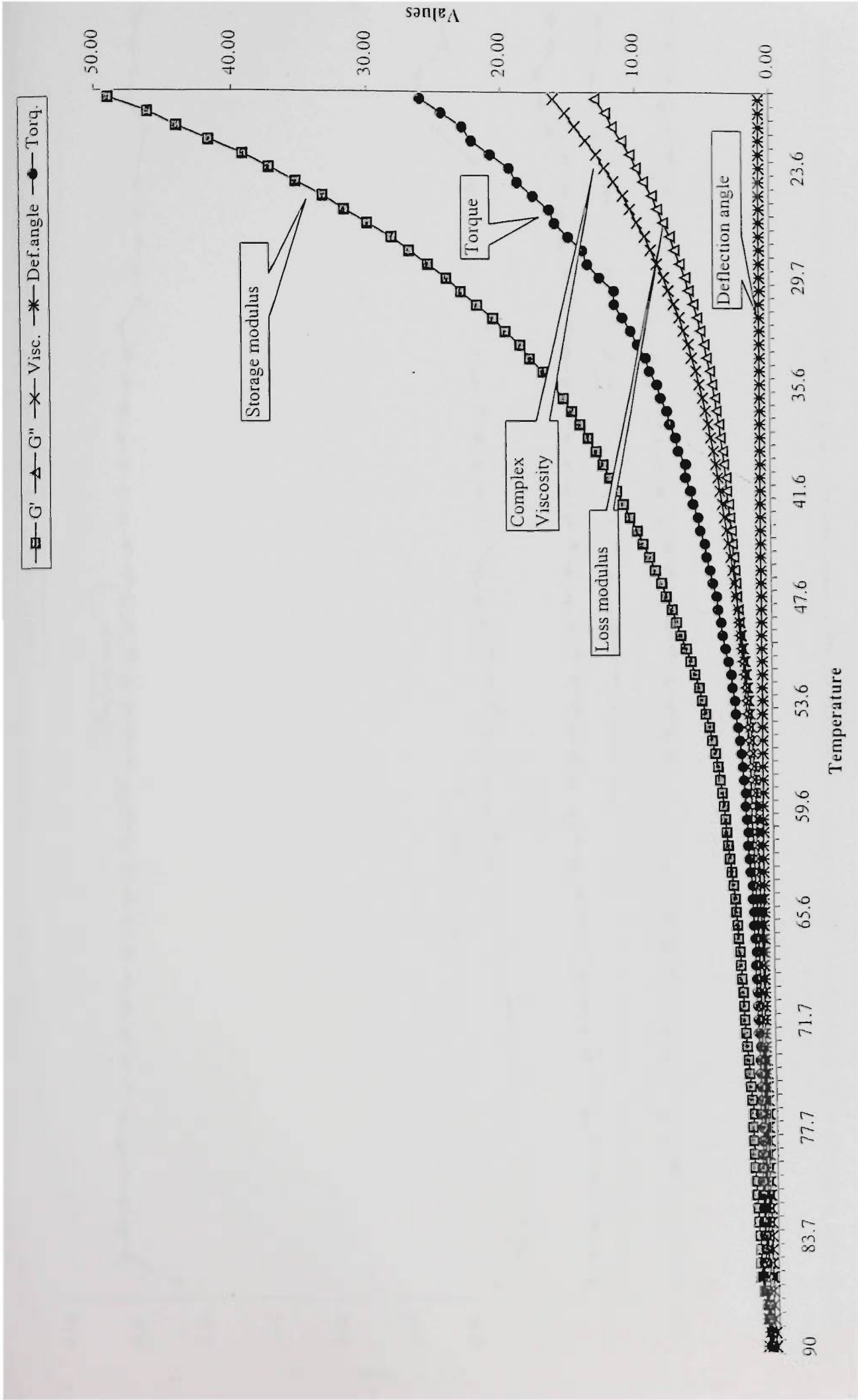


Figure 11.4.19 Deflection angle, loss modulus, torque, complex viscosity and storage modulus over 90° - 20 °C for Maltrin M100 fat replacer at 0.25%

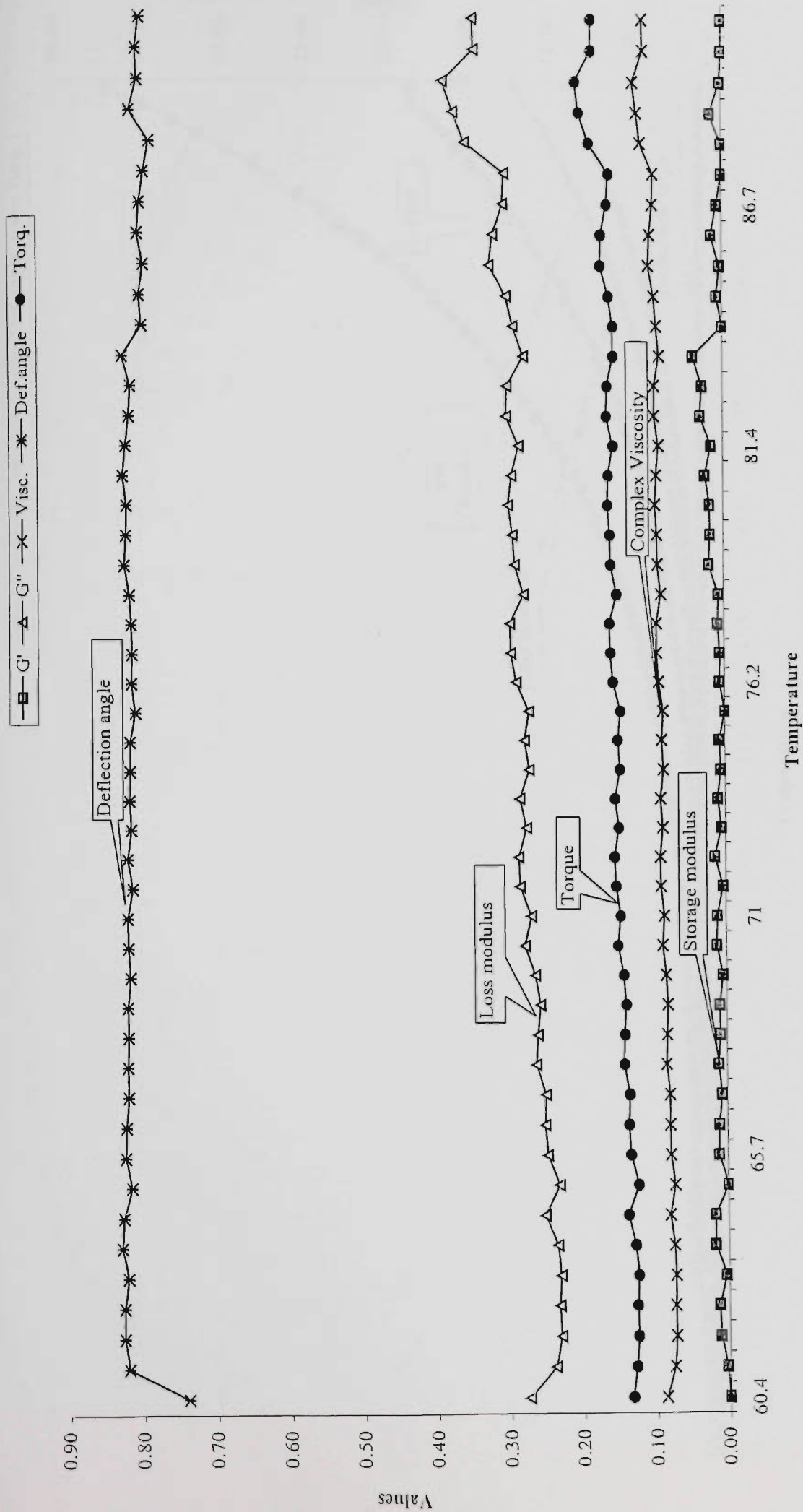


Figure 11.4.20 Deflection angle, loss modulus, torque, complex viscosity and storage modulus over 20 °- 90°C for Versagel and Maltrin M100 fat replacers at 0.25% of each

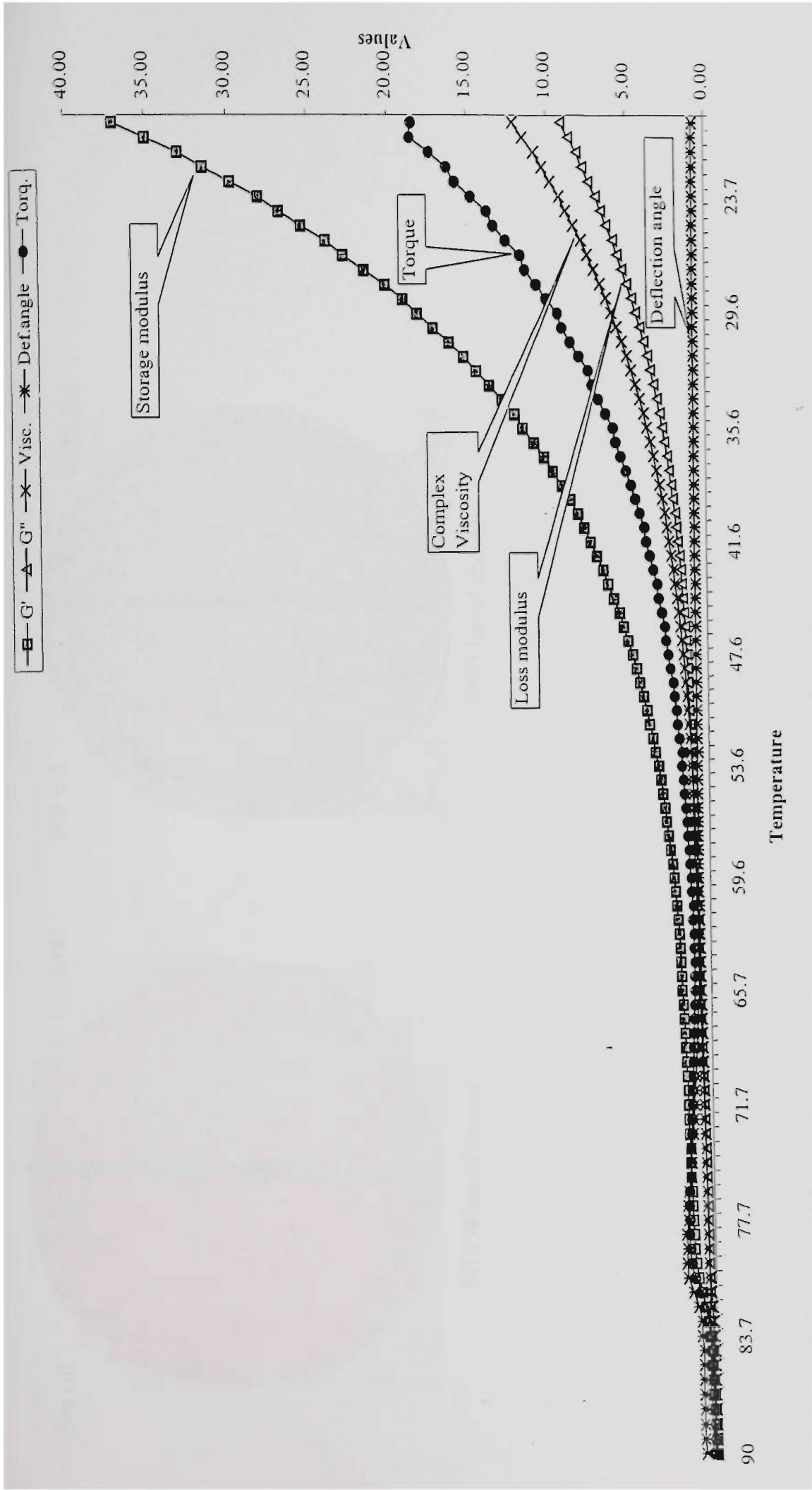


Figure 11.4.21 Deflection angle, loss modulus, torque, complex viscosity and storage modulus over 90° - 20 °C for Versagel and Maltrin M100 fat replacers at 0.25% of each

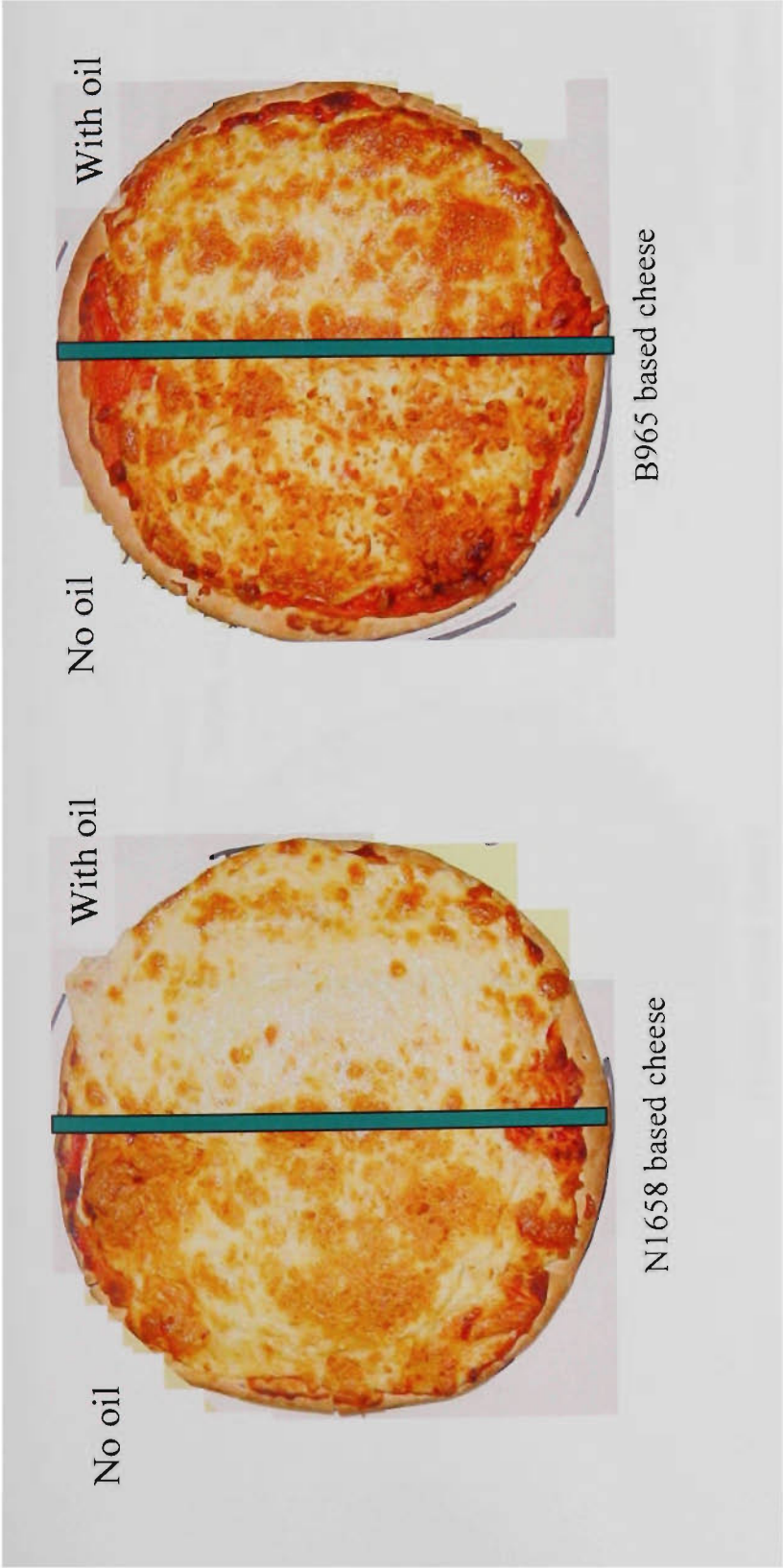


Figure 11.4.22 Pizza bake characteristics of freshly baked pre-acidified cheeses (pH 6.1 using citric acid) added with either N1658 or B965 and stored for 30 d at refrigeration temperature.

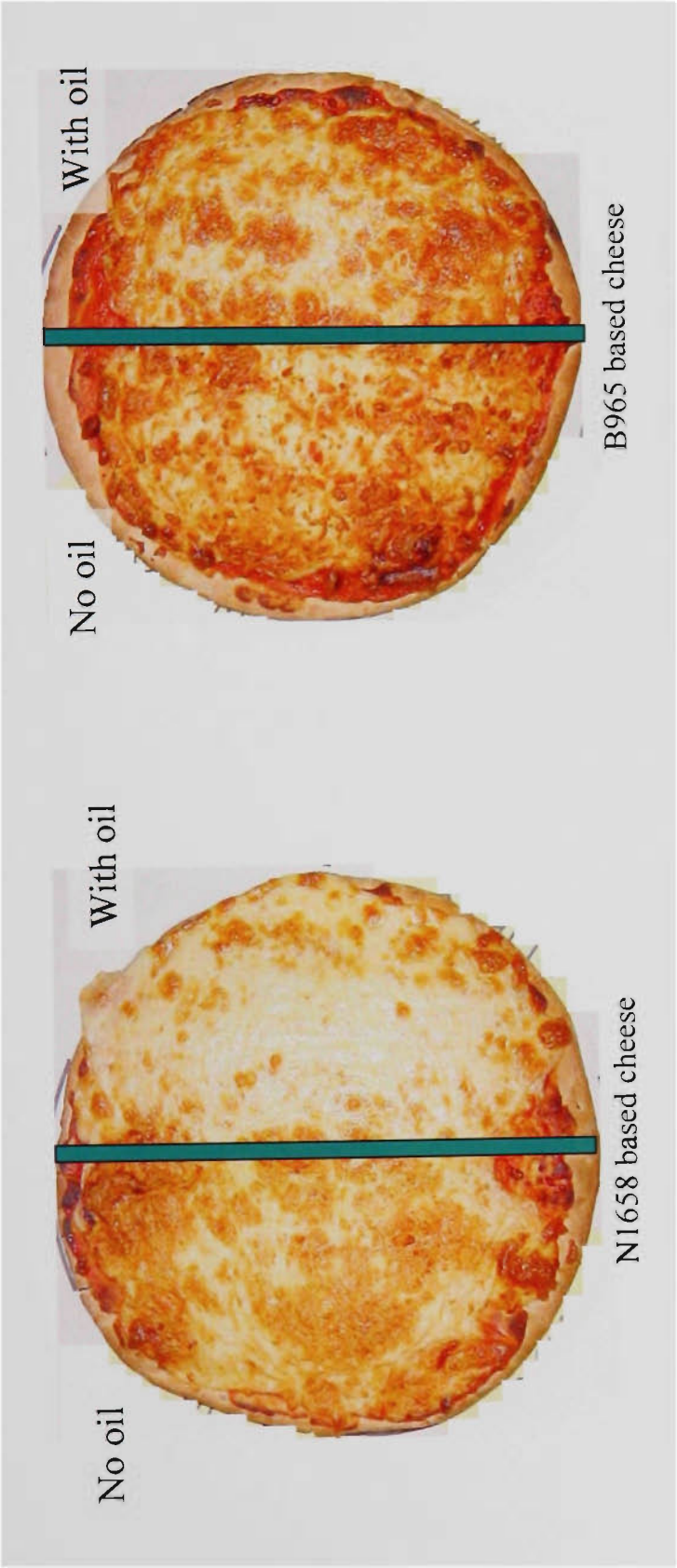


Figure 11.4.23 Pizza bake characteristics of baked and cooled pre-acidified cheeses (pH 6.1 using citric acid) added with either N1658 or B965 and stored for 30 d at refrigeration temperature.

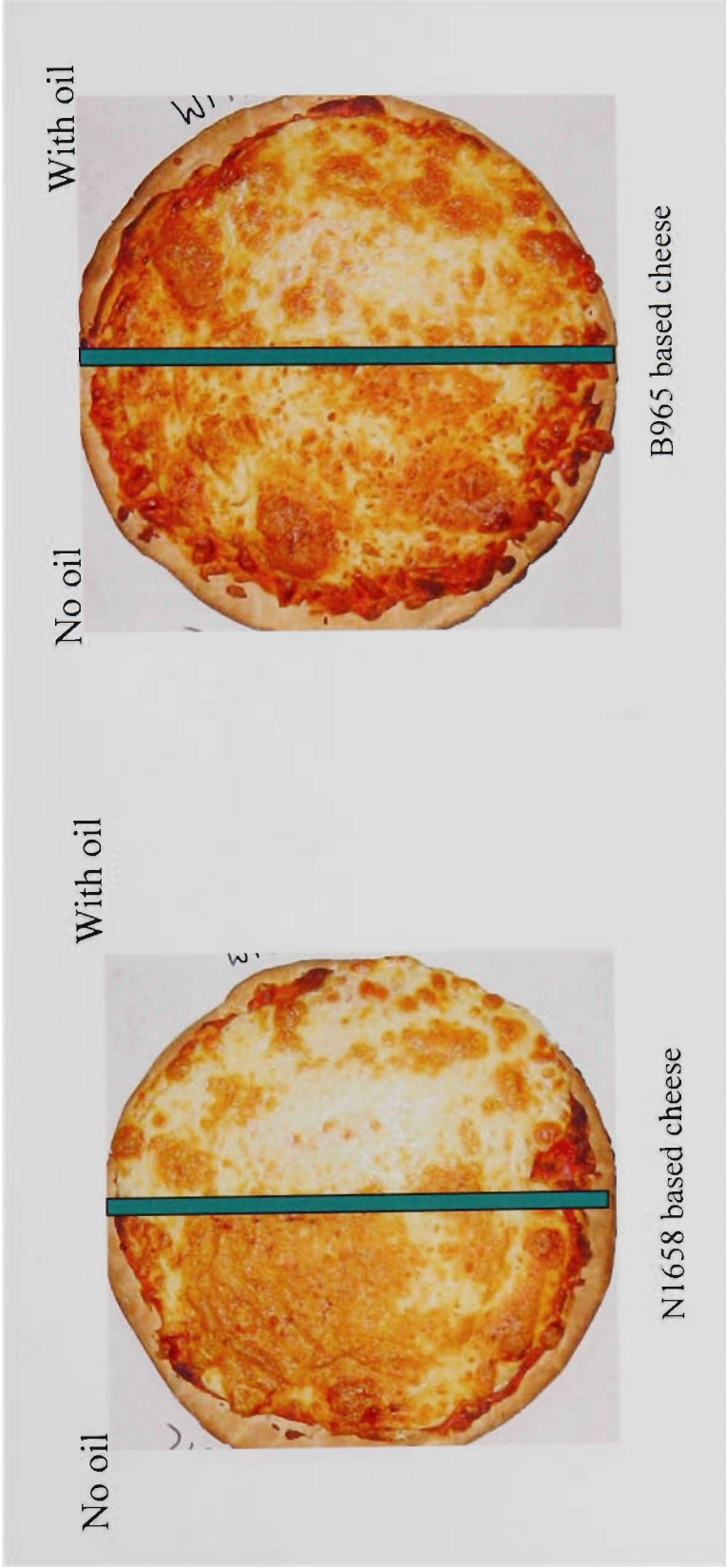


Figure 11.4.24 Pizza bake characteristics of freshly baked pre-acidified cheeses (pH 6.1 using citric acid) added with either N1658 or B965 and stored for 45 d at refrigeration temperature.

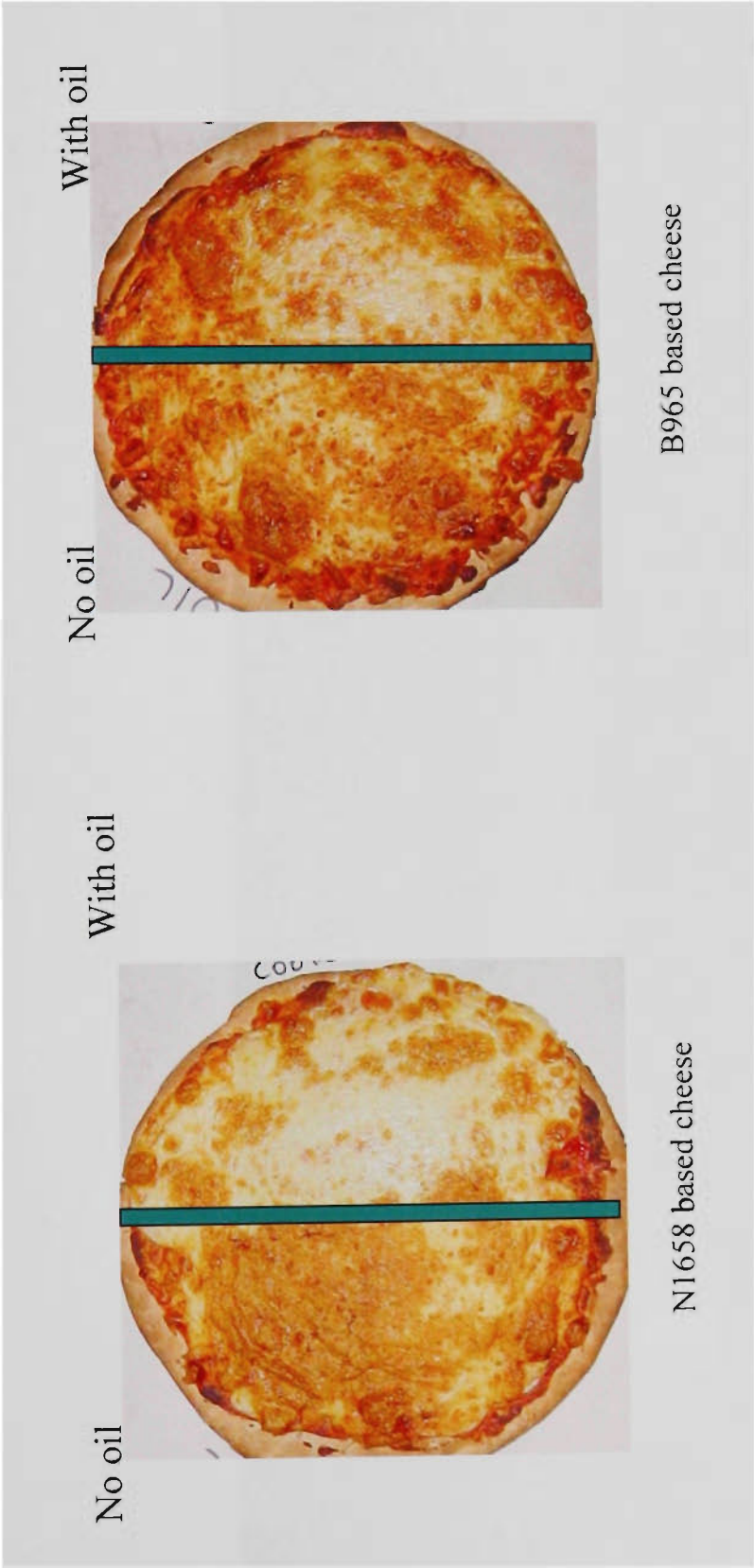


Figure 11.4.25 Pizza bake characteristics of baked and cooled pre-acidified cheeses (pH 6.1 using citric acid) added with either N1658 or B965 and stored for 45 d at refrigeration temperature.



Figure 11.4.26 An image of mozzarella cheese showing the stretched strands when the developed stretch test was performed.

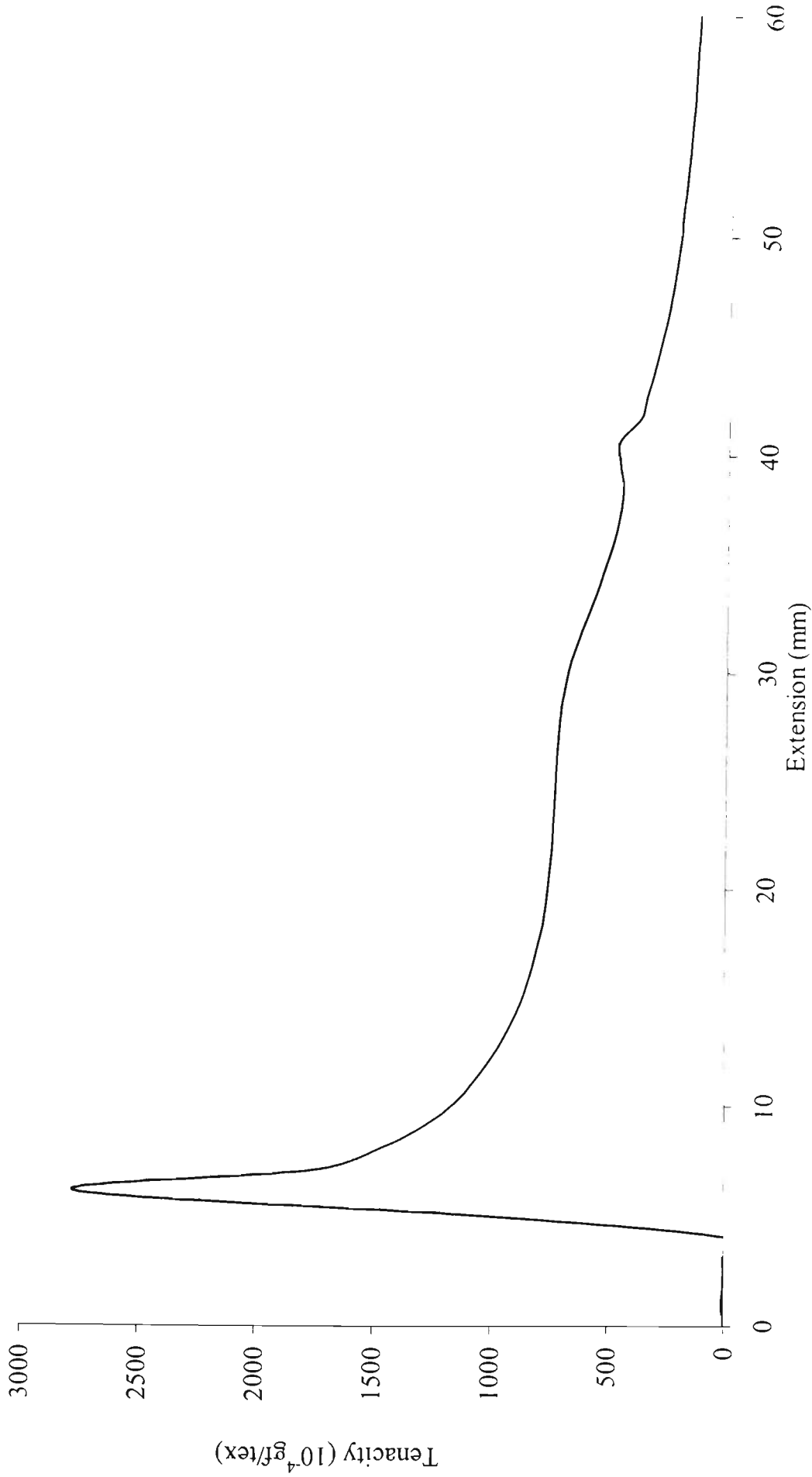


Figure 11.4.27 A typical curve obtained when mozzarella cheese was stretched and the tenacity versus extension was plotted.

12.0 OVERALL CONCLUSIONS

The cheeses made using DS method showed higher moisture retention, yield, FDM and M:P and salt content. There was more salt available per unit of moisture in the DS cheeses compared to TB cheeses. DS cheeses showed similar hardness and cohesiveness values at 2, 16, 23, 34 and 44 d of storage but had significantly higher hardness and lower cohesiveness on 9 d and 37 d of storage compared to TB cheeses. The reduced hardness of TB cheeses could be due to more moisture content and an open microstructure of such cheeses. The mozzarella cheeses took about 21 d to stabilise wherein most of the moisture was re-absorbed into the protein strands and such changes were reflected in cohesiveness values, which were found to be lower before and after 16 to 23 d of storage. The cohesiveness values for DS cheeses were the least, soon after manufacture and highest towards the end of storage period. The springiness values were similar for TB and DS cheeses throughout storage and both cheeses showed the lowest springiness values at 16 d and 23 d of storage and had the highest springiness values at 44 d. Thus increase in storage increased springiness of the cheeses probably due to expansion of protein strands in the cheese body and decrease in the size and number of voids within the protein matrix.

TB and DS cheeses showed reduced melt initially up to 16 d followed by higher values of melt distance by TB cheeses compared to DS cheeses. The ultra low fat content caused both TB and DS cheeses to show burning and browning when baked on a pizza. Differences in pizza bake characteristics could not be observed between TB and DS cheeses due to excessive scorching. Pizza bake results showed that such low fat cheeses required a longer storage period (about 75 d) to improve their functionality. Application of oil did not seem to improve TB and DS cheeses.

Enumeration of starter bacterial counts from 0 d old cheeses showed that the DS cheeses had a log cycle higher count of cocci and rods compared to TB cheeses. In both the cheeses, the total bacterial population increased until 16 d of storage after which they decreased in numbers. The differences in starter bacterial populations were mainly due to the method of salting because both the cheeses were made in 5.5 h from the time starter cultures were added until the end of milling. The manufacturing steps had a significant impact on the starter bacterial population and the numbers of cocci and rods increased during cheddaring and reduced during stretching, but again increased until 16 d of storage in both varieties of cheeses. Both TB and DS cheeses showed that during storage there was increase in degradation of β -casein and α -casein. The κ -casein was evident at 1, 8 and 15 d of storage in TB and DS cheeses and its concentration decreased during storage. TB and DS cheeses had similar extent of proteolysis during storage and results suggested that method of salting was not important in determining the proteolytic pattern of unripened cheeses; rather the enzymes released by the starter bacteria and the amount of salt in cheese could play an important role.

Cheeses made using *L. helveticus* showed increased moisture, FDM and M:P contents compared to those made using *L. delbrueckii* ssp. *bulgaricus*. Also the latter cheeses had higher protein content compared to the former cheeses. The higher moisture content and increased proteolysis of cheeses made using *L. helveticus* may be the reason for their reduced hardness (increased softness) while lower cohesiveness of such cheeses could be due to their protein content. The Lh based cheeses showed significantly higher springiness values throughout storage compared to Lb based cheeses. The higher springiness values could be due to their higher FDM values, which gave resilience to the Lh based cheeses. Meltability of the Lh based cheeses was significantly higher throughout storage and these cheeses also had better functionality when baked on pizzas with an increased whiteness (L values), reduced redness (a values) and increased yellowness (b values) compared to their fresh cheeses. The Lb based

cheeses had poor melt characteristics and did not fuse completely when baked, had excessive blistering and browning. All the ULFM cheeses showed improvement in pizza bake characteristics due to application of hydrophobic material (oil).

Calcium estimation using AAS showed differences in the absorbance values of all samples due to sample temperature. The skim milk and whey samples showed increased absorbance values with higher sample temperature. In contrast, the absorbance values of cheese samples increased with warming of the samples up to 40°C and then decreased at 50°C. Stretch water having higher calcium ion concentrations showed an indefinite trend to an increase in sample temperature. The differences in measured absorbance values for samples due to effect of temperature are suggested to be because of change in solution characteristics such as surface tension and viscosity, which could alter the aspiration rates and also could be due to level of excitation energy available to the calcium ions.

Thus the results showed that it was important to maintain uniform sample treatment temperature and similar dilution levels in order to obtain consistent results. Calcium contents measured at several stages of mozzarella cheese manufacture were important to ascertain the amount of calcium that would be present in the stretched cheese. The variations in calcium content at the various stages during mozzarella cheese manufacture seemed to affect the characteristics of the finished cheeses. Increase in level of pre-acidification decreased the calcium content retained in the cheeses. Control cheeses made without pre-acidification had the highest levels of calcium and most of the calcium was observed to be chelated out into the whey at whey draining stage mainly due to bacterial activity, and later into stretch water at stretching stage due to exchange of calcium with sodium ions. The shear forces of cutting and stretching are also suggested to possibly have an effect on the rate of bacterial acidification and thus negatively influence the amount of calcium retained by the mozzarella cheeses.

Pre-acidification studies conducted using lactic, acetic and citric acids to pH 6.3 showed improvement in some of the characteristics of pre-acidified cheeses compared to control. The PAC6.3, PAA6.3 and control cheeses had similar yield while PAL6.3 cheese showed reduced yield. Control and PAA6.3 cheeses had higher moisture; FDM and M:P contents but lower protein content compared to PAC6.3 and PAL6.3 cheeses. The hardness values for control and PAA6.3 cheeses were lower compared to PAC6.3 and PAL6.3 cheeses throughout storage. The PAL6.3 cheeses were significantly hard due to their higher protein content compared to control, PAA6.3 and PAC6.3 cheeses. All the cheeses showed a significant decrease in hardness values during storage. The pre-acidified cheeses showed a delayed decrease in hardness values and decreased proteolysis due to less ripening time that was available for the starter cultures and thus took longer to soften. PAL6.3 cheeses had the highest cohesiveness values at 15 d followed by PAC6.3 cheeses and then control and PAA6.3 cheeses. At 22 and 29 d, PAL6.3 cheeses had significantly higher cohesiveness compared to control and PAA6.3 cheeses. PAA6.3 cheeses had higher springiness, control and PAC6.3 cheeses had moderate springiness, and PAL6.3 cheeses had the least springiness. PAL6.3 cheeses had the least M:P content and also showed the least springiness values. Thus PAA6.3 cheeses showed improved cohesiveness and springiness than the other cheeses.

The meltability values for control and PAC6.3 cheeses were similar throughout storage and their melt distance was significantly greater than PAA6.3 and PAL6.3 cheeses. The PAL6.3 cheeses showed the least meltability throughout storage. PAC6.3 cheeses showed improved melt characteristics compared to other cheeses. PAC6.3 and PAA6.3 cheeses showed increased whiteness upon baking while PAL6.3 cheeses were slightly better than the control cheeses. The PAC6.3 (with oil) and PAC6.3 (no oil) cheeses showed increased shred fusion, increased whiteness, better melt and flow characteristics compared to control, PAA6.3 and PAL6.3

cheeses. Storage of PAL6.3 cheeses to 45 d increased the size of blisters and improved shred fusion characteristics which were indicated as improved whiteness (L values), decreased redness (a values), which are desirable but their b values were not affected. The PAL6.3 cheeses did not show acceptable pizza bake characteristics even after 45 d of storage due to excessive burning and scorching, although its shred fusion characteristics were improved during storage. Application of oil to cheese shreds improved the shred fusion, melt and flow characteristics of all cheeses during baking.

The effects of pre-acidification using citric acid to pH 6.3, 6.1 or 5.9 on the characteristics of ULFM cheeses were studied. Pre-acidification with citric acid to pH 6.3, 6.1 and 5.9 did not show a significant effect on the yield of the cheeses as expected by the authors. The control and pre-acidified cheeses had similar yield and the level of pre-acidification did not seem to decrease the yield. The moisture, FDM and M:P contents of control, PAC6.1 and PAC5.9 cheeses were similar and significantly higher than for PAC6.3 cheeses. The protein content of the PAC6.3 cheeses was significantly higher than those for the control, PAC6.1 or PAC5.9 cheeses and the latter cheeses had similar protein contents. Increase in pre-acidification to pH 6.1 using citric acid increased the meltability of the cheeses although PAC5.9 cheeses had the least melt. PAC5.9 cheeses showed lower hardness values compared to PAC6.3 and PAC6.1 cheeses. Control cheeses were less hard than the pre-acidified cheeses probably due to increase in ripening time of starter culture activity during cheese making. The pre-acidified cheeses required prolonged storage in order to show reduced hardness values. Pre-acidification was found to increase the cohesive forces within the cheese matrix while storage did not show a marked effect on this characteristic. Springiness values for all the cheeses except PAC6.1, increased with increase in storage time although a definite correlation could not be established between the level of pre-acidification and springiness values of the cheeses.

PAC6.1 cheeses showed good melt, shred fusion and flow of the melted cheeses while control and PAC6.3 cheeses did not show shred fusion or good melt and flow characteristics. Further pre-acidification to pH 5.9 did not markedly improve the melt and flow characteristics but only improved the whiteness of the cheese. Application of oil was necessary for PAC6.1 cheeses to improve pizza bake characteristics. PAC6.3 cheeses when baked showed increased burning and formed large blisters. These cheeses also had the least b values before and after baking while PAC6.1 and PAC5.9 cheeses had higher b values indicating more yellow coloured shreds. Application of oil reduced the redness (a values) for baked cheeses indicating that oil layer on the surface of the cheese shreds had a protective function.

Cheeses made using pre-acidification with acetic acid to pH 6.3, 6.1 and 5.9 showed reduced yield compared to control. The yield of the cheeses decreased with increase in level of pre-acidification i.e. control cheeses made without pre-acidification had the highest yield (6%) followed by PAA6.3, PAA6.1 and PAA5.9 cheeses in decreasing order. PAA5.9 cheeses had the lowest moisture content and the highest protein content and such cheeses showed higher hardness values compared to control, PAA6.3 and PAA6.1 cheeses. The FDM and M:P contents of control, PAA6.3 and PAA6.1 cheeses were statistically similar and significantly higher than for PAA5.9 cheeses. These results showed that lower the pH of pre-acidification using acetic acid, lower was the hydration of casein. All the cheeses showed decrease in hardness values during storage. The pre-acidified cheeses showed a delayed decrease in hardness values, which was affected by the level of pre-acidification. The hardness values of pre-acidified cheeses reduced with decrease in pH of pre-acidification probably due to reduction in calcium content. Cohesiveness values for PAA5.9 cheeses were the least among control, PAA6.3 and PAA6.1 cheeses. Storage did not seem to affect the cohesiveness characteristics of the cheeses. The control and pre-acidified mozzarella cheeses showed similar springiness values and these values increased up to 15 or 22 d and then decreased, which could

be due to the dynamic interactions within the cheese matrix and modifications in the cheese microstructure.

Control cheeses had higher meltability compared to the pre-acidified cheeses. The meltability of the cheeses seemed to decrease with increase in pre-acidification at 11 and 25 d. Meltability of pre-acidified cheeses also showed a negative correlation with the storage period. All the pre-acidified cheeses showed better shred fusion, melt and flow characteristics when baked compared to control. Control cheeses showed intact cheese shreds, which did not fuse together, lacked melt and flow characteristics. The control cheeses were scorched and unappealing. PAA6.1 cheeses showed increased whiteness during baking with very little browning and were similar to a full fat mozzarella cheese except that all the cheese shreds had not melted during baking. PAA6.3 and PAA5.9 cheeses showed more browning than PAA6.1 cheeses although both the cheeses had melted and flowed similar to PAA6.1 cheeses on the pizza base. Application of oil improved functionality of all the cheeses upon baking. The PAA5.9 cheese shreds applied with oil had increased whiteness, reduced browning, but all the cheeses applied with oil had improved shred fusion, melt and flow characteristics.

The pilot scale cheese manufacture showed that the yield was not increased for fat replaced cheeses due to addition of 0.25% of Maltrin M100 or Versagel. The Maltrin based and control cheeses were similar in composition including moisture, FDM and M:P contents. Versagel based cheeses had higher moisture and FDM contents compared to Maltrin based cheeses. The protein contents of Maltrin based and control cheeses were higher than that for Versagel based cheeses. The Versagel based cheeses showed lower protein content but these cheeses were able to retain more moisture (M:P) compared to control and Maltrin based cheeses. The increased moisture content of the Versagel based cheeses could have caused the decrease in protein content of these cheeses.

A method for sample preparation was developed for estimating the amount of starch remaining in the curd using HPLC analysis. Although the starch added to cheese milk contained 80% of oligosaccharides having greater than DP7 the HPLC column was unable to determine oligosaccharides greater than DP7. Estimation of DP7 (maltoheptaose) showed these oligosaccharides to be very low in concentration (2.7 µg/g) in the Maltrin based cheeses. The results showed that most of the oligosaccharides having DP7 or lower were lost into whey while DP10 and above oligosaccharides could remain in the cheese trapped within the protein matrix.

All the cheeses expressed measurable amounts of serum at 22 d of manufacture. Maltrin based and control cheeses showed negligible amounts of expressible serum at 35 d while Versagel based cheeses continued to show higher amounts of expressible serum. The amount of expressible serum decreased with storage for all the cheeses. The Maltrin based cheeses showed higher proteolysis at 45 d compared to control and Versagel based cheeses. At 60 d both control and Maltrin based cheeses had similar extent of proteolysis and Versagel based cheeses continues to show lower proteolysis. At 90 d the Maltrin based cheeses had the highest proteolysis compared to control and Versagel based cheeses. Versagel based cheeses showed the least amount of proteolysis through out storage compared to Maltrin based and control cheeses. Versagel based cheeses showed reduced hardness compared to control and Maltrin based cheeses through out storage and the hardness values for the three cheeses decreased with storage. Control cheeses showed significantly higher cohesiveness at 29 and 44 d compared to Maltrin based cheeses, while the latter had similar cohesiveness values as Versagel based cheeses. Cohesiveness values for Maltrin based, control and Versagel based cheeses were similar at 56 and 80 d. These values increased with storage period for the three varieties of

cheeses. Springiness values for Versagel based cheeses were higher than those for control and Maltrin based cheeses and Maltrin based cheeses showed the least spring.

Maltrin based cheeses showed greater meltability thorough out storage than Versagel based cheeses and the former cheeses also had greater meltability than control cheeses at 45 and 60 d of storage. Pizza bake analyses showed Maltrin based cheeses to have better melt, shred fusion and flow characteristics compared to control and Versagel based cheeses. The latter cheeses showed burning and had several brown blisters on their surface at 29 d. Application of oil was observed to be necessary for proper shred fusion. Versagel based cheeses showed the least shred fusion at 29 d and the shreds did not melt completely even by 44 d. Maltrin and control cheeses also showed browning although to a lesser extent compared to Versagel based cheeses at 44 d. Storage seemed to improve the pizza bake characteristics of Versagel based cheeses and these cheeses showed better functionality at 56 d. The control and Maltrin based cheeses did not show any improvement due to prolonged storage up to 56 d. Application of oil did not produce any defects in the stored cheeses. Prolonged storage to 80 d improved the melt and shred fusion characteristics of Maltrin based cheeses but the cheeses showed browning which was not desirable. Control cheeses did not show sufficient shred fusion and melt and intact shreds caused scorching of the cheese. Unlike control and Maltrin based cheeses, the Versagel based cheeses that were applied with oil had improved melt and shred fusion. The cheeses had improved flow and showed increased whiteness after 80 d of storage. Versagel based cheeses required prolonged storage of 80 d along with oil application to show improved cheese characteristics.

Maltrin based cheeses showed reduced stretch distance at 29 d and this characteristic was improved at 40 d. The Maltrin based cheeses had excellent stretch at 55 d of storage, which decreased with further increase in storage. Control cheeses also showed a similar stretchability

as Maltrin based cheeses and had the highest stretchability at 55d of storage, which decreased at 75 d. Versagel based cheeses showed reduced stretch distance at 29 d but the cheeses achieved maximum stretchability as early as 40 d. These cheeses showed reduced stretchability at 55 and 75 d of storage compared to 40 d old cheeses.

Maltrin based and Versagel based cheeses were made from standardised milk mixed with 0.5% of fat replacer. Such cheeses contained 6.37% fat on wet basis, 55% moisture and 34% protein content. Addition of Maltrin decreased the moisture content and increased the total solids of the milk. The protein content of the fat replaced milk slightly decreased because Maltrin consists of mainly starch and negligible amounts of protein. At whey draining stage (pH 6.1) about 1% protein was lost while 0.13% of protein was lost along with the stretch water. Addition of Maltrin M100 increased the total solids of the cheeses but did not increase their moisture or protein contents. Versagel based cheeses showed greater loss of protein in whey during manufacture. Stretch water used for stretching the Maltrin based and Versagel based cheeses had similar total solids and proteins contents. Negligible amounts of proteins were lost in stretch water for any of the cheeses. Versagel based cheeses had higher moisture content than for Maltrin based cheeses. Addition of 0.25% or 0.1% of Maltrin did not alter the composition of whey but addition of 0.25% Versagel to milk causes an increase in protein content of whey at whey drain. The curd samples for all the fat replaced cheeses showed similar moisture content. During cheddaring some of the Versagel could have been hydrated and thus helped the cheddared curd retain more moisture. Milling carried out at pH of 5.25 for all cheeses showed that the curd made from cheese milk mixed with 0.25% Versagel based milled curd had the highest moisture retention while control samples had the lowest moisture content.

Addition of Versagel at 0.25% was necessary to cause an increase in the moisture retained in the curd. The cheeses made from 0.25% Versagel addition to cheese milk showed higher moisture content after they were stretched. Control and Maltrin based cheeses had similar moisture content. The increase in moisture content of Versagel based cheeses probably caused a decrease in their protein content. Calcium content of curd and cheese samples obtained from fat replaced milks was higher than for control cheeses. About 600- 700 mg of calcium per kg of whey was lost for control and Maltrin based cheeses while 550- 580 mg of calcium per kg of whey was lost for Versagel based cheeses. This could be due to binding of calcium to the proteins available from Versagel. Losses in calcium for all the cheeses seemed to be similar and ranged between 250 to 350 mg calcium per kg of stretch water. Versagel based cheeses retained more amounts of calcium than Maltrin based or control cheeses. Addition of fat replacers reduced the amount of calcium lost into whey and stretch water during manufacture of the cheeses. The pizza bake colour measurements showed increased whiteness for Versagel based cheeses compared to Maltrin based or control cheeses. Application of oil to the fat replaced cheeses seemed to reduce the browning.

Cheeses made from 0.1% Versagel added to cheese milk showed pizza bake characteristics similar to the 0.25% Maltrin based cheeses. Addition of fat replacers along with application of a protective coating of oil to the shredded cheeses reduced the browning of ULFM cheeses. The concentration of sodium in milk was measured to be about 247- 271 mg/kg while that of potassium was about 1632- 1656 mg/kg and magnesium was about 71- 114 mg/kg. Most of the sodium, potassium and magnesium salts were lost in whey and reduced amounts of these minerals were retained per unit of curd weight compared to per unit weight of whey probably due to the soluble nature of sodium and potassium ions. Magnesium was retained to a greater extent than was sodium or potassium in the curd. Increased losses of sodium, potassium and magnesium into whey reduced their concentration in the curd. Stretching the curd in brine

caused increased loss of 8- 14 mg of magnesium and 127- 160 mg of potassium lost per kg of stretch water from the curd. The control mozzarella cheese samples contained about 2950 mg of sodium; Maltrin based cheeses had about 3560- 3945 mg and Versagel based cheeses had 4095- 4118 mg of sodium per kg of cheeses. The exchange of calcium from its colloidal state with the sodium could have caused the increased amounts of sodium in Versagel based cheeses.

Size of starch granules, the extent of the starch modifications and their origin caused several differences in their hydration characteristics. The starches showing increased hydration also gelatinised to a greater extent. Heating the starches to 90°C caused a reduction in their refractive index possibly due to exudation of the granular constituents. The storage modulus and loss modulus values were significantly lower for Lite milk mixed with Maltrin compared to that mixed with Versagel. This could be due to the early disruption of maltodextrin granules due to application of heat in comparison to Versagel, which consists of β -lactoglobulin, and carageenan and xanthan gums. The rate of addition (0.25%) was too low for both Maltrin and Versagel to form gels but when added together to the Lite milk, they were able to form a firm gel. N1658 based cheeses when applied with oil showed excellent melt, shred fusion and flow characteristics upon baking on a pizza base. During storage the N1658 based cheeses showed good pizza bake characteristics without burning and the characteristics remained the same after the pizzas were cooled. These cheeses had the desired characteristics similar to that of a full fat mozzarella cheese.

13.0 FUTURE RESEARCH DIRECTION

The demand for low fat and reduced fat mozzarella cheeses is increasing and these products are bound to capture a major market share only if they meet the characteristics of full fat mozzarella cheeses. The work carried out as part of this project as well as other projects on the anvil show that there are ways in which a low fat product or rather a no fat product can be developed. The two major criteria for developing a fat free mozzarella, are firstly, increase in the cheese moisture, which increases cheese yield and the fat free cheese realises better profits, and secondly, to reduce the calcium content of cheese using means such as pre-acidification. Salting has been found to increase the meltability of mozzarella cheeses as it substitutes calcium. But details of the functions of salt in cheese are not completely understood. A few reports have suggested addition of salt to milk during cheese manufacture to have beneficial effect on the full fat mozzarella cheese functionality. Thus the stage for salting needs to be understood as well as studies on the innate details of salt transfer mechanisms needs to be undertaken. In low fat and reduced fat mozzarella cheeses protein plays a more important role because of a lack of fat. Proteolysis during prolonged storage improves the pizza bake characteristics of low fat mozzarella cheeses. But controversies surrounding the extent of proteolysis due to plasmin and un-denatured rennet require more studies to be carried out.

Pre-acidification and direct acidification methods have been proven to decrease calcium content in mozzarella cheeses. Studies need to be carried out as to the extent of calcium to be removed from the cheese to obtain a good quality low fat or fat free mozzarella cheese. Also due to pre-acidification there is a loss in proteins into the whey. Studies are needed to determine a way to stop leaching of proteins thereby increasing the yield of cheese. Addition of starch has shown a definite scope in developing a fat free mozzarella cheese and more work needs to be carried out to ascertain interactions between fat replacers and casein network to

examine the functionality of starches. There is a need to understand hydration of starches that are added to the cheese milk and more starches may be examined to improve functionality of low fat and fat free mozzarella cheese. Similar studies could also be carried out in low fat and fat free cheddar cheeses and other varieties of cheeses.

14.0 REFERENCES

- ADC. (1996). *Compendium and written communication*.
- ADC. (2002). *Compendium and written communication*.
- Ahmad, M., Riaz-ur-Rehman., Khan, A. G. (1978). Effect of salt concentration and lactic starter in the manufacture of Domiati cheese. *J. Agric. Res. Pakistan* 16 (2): 241-246.
- Aiyar, K. R. and Wallace, G. M. (1970). The significance of calcium in the synthesis of rennet-casein gels. *Proc. 18th Intl. Dairy Congress*. Abstract no. E:47
- Ali, S. S., Kazi, G. H. and Kazi, T. G. (1997). Use of comparative sample preparation methods for the extraction of metal ions in milk as determined by atomic absorption spectroscopy. *ACGC Chem. Commun.* 6: 9-12
- Anderson, M. E., Brooker, D. B., Fischer, J. R., Ruiz, E. L. and Marshall, R. T. (1973). Measurement of calcium of milk by atomic absorption spectrophotometry in the presence of major ingredients of detergents. *J. Milk Food Technol.* 36 (11): 554- 558
- Anon. (1992). Soft options. German survey. *Dairy Ind. Intl.* 57 (6): 29-30.
- Anon. (1995). Cheese manufacturers see market for healthy cheese having most potential. *Cheese Reporter.* 120 (15): 16.
- Anon. (2002). The specialist cheesemakers code of best practice. Internet source, http://www.specialistcheesemakers.co.uk/best_practice/Glossary_of_terms.htm.
- Apostolopoulos, C. (1994). Simple empirical and fundamental methods to determine objectively the stretchability of mozzarella cheese. *J. Dairy Res.* 61: 405-413
- Apostolopoulos-C; Bines-VE; Marshall-RJ 1994 Effect of post-cheddaring manufacturing parameters on the meltability and free oil of Mozzarella cheese. *J. Society Dairy Technol.* 47 (3): 84- 87.
- Ardo, Y., and Pettersson, H. E. (1988). Accelerated cheese ripening with heat treated cells of *Lactobacillus helveticus* and a commercial proteolytic enzyme. *J. Dairy Res.* 55: 239- .

- Athar, I. H., and Anwar, M. (1992). Comparison of Mozzarella cheese: prepared from buffalo milk by starter culture and direct acidification techniques. *Pakistan J. Scientific Industrial Res.* 35 (3): 108-111.
- Babad, J., and Shtrikman, N. (1951). The estimation of citric acid in dairy products. *J. Dairy Res.* 18 (1): 72- 76.
- Barbano, D. M. (1999). Controlling functionality of mozzarella cheese through process control. A paper presented for 36th Annual Marschall Italian and Speciality Cheese Seminar, held in Madison, Wisconsin.
- Barbano, D. M., Lynch, J. M., Fleming, J. R. (1991). Direct and indirect determination of true protein content of milk by Kjeldahl analysis: collaborative study. *J. AOAC.* 74 (2): 281-288.
- Barbano, D. M., Yun, J. J. and Kindstedt, P. S. (1994). Mozzarella cheese making by a stirred-curd method, no-brine procedure. *J. Dairy Sci.* 77: 2687- 2694.
- Barr, A. (1990). Consumer motivational forces affecting the sale of light dairy products. *Food Technol.* 44: 97-98.
- Barz, R. L. and Cremer, C. P. (1993). Process of making acceptable mozzarella cheese without ageing. *U. S. Patent* 5: 200- 216
- Baxter, B. L., White, C. H., and Coggins, P. C. (1997). The use of adjunct cultures and elevated temperatures in low fat cheddar cheese. *J. Dairy Sci.*, 80 (Suppl. 1): pg113.
- Bhaskaracharya, R. K. (2000). The texture and microstructure of mozzarella cheese as affected by fat content, EPS producing starter culture and fat replacers. M. Sc. *Thesis*, School of Life Sciences, Victoria Univ. Technol., Melbourne, Australia.
- Bhaskaracharya, R. K. and Shah, N. P. (1999). Texture evaluation of commercial mozzarella cheeses. *Aust. J. Dairy Technol.* 54 (1): 36- 40.

- Bhaskaracharya, R. K., and Shah, N. P. (2000a). A simplified method for examination of microstructure of mozzarella cheeses with scanning electron microscopy. *Aust. J. Dairy Technol.* 55 (1): 28- 32.
- Bhaskaracharya, R. K., and Shah, N. P. (2000b). Texture characteristics and microstructure of skim milk mozzarella cheese made using exopolysaccharide or non-exopolysaccharide producing starter cultures. *Aust. J. Dairy Technol.* 55(3): 132- 138.
- Bhaskaracharya, R. K., and Shah, N. P. (2001a). Cultures and Coagulants- Texture and microstructure of mozzarella. *Dairy Ind. Intl.* 66(2): 42- 45.
- Bhaskaracharya, R. K., and Shah, N. P. (2001b). Mozzarella without the fat. *Dairy Ind. Intl.* 66(12): 22- 26.
- Bhaskaracharya, R. K., and Shah, N. P. (2001c). Texture and microstructure of skim milk mozzarella cheeses made using fat replacers. *Aust. J. Dairy Technol.* 56 (1): 9- 14.
- Breene, W. M., Price, W. V., Ernstrom, C. A. (1964). Manufacture of pizza cheese without starter. *J. Dairy Sci.* 47 (11): 1173- 1180.
- Brendehaug, J. and Abrahamsen, R. K. (1986). Chemical composition of milk from a herd of Norwegian goats. *J. Dairy Res.* 53 (2): 211- 221.
- Brooks, I. B., Luster, G. A., Easterly, D. G. (1970). A procedure for the rapid determination of the major cations in milk by atomic absorption spectrophotometry. *Atomic Absorption Newsletter* 9 (4): 93- 94.
- Broome, M. C., and Limsowtin, G. K. Y. (1998). Starter peptidase activity in maturing cheese. *Aust. J. Dairy Technol.*, 53 (2): 79-82.
- Brown, N. D. (1989). Manufacture of cornhusker cheese with reduced fat content. *Cult. Dairy Prod. J.* 24 (3): 23.
- Burros, M. (1992). If butter fat is cut, cheese doesn't cut it. *New York Times*, June 3, pC1.
- Bynum, D. G. and Barbano, D. M. 1985. Whole milk reverse osmosis retentates for cheddar cheese manufacture: Chemical changes during ageing. *J. Dairy Sci.* 68: 1-10.

- Cabanis, M. T., Cassanas, G., Cabanis, J. C. and Brun, S. (1988). Comparison of four methods for digesting food samples for determination of trace levels of cadmium by flameless atomic absorption spectrophotometry. *J. AOAC*. 71 (5): 1033- 1037.
- Case, R. A., Bradley, R.L. Jr., Williams, R. R. (1985). Chemical and physical methods in *Standard methods for the examination of dairy products*, 15th edition. Ed. Richardson, G. H., Publ. American Public Health Association, Baltimore, Maryland, USA.
- Cavella, C., Chemin, S., and Masi, P. (1992). Objective measurement of the stretchability of mozzarella cheese. *J. Text. Studies* 23: 185- 194
- Cerning, J., Bouillanne, C., Desmazeaud, M. J., and Landon, M. (1988). Exocellular polysaccharide production by *Streptococcus thermophilus*. *Biotechnol. Letters*, 10 (4): 255-260.
- Cerning, J., Bouillanne, C., Desmazeaud, M. J., and Landon, M. (1986). Isolation and characterization of exo-cellular polysaccharide produced by *Lactobacillus bulgaricus*. *Biotechnol. Letters*, 8 (9): 625-628.
- Chen, A. H., Larkin, J. W., Clark, C. J. and Irwin, W. E. (1979). Textural analysis of cheese *J. Dairy Sci.* 62 (6): 901-907.
- Clunies, P. E., Kakuda, Y., Mullen, K., Arnott, D. R. and de Man, J. M. (1986). Physical properties of yogurt: a comparison of vat versus continuous heating systems of milk. *J. Dairy Sci.* 69: 2593.
- Coffey, A., Stokes, D., Fitzgerald, G. F., and Ross, R. P. (2002) Traditional and molecular approaches to improving bacteriophage resistance of Cheddar and mozzarella cheese starters. *Irish J. Agric. Food Res.*, 40 (2): 239-270.
- Coppola, S., Villani, F., Coppola, R., and Parente, E. (1990). Comparison of different starter systems for water-buffalo mozzarella cheese manufacture. *Lait*, 70 (5/6): 411-423.
- Creamer, L. K. (1976). Casein proteolysis in mozzarella type cheese. *New Zealand J. Dairy Sci. Technol.*, 11: 130-

- Creamer, L. K. and Waugh, D. F. (1966). Calcium binding and precipitate solvation of C- α_s -caseinates. *J. Dairy Sci.* 49: 706 (Abstr. M51).
- Dahmen, J., Fuchs-Pohl, G. Merck, E. and Pohl, B. (1989). A universal calibration method for flame atomic absorption analysis, Publ. *Varian Instruments At Work* June AA-88.
- Dalgleish, D. G., and Law, A. J. R. (1988). pH induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. *J. Dairy Res.* 55 (4): 529- 538.
- Dalgleish, D. G., and Law, A. J. R. (1989). pH induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. *J. Dairy Res.* 56 (5): 727- 735.
- Dave, R. I., and Shah, N. P. (1996). Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium* ssp. *J. Dairy Sci.* 79: 1529- 1536.
- Davies, D. T., and Law, A. J. R. (1983). Variation in the protein composition of bovine casein micelles and serum casein in relation to micellar size and milk temperature. *J. Dairy Res.* 50: 67- 75.
- de Jong, L. and de Groot-Mostert, A. E. A. (1977). The proteolysis action of rennet on different casein substrates under various conditions. *Neth. Milk Dairy J.* 31: 296.
- de Ruig, W. G. (1986). Atomic absorption spectrometric determination of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc in animal feeding stuffs: Interlaboratory collaborative studies. *J. AOAC.* 69 (6): 1009- 1013.
- del Prato, S. O. (1993). Italian mozzarella. *Dairy Ind. Intl.* 58 (4): 26-27, 29.
- del Prato, S. O. (1993). Mozzarella cheesemaking. *Latte*, 18 (5): 594-604.
- Demott, B. J. (1988). Determination of calcium in cottage cheese by titration. *J. Food Protection*, 51 (10): 802- 803.
- Diefes, H. A., Rizvi, S. S. H. and Bartsch, J. A. (1993). Rheological behaviour of frozen and thawed low moisture, part skim mozzarella cheese. *J. Food Sci.* 58 (4): 764-769.

- do Valle, J. L. E., and de Leitaó, M. F. (1995). Utilization of citric acid in the manufacture of Mozzarella cheese. *Coletanea Instituto Tecnologia Alimentos*, 25 (2): 143-151.
- Downey, W. K., and Murphy, R. F. (1970). The temperature-dependent dissociation of β -casein from bovine casein micelles and complexes. *J. Dairy Res.* 37: 361- 372.
- Dryer. (1994). Good news, bad news about light cheeses. *Dairy foods@95* (6): 20.
- Dudley, E. G., Husgen, A. C., Wen-He, and Steele, J. L. (1996). Sequencing, distribution, and inactivation of the dipeptidase A gene (pepDA) from *Lactobacillus helveticus* CNRZ32. *J. Bacteriology*, 178 (3): 701-704.
- Dulay, T. A., Gonzaga, J. O., and Borromeo, A. M. (1986) The influence of cheese cultures on the quality of soft cheese. *Philippine Agriculturist*, 69 (1): 9-14.
- El Soda, M., Madkor, S. A., and Tong, P. S. (2000). Evaluation of commercial adjuncts for use in cheese ripening. IV. Comparison between attenuated and not attenuated lactobacilli. *Milchwissenschaft* 55 (5): 260-263.
- Elwell, W. T., and Gidley, J. A. F. (1966). Sources of interference in atomic absorption spectrophotometry, Second ed., Publ. Pergamon Press Ltd., Oxford, London.
- Euber, J. R., and Brunner, J. R. (1978). Determination of lactose in dairy products by HPLC. *J. Dairy Sci.* 61 (Suppl. 1): 103.
- FAO. (1969). Toxicological evaluation of some food colours, emulsifiers, stabilizers, anti-caking agents and certain other substances. *FAO Nutrition Meetings Report Series*, Rome, 27 May - 4 June 1969 No. 46A WHO/FOOD ADD/70.36.
- FAO. (1997). The role of carbohydrates in nutrition. Chapter 1 in report presented at *The Joint FAO/WHO Expert Consultation* on "Carbohydrates in Human Nutrition" held from 14 to 18 April 1997, Rome, Italy.
- Farkye, N. Y., Madkor, S. A., and Atkins, H. G. (1995). Proteolytic abilities of some lactic acid bacteria in a model cheese system. *Intl. Dairy J.* 5 (7): 715-725.

- Farkyne, N. Y., and Fox, P. F. (1990). Observations of plasmin activity in cheese. *J. Dairy Res.* 57: 413.
- Farre, R., Lagarda, J. M. and Montoro, R. (1986). Atomic absorption Spectrophotometric determination of chromium in foods. *J. AOAC.* 69 (5): 876- 879.
- Fecera, R. M., McMahon, D. J. and Oberg, C. J. (1995). Effect of exopolysaccharide producing cultures on moisture retention in low fat mozzarella cheese. Technical Session. *IFT Annual Meeting* p134.
- Feldman, C. (1974). Perchloric acid procedure for wet-ashing organics for the determination of mercury (and other metals). Publ. American Chemical Society Labguide, Washington ACS. *Anal. Chem.* 46 (11): 1606- 1609.
- Fernandez, A. (1985). Ultrafiltration and evaporation in the manufacture of Mozzarella cheese. *Dissertation Abstr. Intl.*, B 45 (9) 2739, 192pp.
- Fernandez, A., Kosikowski, F. V. (1986). Physical properties of direct acidified Mozzarella cheese from ultrafiltered whole milk retentates. *J. Dairy Sci.*, 69 (3): 643-648.
- Fife, R. L., McMahon, D. J., Oberg, C. J. and Alleyne, M. C. (1995). Functionality of low fat mozzarella cheese made with commercial fat replacers. Technical Session. *IFT Annual Meeting* p133.
- Fiske, C. H. and Subbarow, Y. (1925). The colorimetric determination of phosphorous. *J. Biol. Chem.* 66: 375-
- Fox, C. W., and Ernstrom, C. A. (1969). Role of calcium in the low-temperature acidification of skim milk and the formation of direct-acid curd. A paper presented at the 52nd ADSA Annual meeting, Oklahome, USA. *J. Dairy Sci.* 52: 885 (Abstr. M32)
- Fox, P. F. (1987). Significance of salt in cheese ripening. *Dairy Inustries Intl.*, 52 (9):19, 21-22.

- Fox, P. F., McSweeney, P. L. H. and Singh T. K. (1995). Methods for assessing proteolysis in cheese during maturation. In *Chemistry of Structure Function Relationships in Cheese* Malin, E. L., Tunick, M. H., Plenum press, New York 367: 161- 194.
- Furtado, M. M., Chandan, R. C., Brunner, J. R., Wishnetsky. T. (1982). [Effect of salt concentration on the maturation of a cheese ripened by *Penicillium caseicolum*]. *Revista Instituto Laticinios Candido Tostes* 37 (224) 15-18, [CDROM] Available: In *Food Sci. Technol. Abstracts*, Item: 84-11-P2364.
- Gasperi, F., Gallerani, G., Boschetti, A., Biasioli, F., Monetti, A., Boscaini, E., Jordan, A., Lindinger, W., and Iannotta, S. (2001). The Mozzarella cheese flavour profile: a comparison between judge panel analysis and proton transfer reaction mass spectrometry. *J. Sci. Food Agric.*, 81 (3): 357-363.
- Georgakis, S. A. (1973). Studies on the manufacture of Greek Feta pickled cheese. I. Relation between the amount of salt used and duration of salting. *Milchwissenschaft* 28 (8): 500-502.
- Geurts, T. J., Walstra, P. and Mulder, H. (1972). Brine composition and the prevention of the defect 'soft rind' in cheese. *Neth. Milk Dairy J.* 26: 168- 179.
- Ghosh, B. C., Kulkarni, S. (1996). Low cholesterol Mozzarella cheese - technology standardization. *J. Food Sci. Technol. India* 33 (6): 488-492.
- Ghosh, B. C., Singh, S. (1991). Effect of brining on the quality of Mozzarella cheese manufactured from buffalo milk. *Cheiron* 20 (1): 19-22.
- Girgis-ES; Shehata-TE; Naghmoush-MR; Khalid-MA 1981 Effect of salt on some defectuous [spoilage] microorganisms in soft cheese. *Res. Bulletin*, Faculty of Agriculture, Ain-Shams Univ., No. 1500, 8pp.
- Green, M. L., Marshall, R. J. and Brooker, B. E. (1985). Instrumental and sensory texture measurement and fracture mechanisms of cheddar and cheshire cheeses. *J. Texture Stud.* 16: 351.

- Grigg, D. (1993). International variations in food consumption in the 1980's. *Geography* 78: 251- 266.
- Grigg, D. (1996). The starchy staples in world food consumption. *Annals Assoc. Am. Geographers*, 86: 412- 431.
- Guinee, T. P., Auty, M. A. E., Harrington, D., Corcoran, M. O., Mullins, C. and Mulholland, E. O. (1998). Characteristics of different cheeses used in pizza pie. A paper presented at the Cheese Science'98 conference, 1- 3 July 1998, Melbourne, Australia. *Aust. J. Dairy Technol.* 53 (2): 109.
- Guinee-TP; Fox-PF 1986 Influence of cheese geometry on the movement of sodium chloride and water during brining. *Irish J. Food Sci. Technol.* 10 (2) 73-96.
- Gunasekaran, S, and Ak-Mehmet, M. (2000). Dynamic oscillatory shear testing of foods-selected applications. *Trends Food Sci. Technol.* 11: 115- 127.
- Guo, M. R., Kindstedt, P. S., Metzger, L., and Barbano, D. M. (1998). Low-fat Mozzarella cheese from pre-acidified milk. *Aust. J. Dairy Technol.*, 53 (2): 108
- Hankinson, D. J. (1975). Potential sources of copper contamination of farm milk supplies measured by atomic absorption spectrophotometry. *J. Dairy Sci.* 58 (3): 326- 336.
- Hassan, A. N. and Frank, J. F. (1997). Modification of microstructure and texture of rennet curd by using a capsule forming non-ropy lactic culture. *J. Dairy Res.* 64: 115-121.
- Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1995a). Observation of encapsulated lactic acid bacteria using confocal scanning laser microscopy. *J. Dairy Sci.* 78: 2624- 2628.
- Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1995b). Formation of the yogurt microstructure and three dimensional visualization as determined by confocal scanning laser microscopy. *J. Dairy Sci.* 78: 2629-2636.

- Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1996a). Rheological properties of yogurt made with encapsulated non-ropy lactic cultures. *J. Dairy Sci.* 79: 2091-2097.
- Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A. and Shalabi, S. I. (1996b). Textural properties of yogurt made with encapsulated non-ropy lactic cultures. *J. Dairy Sci.* 79: 2098-2103.
- Heinz, J. V., Marshall, R. T. and Anderson, M. E. (1967). Determining cleanliness of milk contact surfaces by analyzing for calcium residual: preliminary studies. *J. Milk Food Technol.* 30: 337- 339.
- Hicks, C. L., and Ibrahim, S. A. (1992). Lactic bulk starter homogenization affects culture agglutination. *J. Food Sci.* 57 (5): 1086-1092.
- Hill, A. R., Bullock, D. H. and Irvine, D. M. (1985). Composition of whey: effect of pH and temperature of dipping. *Can. Inst. Food Sci. Technol. J.* 18: 53- 57.
- Holsinger, V. H., Smith, P. W. and Tunick, M. H. (1995). Overview: Cheese chemistry and rheology. In *Chemistry of Structure Function Relationships in Cheese*. Malin, E. L., Tunick, M. H. Plenum press, New York. 367: 1-6.
- Hutkins, R., Halambeck, S. M., and Morris, H. A. (1986) Use of galactose-fermenting *Streptococcus thermophilus* in the manufacture of Swiss, Mozzarella, and short-method Cheddar cheese. *J. Dairy Sci.*, 69 (1): 1-8.
- Incze, D. S. (1976). Analytical applications of complex equilibria. John Wiley and Sons Inc. New York, NY
- Innocente-N; Sensidoni-A 1996 Use of ionic integrator in cheese salting. *Scienza Tecnica Lattiero Casearia* 47 (4):284- 302 [CDROM] Available: In *Food Sci. Technol. Abstracts* Item: 1997-05-P0136.
- International Dairy Federation (1964). Determination of the ash content of processed cheese products. *Standard FIL- IDF 27*, IDF, Brussels, Belgium.

- International Dairy Federation (1966). Determination of the calcium content of milk. *Standard FIL- IDF 36*, IDF, Brussels, Belgium.
- International Dairy Federation (1980). Milk and milk products: Determination of the copper content, *Standard FIL- IDF 76; 76A*, IDF, Brussels, Belgium.
- International Dairy Federation (1982). Cheese and processed cheese products. Determination of total phosphorous content (photometric method). *Provisional Int. IDF Standard*, 33C, IDF, Brussels, Belgium.
- Jana, A. H., and Upadhyay, K. G. (1991). Mozzarella cheese- a review. *Indian J. Dairy Sci.* 44 (2): 167- 175.
- Johnson, J. A. C., Etzel, M. R., Chen, C. M., and Johnson, M. E. (1995). Accelerated ripening of reduced-fat Cheddar cheese using four attenuated *Lactobacillus helveticus* CNRZ-32 adjuncts. *J. Dairy Sci.*, 78 (4): 769-776.
- Joshi, N. S., Muthukumarappan, K., and Dave, R. I. (2001). Calcium paper. A paper presented for *International Food Technologists* in New Orleans, Louisiana, USA held on June 26, 2001.
- Kahn, H. L. and Kerber, J. D. (1971). Sampling improvements in atomic absorption spectroscopy. *J. Amer. Oil Chemists Soc.* 48 (9): 434- 437.
- Kamal, T. H. (1960). Compleximetric titration of calcium and magnesium in the presence of phosphate in milk and blood plasma. *Agricultural and Food Chemistry* 8 (2): 156- 158
- Katz, S. A., Jenniss, S. N. (1981). *J. Environ. Anal. Chem.* 9: 209- 220.
- Kaya-S 2002 Effect of salt on hardness and whiteness of Gaziantep cheese during short-term brining. *J. Food-Engg.* 52 (2): 155- 159.
- Kaya-S; Kaya-A; Oner-MD 1999 The effect of salt concentration on rancidity in Gaziantep cheese. *J. Sci. Food Agric.* 79 (2): 213- 219.

- Keller, B. (1978). Continuous culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and their use for flavor development in cheese made by direct acidification. *Dissertation Abst. Intl.* 38 (12): 5829, 146pp.
- Keller, B., Olson, N. F., and Richardson, T. (1974). Mineral retention and rheological properties of mozzarella cheese made by direct acidification. *J. Dairy Sci.* 57 (2): 174-180.
- Khalid-AS; Harrigan-WF 1984 Investigation into the effect of salt level on growth of. and toxin production by, *Staphylococcus aureus* in Sudanese cheese. *Lebensmittel Wissenschaft Technol.* 17 (2): 99- 103.
- 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.
- Kim, J. S., Kim, M. N., and Kim, J. O. (1995). Approximate chemical compositions and rheological properties of Mozzarella cheese manufactured from the mixture of cow's milk and soymilk by direct acidification. *Korean J. Dairy Sci.* 17 (1): 51-58.
- Kim, J., Schmidt, K. A., Phebus, R. K., and Jeon, I. J. (1998). Time and temperature of stretching as critical control points for *Listeria monocytogenes* during production of Mozzarella cheese. *J. Food Prot.* 61 (1): 116- 118.
- Kindstedt, P. S. (1991). Functional properties of mozzarella cheese on pizza: a review. *Cult. Dairy Prod. J.* 26: 27- 31.
- Kindstedt, P. S. (1993). Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of mozzarella cheese. *Crit. Revs. Food Sci. Nutr.* 33 (2): 167-187.
- Kindstedt, P. S. and Guo, M. R. (1997). Recent Developments in the science and technology of pizza cheese. *Aust. J. Dairy Technol.* 52 (1): 41- 43.

- Kindstedt, P. S. and Kosikowski, F. V. (1985a). Improved compleximetric determination of calcium in cheese. *J. Dairy Sci.* 68 (4): 806- 809.
- Kindstedt, P. S. and Kosikowski, F. V. (1985b). Alternative test for phosphorous in cheese. *J. Dairy Sci.* 68 (8): 1859- 1864.
- Kindstedt, P. S., and Kiely L. J. (1990). Physical and chemical methods for controlling the body and texture of mozzarella cheese. Oral presentation at the 9th Biennial Cheese Industry Conference, Utah State Univ., Utah, USA.
- Kindstedt, P. S., Kiely, L. J. and Gilmore, J. A. (1992), Variation in composition and functional properties within brine-salted mozzarella cheese. *J. Dairy Sci.* 75 (11): 2913- 2921.
- Kindstedt, P. S., Larose, K. L., Gilmore, J. A., and Davis, L. (1996). Distribution of salt and moisture in mozzarella cheese with soft surface defect. *J. Dairy Sci.* 79 (12): 2278-2283.
- Kindstedt, P. S., Rippe, J. K., and Duthie, C. M. (1989). Measurement of mozzarella cheese melting properties by helical viscometry. *J. Dairy Sci.* 72: 3117- 3122.
- Kindstedt, P. S., Rowney, M., and Roupas, P. (1999). Technology, biochemistry and functionality of pasta filata/pizza cheese. In *Technology of cheese making*, ed. Law, B. A., and Ashurst, P. R., Publ. Sheffield Academic Press, Sheffield, England.
- Kindstedt, P. S., Zielinski, A., Aliste, A. M. G. C. (2001). A post-manufacture method to evaluate the effect of pH on Mozzarella cheese characteristics. *Aust. J. Dairy Technol.* 56 (3): 202-207.
- Kolz, I. L., Henze, G. E., Kaiser, G. (1979). *Talanta* 681- 691
- Koop, J., Klomp, H. and Westerbeck, D. (1986). *Neth. Milk Dairy J.* 40 (4): 337- 350.
- Kosikowski, F. V. (1977). Cheese and fermented milk foods. First ed. Pub. Edward Brothers, Inc., Ann Arbor, Michigan, USA.
- Kosikowski, F. V. (1982). Cheese and fermented milk foods. Second ed. Pub. Kosikowski, F. V. and Associates, Brooktondale, New York, USA.

- Kosikowski, F.V. and Mistry, V.V. (1997). Cheese and Fermented Milk Foods. Third ed., AVI Publishing Company, Westport, Connecticut, USA.
- la Notte, E., Santoro, M., Leone, A. M., and Vitagliano, M. (1980). Behaviour of free amino acids during ripening of pasta filata cheeses. *Fior di Latte. Scienza Tecnica Lattiero Casearia* 31 (1): 19-39.
- Larsen, R. F., and Anon, M. C. (1989). Effect of water activity a_w of milk on acid production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *J. Food Sci.* 54 (4): 917-921.
- Lawrence, R. C., Creamer, L. K. and Gilles, J. (1987). Texture development during cheese ripening. *J. Dairy Sci.* 70: 1748- 1760.
- Lawrence, R. C., Heap, H. A. and Gilles, J. (1984). A controlled approach to cheese technology. *J. Dairy Sci.* 67: 1632.
- Lawrence-RC; Gilles-J 1969 The formation of bitterness in cheese: a critical evaluation. *New Zealand J. Dairy Technol.* 4 (4): 189- 96.
- Leenheer, J. (1988). Milk and milk powder: Determination of the calcium content- Atomic absorption spectrometric method- collaborative study. *Intl. Dairy Fed. Bull.* 235: 34- 39
- LiMaltrin Basedek, B. E. and Rowe, C. J. (1986). Curve correction in atomic absorption. Publ. *Varian Instruments At Work*, NuMaltrin baseder AA- 58
- Low, D., Ahlgren, J. A., Horne, D., McMahon, D. J., Oberg, C. J., and Broadbent, J. R. (1998). Role of *Streptococcus thermophilus* MR-1C capsular exopolysaccharide in cheese moisture retention. *Appl. Envir. Microbiol.*, 64 (6) : 2147-2151.
- Low, D., McMahon, D.J., Oberg, C.J., Hornel, D. and Broadbent, J.R. (1997). Influence of *Streptococcus thermophilus* 1 OJC EPS on the moisture content of low fat mozzarella cheese. *J. Dairy Sci.* 80 (Suppl.1): 107.
- Luyten, H. (1988). The rheological and fracture properties of gouda cheese. *PhD thesis*, Wageningen Agricultural University, The Netherlands.

- Lyster, R. L. J. (1965). The composition of milk deposits in an ultra high temperature plant. *J. Dairy Res.* 32 (2): 203- 208.
- Malin, E. L., Banks, J. M., Tunick, M. H., Law, A. J. R., Leaver, J. and Holsinger, V. H. (1993). Texture enhancement of low fat mozzarella cheese by refrigerated storage. *Int. Dairy J.* 3: 4- 6, 548 poster A4.
- Mann, E. J. (1992). Mozzarella cheese. *Dairy Ind. Int.* 57 (9): 17- 18.
- Marier, J. R. and Boulet, M. (1958). Direct determination of citric acid in milk with an improved pyridine- acetic anhydride method. *J. Dairy Sci.* 41 (2): 1683- 1692.
- Martinez, G. C., Chafer, M., Fito, P., Chiralt, A. (2002) Development of salt profiles on Manchego type cheese during brining. Influence of vacuum pressure. *J. Food Engg.* 53 (1): 67-73.
- Martin-Hernandez, M. C. and Juarez, M. (1989). Retention of main and trace elements in four types of goat cheese. *J. Dairy Sci.* 72 (5): 1092- 1097.
- Masei, P. and Addeo, F. (1986). An examination of some mechanical properties of a group of Italian cheeses and their relationship to structure and conditions of manufacture. *J. Food Engg.* 5: 217- 229.
- McMahon, D. J., Fife, R. L., and Oberg, C. J. (1999). Water partitioning in mozzarella cheese and its relationship to cheese meltability. *J. Dairy Sci.* 82 (7): 1361- 1369.
- McMahon, D. J., Oberg, C. J., and McManus, W. (1993). Functionality of mozzarella cheese. *Aust. J. Dairy Technol.*, 48: 99- 104.
- Merrill, R. K. (1994). A study of factors controlling physical properties of mozzarella cheese and the manufacture of a reduced fat mozzarella cheese. *Disser. Abstr. Int.* 55 (4): 1241- 1242.
- Merrill, R. K., Oberg, C. J. and McMahon, D. J. (1994). A method for manufacturing reduced fat mozzarella cheese. *J. Dairy Sci.* 77 (7): 1783- 1789.

- Metzger, L. E., Barbano, D. M., Rudan, M. A. and Kindstedt, P. S. (2000). Effect of milk pre-acidification on low fat mozzarella cheese. I. Composition and yield. *J. Dairy Sci.* 83 (4): 648- 658.
- Metzger, L. E., Barbano, D. M., Rudan, M. A., Kindstedt, P. S. and Guo, M. R. (2000a). Whiteness change during heating and cooling of mozzarella cheese. *J. Dairy Sci.* 83 (1): 1- 10.
- Mistry, V. V. and Anderson, D. L. (1993a). Composition and microstructure of commercial full fat and low fat cheeses. *Food Structure* 12: 259- 266.
- Mistry, V. V. and Anderson, D. L. (1993b). Improving the sensory characteristics of reduced fat cheese. *Amer. Chem. Soc. Nat. Meeting Abst.* vol. 208 part no. AGFD 93.
- Moffett, J. H. (1988). Instrument zero and blank reading on the Spectra AA instruments. Publ. *Varian Instruments At Work* April, AA-79.
- Moffett, J. H. (1995). Determination of magnesium, calcium and potassium in brines by flame AAS using the SIPS- 10 accessory for automated calibration and online sample dilution. Publ. *Varian Instruments At Work* July, AA- 123.
- Moffett, J. H. (2000). Why calibration graphs curve in atomic absorption spectrometry. Publ. *Varian Instruments At Work* February, AA- 128.
- Mohamed, M. O. and Morris, H. A. (1987). Textural and microstructural properties of rennet induced milk coagulum as affected by the addition of soy protein isolate. *J. Texture Stud.* 18: 137.
- Moneib, A., Hamdy, A., and Safwat, A. (1970). The effect of salt and rennet added to the milk on physico-chemical properties of fresh soft cheese. *Agric. Res. Rev. Cairo*, 48 (4): 131- 138.
- Morgan, K.J., Zabik, M.E. (1981). Amount and food sources of total sugar intake by children ages 5 to 12 years. *Am. J. Clinical Nutr.* 34: 404- 413.

- Morris-HA; Guinee-TP; Fox-PF 1985 Salt differentiation in Cheddar cheese. *J. Dairy Sci.* 68 (8): 1851- 1858.
- Mounsey, J. S., and O'Riordan, E. D. (2001). Characteristics of imitation cheese containing native starches. *J. Food Sci.* 66 (4): 586- 591.
- Muir, D. D., Banks, J. M., and Hunter, E. A. (1992). Sensory changes during maturation of fat-reduced Cheddar cheese: effect of addition of enzymically active attenuated starter cultures. *Milchwissenschaft* 47 (4): 218- 222.
- Mukherjee, K. K., and Hutkins, R. W. (1994). Isolation of galactose-fermenting thermophilic cultures and their use in the manufacture of low browning Mozzarella cheese. *J. Dairy Sci.* 77 (10): 2839- 2849.
- Murthy, G. K. and Campbell, J. E. (1959). A method for the rapid ashing of milk for radionuclide analysis. *J. Dairy Sci.* 42 (8): 1288- 1293.
- Murthy, G. K. and Rhea, M. (1967). Determination of major cations in milk by atomic absorption spectrophotometry. *J. Dairy Sci.* 50 (3): 313- 317.
- Murthy, G. K. and Whitney, R. McL. (1956). A plan for the rapid determination of major cations in milk. *J. Dairy Sci.* 39 (4): 364- 373.
- Murthy, G. K., Coakley, J. E. and Campbell, J. E. (1960). A method for the elimination of ashing in Strontium determination of milk. *J. Dairy Sci.* 43 (2): 151- 154.
- Murthy, G. K., Rhea, U. S. and Peeler, J. T. (1972). Copper, iron, manganese, strontium and zinc content of market milk. *J. Dairy Sci.* 55 (12): 1666- 1674.
- Muthukumarappan, K., Wang, Y. C. and Gunasekaran, S. (1999). Short communication: Modified schreiber test for evaluation of mozzarella cheese meltability. *J. Dairy Sci.* 82: 1068- 1071.
- National Health and Medical Research Council (1987). Food standards code, Australian government publishing service, Canberra. 253- 269.

- Nilson, K. M. (1968). Effects of salt concentration on the quality of Italian cheeses. *Food Product Development* 2 (4): 90, 92 & 94.
- Nilson, K. M. (1969). Solving problems of Mozzarella cheese. *Am. Dairy Rev.* 31 (4): 28, 30 & 72.
- Nilson, K. M., and LaClair, F. A. (1976). Stirred curd mozzarella cheese. A paper presented for the 13th Annual Marschall Italian and Speciality Cheese Seminar, held in Madison, Wisconsin on May 3 and 4, 1976.
- Nilson, K. M., LaClair, F. A. (1975). Cheese - direct salting and automation. *Dairy Ice-Cream Field* 158 (6): 56, 58, 60.
- Noh, B., and Richardson, T. (1989). Incorporation of radiolabeled whey proteins into casein micelles by heat processing. *J. Dairy Sci.* 72: 1724- 1731.
- Noomen, A. (1978). Activity of proteolytic enzymes in simulated soft cheeses (Meshanger type). 2. Activity of calf rennet. *Neth. Milk Dairy J.* 32: 49.
- Ntailianas, N. A. and Whitney, R. L. McL. (1964). Calcein as an indicator for the determination of total calcium and magnesium and calcium alone in the same aliquot of milk. *J. Dairy Sci.* 47 (1): 19- 27.
- Oberg, C. J., Broadbent, J. R., Strickland, M., and McMahon, D. J. (2002). Diversity in specificity of the extracellular proteinases in *Lactobacillus helveticus* and *Lactobacillus debrueckii* ssp. *bulgaricus*. *Letters in Appl. Microbiol.* 34 (6): 455- 460.
- Oberg, C. J., McManus, W. R. and McMahon, D. J. (1993). Microstructure of mozzarella cheese during manufacture. *Food Structure* 12: 251- 258.
- Oberg, C. J., Merrill, R. K., Moyes, L. V., Brown, R. J., and Richardson, G. H. (1991). Effects of *Lactobacillus helveticus* cultures on physical properties of mozzarella cheese. *J. Dairy Sci.* 74: 4101- .
- Okada, M., Chonan, T, and Ho-Gakkai, N. C. (1983). Determination of lactose in milk by high performance liquid chromatography. *Chem. Abstracts* 54 (9): 562- 564.

- Olson, N. F. (1990). Measuring cheese acidity- The relationship of titratable acidity and pH in cheesemaking. *Univ. Wisconsin Dairy Pipeline Newsletter* 2 (3): 1- 3, 7.
- Olson, N. F. and Johnson, M. E. (1990). Light cheese products: characteristics and economics. *Food Technol.* 44 (10): 93- 96.
- Other, K. (1999). Sulfonation and sulfation to thorium and thorium compounds in *Encyclopedia of Chemical Technology* Vol. 22, third edition. Publ. John Wiley and Sons, New York, USA.
- Pagliarini, E. and Beatrice, N. (1994). Sensory and rheological properties of low fat filled 'Pasta filata' cheese. *J. Dairy Res.* 61: 299-304.
- Pappas, C. P., Kondyli, E., Voutsinas, L. P., Mallatou, H. (1996). Effects of salting method and storage time on composition and quality of Feta cheese. *J. Society Dairy Technol.* 49 (4): 113- 118.
- Patel, A. M., Dave, J. M., and Sannabhadti, S. S. (1983). Acid production by yoghurt starters and their effect on proteolysis and lactose utilization in buffalo skim milk. *J. Food Sci. Technol. India* 20 (6): 317- 319.
- Patel, G. C., Vyas, S. H., and Upadhyay, K. G. (1986). Evaluation of Mozzarella cheese made from buffalo milk using direct acidification technique. *Indian J. Dairy Sci.*, 39 (4): 394- 403.
- Patel, S. S., and Wilbey, R. A. (1989). *J. Society Dairy Technol.* 42:79- 80.
- Paulson, B. M., McMahon, D. J. and Oberg, C. J. (1998). Influence of sodium chloride on appearance, functionality and protein arrangements in non-fat mozzarella cheese. *J. Dairy Sci.* 81 (8): 2053- 2064
- Pavia, M., Trujillo, A. J., Guamis, B., and Ferragut, V. (2000). Effectiveness of high-pressure brining of Manchego-type cheese. *Lebensmittel Wissenschaft Technol.* 33 (5): 401- 403.
- [CDROM] Available: In *Food Sci. Technol. Abstracts* Item: 2000-11-P1804.

- Perry, D. B., McMahon, D. J. and Oberg, C. J. (1998). Manufacture of low fat mozzarella cheese using EPS producing starter cultures. *J. Dairy Sci.* 81: 563- 566.
- Perry, D. B., McMahon, D. J., and Oberg, C. J. (1997). Effect of exopolysaccharide-producing cultures on moisture retention in low fat Mozzarella cheese. *J. Dairy Sci.* 80 (5): 799- 805.
- Poduval, V. S., and Mistry, V. V. (1999). Manufacture of reduced fat mozzarella cheese using ultrafiltered sweet buttermilk and homogenized cream. *J. Dairy Sci.* 82 (1): 1- 9.
- Pollman, R. M. (1991). Atomic absorption spectrophotometric determination of calcium and magnesium and colorimetric determination of phosphorous in cheese: collaborative study. *J. AOAC* 74 (1): 27- 31.
- Pomeranz, Y. (1982). Grain structure and end-use properties. *Food Microstructure* 1 (2): 107- 124.
- Prasad, N., and Alvarez, V. B. (1999). Effect of salt and chymosin on the physico-chemical properties of Feta cheese during ripening. *J. Dairy Sci.* 82 (6): 1061- 1067.
- Prentice, J. H. (1987). Cheese: Chemistry, Physics and Microbiology. Vol 1, Fox, P. F. ed., Elsevier applied science publishers, London, 299- 344.
- Puchyr, R. F. and Shapiro, R. (1986). Determination of trace elements in foods by HCl, HNO₃ leaching and flame atomic absorption spectroscopy. *J. AOAC.* 69 (5): 868- 870.
- Ramanauskas, R. (1971). [Effect of salt on the development of cheese consistency. (In Proceedings of Inter-University Dairy Conference.)) In *Sbornik dokladov mezhvuzovskoi konferentsii po molochnomu delu.* 271- 273. [CDROM] Available: In *Food Sci. Technol. Abstracts* Item: 72-05-P0807.
- Ramanauskas, R., and Pesetskias, D. (1976). [Effect of salt on the ripening of Dutch cheese.] Trudy,-Litovskii-Filial-Vsesoyuznogo-Nauchno-issledovatel'skogo-Instituta-Maslodel'noi-i-Syrodel'noi-Promyshlennosti, 10: 107- 120. [CDROM] Available: In *Food Sci. Technol. Abstracts* Item: 77-07-P1140.

- Ramazanov, I. U., Adzhiev, S. A., Durova, E. I., El'kanova, S. M. (1982). [Intensification of lactic fermentation in pickled cheeses]. Severo-Kavkazskii Filial Vses. Nauchno-issled. Inst. Maslodel'noi i Syrodel'noi Promyshlennosti, Stavropol', USSR pp. 15-18, 97. [CDROM], Available: In *Food Sci. Technol. Abstracts* Item: 85-08-P0077.
- Reddy, M. S. (1984). Method of growing cheese starter microorganisms. *PCT-Intl. Patent Appl.* No. WO8404106A1.
- Renaud, S. and de Lorgeril, M. (1992). Wine, alcohol platelets and the french paradox for coronary heart disease. *Lancet* 339: 1523.
- Richardson, G. H. (1985). Standard methods for the examination of dairy products. Fifteenth ed., Publ. *Amer. Public Health Assoc. Inc.*, Washington, DC.
- Richmond, M. L., Barfuss, D. L., Harte, B. R., Gray, J. I., and Stine, C. M. (1982). Separation of carbohydrates in dairy products by high performance liquid chromatography. *J. Dairy Sci.* 65 (8): 1394- 1400.
- Robinson, R. K. (2002). Dairy microbiology handbook, ed. Robinson, R. K., Publ. Wiley Interscience, NY, USA.
- Rowney, M., Roupas, P., Hickey, M. W., and Everett, D. W. (1999). Factors affecting the functionality of mozzarella cheese. *Aust. J. Dairy Technol.* 54 (2): 94- 102.
- Rudan, M. A., and Barbano, D. M. (1998). A model of mozzarella cheese melting and browning during pizza baking. *J. Dairy Sci.* 81: 2312- 2319.
- Rudan, M. A., Barbano, D. M., Guo, M. R., and Kindstedt, P. S. (1998). Effect of the modification of fat particle size by homogenisation on composition, proteolysis, functionality and appearance of reduced fat mozzarella cheese. *J. Dairy Sci.* 81: 2065- 2076.
- Salih, Y., McDowell, L. R., Hentges, J. F., Mason Jr, R. M., and Wilcox, C. J. (1987). Mineral content of milk, colostrums, and serum as affected by physiological state and mineral supplementation. *J. Dairy Sci.* 70 (3): 608- 612.

- Scott, F. W. (1992). HPLC determination of carbohydrates in *Food analysis by HPLC*. ed. Nollet, L. M. Publ. Marcel Dekker Inc., New York, USA.
- Scott, R. (1998). Cheese making practice. Third ed., Robinson, R. K., and Wilbey, R. A., ed., Publ. Aspen Publishers, Inc., Maryland, USA.
- Shehata, A. E., Iyer, M., Olson, N. F., and Richardson, T. (1967). Effect of type of acid used in direct acidification procedures on moisture, firmness and calcium levels of cheese. *J. Dairy Sci.* 50 (6): 824- 827.
- Shehata, A. E., Magdoub, M. N. I., Fayed, E. O., and Hofi, A. A. (1984). Effect of salt and capsicum tincture on the properties of pickled Domiati cheese. *Egyptian J. Dairy Sci.* 12 (1): 47- 54.
- Shekar, S., and Bhat, G. S. (1983). Influence of dissolved oxygen on acid production in buffalo milk by lactic cultures. *J. Food Protect.* 46 (4): 321- 324.
- Shepherd, R. and Stockley, L. (1987). Nutrition knowledge, attitudes, and fat consumption. *J. Amer. Diet. Assoc.* 87 (5): 615- 619.
- Shere, L. (1942). How to prevent and remove milk deposits. *Food Ind.* 14: 44- 46.
- Shive Kumar, and Mathur, B. N. (1989). Studies on the manufacture of yogurt and Mozzarella cheese from milk preserved by LP-system. *Indian J. Dairy Sci.* 42 (2): 194- 197.
- Shukla, D. C., Ladkani, B. G. (1989a). Process optimisation of direct acid Mozzarella cheese from buffalo milk. *Asian J. Dairy Res.* 8 (2): 90- 94.
- Shukla, D. C., Ladkani, B. G. (1989b). Quality of direct acid Mozzarella cheese from buffalo milk. *Indian J. Dairy Sci.* 42 (3): 601- 605.
- Sigsgaard, P. (1994). Defined cultures for pizza cheese. *Scand. Dairy Info.* 8 (3): 36- 38.
- Singh, H., and Fox, P. F. (1987). Heat stability of milk: role of β -lactoglobulin in the pH dependent dissociation of micellar κ -casein. *J. Dairy Res.* 54: 509- 521.
- Solarza, F. J. and Bell, A. E. (1995). Effect of calcium, fat and total solids on the rheology of a model soft cheese system. *J. Soc. Dairy Technol.* 48: 133- 139.

- Solchaga, M., Montoro, R. and de la Guardia, M. (1986). Flame absorption spectroscopic determination of cadmium, copper, iron, lead and zinc in mussels. *J. AOAC*. 69 (5): 874-876.
- Stephen, A. M. (1995). Are high carbohydrate intakes healthy? *Carbohydrates and Health-New Insights*. Sydney, Australia: ILSI, 1995.
- Stephen, A. M., Sieber, G. M., Gerster, Y. A., Morgan, D. R. (1995). Intake of carbohydrate and its components - international comparisons, trends over time and effects of changing to low fat diets. *Am. J. Clinical Nutr.* 62: 851S- 867S.
- Subramanian, P., Malik, R. K., and Mathur, D. K. (1992a). Protein breakdown in Cheddar cheese made from buffalo milk. I. Total and soluble nitrogen fractions. *Indian J. Dairy Sci.* 45 (7): 360- 365.
- Subramanian, P., Malik, R. K., and Mathur, D. K. (1992b). Protein breakdown in Cheddar cheese made from buffalo milk. II. Polyacrylamide gel electrophoretic profile. *Indian J. Dairy Sci.* 45 (7): 366- 369.
- Summerkamp, B. and Hisser, M. (1990). Fat substitute update. *Food Technol.* (3): 92- 97.
- Swaisgood, H. E. (1992). Chemistry of the caseins. In *Advanced Dairy Chemistry*, Vol. 1. Proteins, ed. Fox, P. F., Publ. Elsevier Sci. Publishers, Essex, England.
- Szczesnial, A. S. (1963). Classification of textural characteristics. *J. Food Sci.* 28 (4): 385-389.
- Taylor, C. M., Macleod, G., Rose, M. S. (1956). Foundation of nutrition, MacMillon Co., USA, Fifth ed., pp. 390- 391.
- Toppino, P. M., Nani, R., Contarini, G., Emaldi, G. C. (1988). Cheese product similar to stretched curd cheeses. *Industria Latte* 24 (1): 45- 53.
- Tranchant, C. C., Dalgleish, D. G. and Hill, A.R. (1998). Coagulation behaviour of differently acidified and renneted milk and the effects of pre-treatment of milk. A poster presented at *Food Science at work 1999*, Univ. of Guelph, Ontario, Canada.

- Tranchant, C. C., Dalglish, D. G. and Hill, A.R. (2002). Milk gel formation by combined acidification and renneting: New insights into coagulation dynamics, gelation phenomena and gel properties. A poster presented at the *IFT Annual Meeting* in June 2002, Anaheim, California, USA.
- Tunick, M. H. (1994) Effects of homogenisation and proteolysis on free oil in mozzarella cheese. *J. Dairy Sci.* 77 (9): 2487- 2493.
- Tunick, M. H., Holsinger, V. H., Mackey, K. L. and Smith, P. W. (1991). Effects of composition and storage on the texture of mozzarella cheese. *Neth. Milk Dairy J.* 45: 117- 125.
- Tunick, M. H., Mackey, K. L., Shieh, J. J., Smith, P. W., Cooke, P. and Malin, E. L. (1993a). Rheology and microstructure of low fat mozzarella cheese. *Int. Dairy J.* 3: 649- 662.
- Tunick, M. H., Malin, E. L., Shieh, J. J., Smith, P. W., Sullivan, B. C., Mackey, K. L. and Holsinger, V. H. (1993b). Proteolysis and rheology of low fat and full fat mozzarella cheeses prepared from homogenised milk. *J. Dairy Sci.* 76 (12): 3621- 3628.
- Tunick, M. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. (1995a). School lunch pizzas topped with low fat mozzarella cheese. *Cult. Dairy Prod. J.* 30 (2): 6- 9.
- Tunick, M. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. (1995b). Effects of skim milk homogenisation on proteolysis and rheology of mozzarella cheese. *Int. Dairy J.* 5: 483- 491.
- Tunick, M. H., Malin, E. L., Smith, P. W., Cooke, P. H. and Holsinger, V. H. (1995c). Influence of rennet source on proteolysis, rheology and microstructure of low fat mozzarella cheese. *J. Dairy Sci.* 78 (suppl.1): 101.
- Turner, K. W., Thomas, T. D. (1980) Lactose fermentation in Cheddar cheese and the effect of salt. *New Zealand J. Dairy Sci. Technol.* 15 (3): 265- 276.
- F.D.A (1989). *Code of Federal Regulations*, Title 21, Parts 100-169, US Department of Health and Human Sciences, Washington, DC.

- USDA (1991). Dairy situation and outlook yearbook. *US government printing office*. Washington, DC, USA, 17.
- van Hooydonk, A. C. M., Boerrigter, I. J. and Hagedoorn, H. G. (1986). pH induced physico-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physico-chemical properties. *Neth. Milk Dairy J.* 40: 281- 296.
- van Hooydonk, A. C. M., Boerrigter, I. J. and Hagedoorn, H. G. (1986). pH induced physico-chemical changes of casein micelles in milk and their effect on renneting. 2. Effect of pH on renneting of milk. *Neth. Milk Dairy J.* 40: 297- 313.
- Van Slyke, L. L. (1928). Chemistry of sour milk. *Technical Bulletin* no. 140, NY Expt. Station.
- Varnam, A. H., and Sutherland, J. P. (1994). Milk and milk products: Technology, chemistry and microbiology. Vol. 1. Publ. Chapman and Hall, London, U.K.
- Verruma-Bernardi, M. R., Damasio, M. H., do Valle, J. L. E., and de Oliveira, A. J. (2000). Mozzarella cheese of buffalo's milk elaborated by traditional and direct acidification methods. *Ciencia Tecnologia Alimentos* 20 (2): 138- 144.
- Vogel, A. 1989. Textbook of quantitative chemical analysis. Fifth ed., Publ. Longman and John Wiley and Sons, NY, USA.
- Walstra, P., Guerts, T. J., Noomen, A., Jellema, A., and van Boekel, M. A. J. S. (1999). Dairy technology: Principles of milk properties and processes. Publ. Marcel Dekker, Inc., New York, USA.
- Walstra, P., Noomen, A. and Guerts, T. J. (1987). Cheese: Chemistry, Physics and Microbiology, vol. 2, Fox, P. F. ed. *Elsevier applied science publishers*, London, 45-92.
- Welz, B. (1985). Atomic absorption spectrometry, VCH publishers, Weinheim, FRG.
- Wenner, V. R. (1958). Rapid determination of milk salts and ions. I. Determination of sodium, potassium, magnesium and calcium by flame spectrophotometry. *J. Dairy Sci.* 41 (6): 761- 768.

- Whelan, R. H., and Conant, F. R. (1979). Cheese food product. *US Patent*. No. 4143175. CPC Intl. Inc.
- Willis, J. B. (1965). Nitrous oxide- acetylene flame in atomic absorption spectroscopy. *Nature* 207: 715- 716.
- Winterer, H. (1986). Coliforms in semi-hard cheese. *Milchwissenschaft* 37 (22): 661- 664, 669- 670.
- Wong, N. P., Lacroix, D. E. and Vestal, J. H. (1977). Trace minerals in cottage cheese. *J. Dairy Sci.* 60 (10): 1650- 1652.
- Wong, N. P., Lacroix, D. E. Mattingly, W. A., Vestal, J. H. and Alford, J. A. (1975). The effect of manufacturing variables on the mineral content of cottage cheese. *J. Dairy Sci.* 59 (1): 41- 44.
- Woolfe, J. A. (1987). The potato in the human diet. Publ. Cambridge Univ. Press, Cambridge, U.K.
- Wursch, P. (1989). Starch in human nutrition. *World Rev. Nutr. Diet.*, 60: 199- 256.
- Yo Shen, C., Steele, J. L. (1998). Genetic characterization and physiological role of endopeptidase O from *Lactobacillus helveticus* CNRZ32. *Appl. Envir. Microbiol.* 64 (9): 3411- 3415.
- Youn. H. H., Yun, J. J., Barbano, D.M., and Kindstedt, P. S. (1998). Impact of three commercial *Lactobacillus* culture strains on Mozzarella cheese yield. *Aust. J. Dairy Technol.* 53 (3): 170- 174.
- Yuksel, G. U., and Steele, J. L. (1996). DNA sequence analysis, expression, distribution, and physiological role of the Xaa-prolyldipeptidyl aminopeptidase gene from *Lactobacillus helveticus* CNRZ32. *Appl. Microbiol. Biotechnol.* 44 (6): 766- 773.
- Yun, J. J., Barbano, D. M. and Kindstedt, P. S. (1993). Mozzarella cheese: Impact of milling pH on chemical composition and proteolysis. *J. Dairy Sci.* 76 (12): 3629- 3638.

- Yun, J. J., Barbano, D. M., Kiely, L. J., and Kindstedt, P. S. (1995). Mozzarella cheese: impact of rod:coccus ratio on composition, proteolysis, and functional properties. *J. Dairy Sci.* 78 (4): 751- 760.
- Yun, J. J., Barbano, D. M., Kindstedt, P. S. and Larose, K. L. (1995). Mozzarella cheese: Impact of whey pH at draining on chemical composition, proteolysis and functional properties. *J. Dairy Sci.* 78 (1): 1- 7.
- Yun, J. J., Hsieh, Y. L., Barbano, D. M. and Kindstedt, P. S. (1994). Draw pH and storage affect rheological properties of mozzarella cheese. *J. Food Sci.* 59: 1302- 1304.
- Zerfiridis, G. K., Alichanidis, E., Tzanetakis, N. M. (1989). Effect of processing parameters on the ripening of Teleme cheese. *Lebensmittel Wissenschaft Technol.* 22 (4): 169- 174.
- Zittle, C. A. (1957). Binding of calcium and phosphate ions to casein at pH 5 to 7.5. *J. Dairy Sci.* 40: 597 (Abstract).
- Zucchetti, S. and Contarini, G. (1993). AAS determination of calcium, sodium and potassium in dairy products using TCA [trichloroacetic acid] for extraction. *Atomic Spectroscopy* 14 (2): 60- 64.

15.0 APPENDICES

Quality Assurance Report

Type	Naturen 145
Batch	1453499
Activity	140.52 IMCU/ml
Nata Certificate	125

Micro Test Methods Used

Total plate count	A.S. 1766 2.1.1
Coliforms	A.S. 1766 2.3
Yeast & Mould	A.S. 1766 2.2
Salmonella	A.S. 1766 2.5
C.P. Staphylococci	A.S. 1766 2.4
Listeria Monocytogenes	A.S. 1766.2.15
Lactobacilli	A.S. 1766.2.6
Salt Tolerant Bacteria	Internal Procedure E09

Microbiological Results

	<u>Count</u>
Total Count per mL on PCA	< 01
Yeast per mL on MA	< 10
Mould per mL on MA	< 10
Coliforms per mL	Absent
Salt Tolerant Bacteria per mL	< 10
Gas Producing Lactobacilli per mL on ROGOSA	< 10
* Listeria Monocytogenes per 25mL	Absent
* Salmonella per 25mL	Absent
* Staph. Aureus per mL	Absent

Chemical Composition

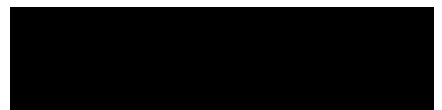
Salt	18.0%
Sodium Benzoate	0.5%
pH Value	5.59
Chymosin: Bovine Pepsin	92.8:7.2%
Density	1.144

Halal Vell Origin & Storage

Abattoir	S.B.A Foods Pty Ltd
Est Number	761
AFIC Personnel	Arif Uyanik
Date of Despatch	15/11/96, 17/02/97
Storage Location :	SA Cold Stores

This is to certify that the above product has complied with established Quality Standards and that an independant NATA laboratory has verified pathogenic results indentified by *

Tested by :



Date Release : 24/12/1999

Best Before Date : 15/12/2000

CHR HANSEN

1453499

Chr. Hansen Pty Ltd
49 Barry Street
PO Box 591
Bayswater Vic 3153

Phone : (03) 9 762 9600
Fax : (03) 9 762 9700

THE AUSTRALIAN FEDERATION OF ISLAMIC COUNCILS INC.

66-68 Jeffcott Street, West Melbourne, Victoria 3003.

P.O. Box 4319 MELBOURNE, 3001.

Telephones: (03) 329 1228 (03) 328 2067

HALAL MEAT TRANSFER CERTIFICATE

TOIR TO BONING ROOM, COLD STORAGE OR PROCESSING WORKS

To: South Aust cold Store Est 1467

Dated: 15/11/96

Number and weight of carcasses/cartons.

Number	Weight	Description	Aust. Approved Stamp
<u>14</u>	<u>340.6</u>	<u>CALF VEAL</u>	

R. J. Gilbertson (Southern)

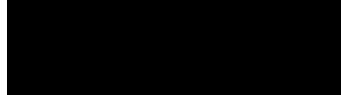
Est No 761

Slaughter 03/10/96 to 23/10/96

Container No.

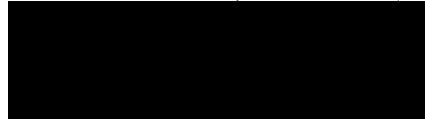
Seal No next applicable

John Mason

Signature: 

Personnel: Abattoir

Mr. Uxmark

Signature: 

ID Card No. 158

Date 15/11/96

RECEIVED BY:

Personnel Boning Room/Cold Storage/

Card No.

Signature

Time

Date

This certificate is required by A.F.I.C. Office accompanying the Interim Certificate when loading out the product from the above mentioned Halal establishment.

Any discrepancy in this certificate will result in the rejection of the endorsement.



AMYLOGLUCOSIDASE (*A. niger*) (Cat. No. E-AMGDF)

PROPERTIES

1. ELECTROPHORETIC PURITY

- Single band on Isoelectric focusing (pI ~ 4.0).
- Single band on SDS-gel electrophoresis (MW ~ 118,000)

2. SPECIFIC ACTIVITY AND LEVELS OF OTHER ACTIVITIES

SUBSTRATE	ACTIVITY (U/ml)
Starch (amyloglucosidase)	3260
p-Nitrophenyl β -maltoside	200
Maltose	380
Ceralpha Reagent (for the measurement of α -amylase)	120
Barley Beta-Glucan (cellulase)	0.05
Wheat arabinoxylan (β -xylanase)	0.008

3. PHYSICOCHEMICAL PROPERTIES

pH Optima	4.0
pH Stability	4.-5.5
Temperature Optima	70°C
Temperature Stability	<60°C

4. STORAGE CONDITIONS

The enzyme is supplied in vials of 40 ml, as a solution in 50% glycerol plus sodium benzoate (0.2%), and should be stored at 4°C.

This enzyme is recommended for use in **Total Dietary Fibre** analytical procedures and the **Megazyme Total Starch** test method. The preparation is effectively devoid of cellulase and is free of catalase.

Certificate of Analysis

SIGMA ALDRICH PTY LTD (T)
#2, 10 ANELLA AVENUE
CASTLE HILL 2154

PO NBR: 6394

PRODUCT NUMBER: W261106-SPEC

LOT NUMBER: 14115AU

PRODUCT NAME: LACTIC ACID, 85%, FCC

FORMULA: C₃H₆O₃

FORMULA WEIGHT: 90.08

APPEARANCE

VISCOUS COLORLESS LIQUID

REFRACTIVE INDEX AT
20 DEG C

1.4260

INFRARED SPECTRUM

CONFORMS TO STRUCTURE AND STANDARD AS
ILLUSTRATED ON PAGE 517C OF EDITION I,
VOLUME 1 OF "THE ALDRICH LIBRARY OF FT-IR
SPECTRA".

TITRATION

87.4 % (WITH HCL)

MISCELLANEOUS ASSAYS

PASSES SUGARS TEST

RESIDUE ON IGNITION

0.05% (15 MINUTES, 800 DEGREES C)

COLOR TEST

0<APHA<10

ACIDITY

PASSES TEST

CHLORIDE

<0.1%

IRON

<10 PPM

TRACE HYDROCYANIC ACID

<5 PPM

HEAVY METALS

<10 PPM

CONTINUED ON NEXT PAGE

ALDRICH CHEMICAL COMPANY
DAVID SWESSEL

JULY 12, 2000

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.

Aldrich brand products are sold exclusively through Sigma-Aldrich, Inc.

Organics and Inorganics for Chemical Synthesis.

We are Committed to the Success of our Customers through Science, Technology and Service.

Certificate of Analysis

SIGMA ALDRICH PTY LTD (T)
#2, 10 ANELLA AVENUE
CASTLE HILL 2154

PO NBR: 6394

PRODUCT NUMBER: W261106-SPEC

LOT NUMBER: 14115AU

PRODUCT NAME: LACTIC ACID, 85%, FCC

FORMULA: C₃H₆O₃

FORMULA WEIGHT: 90.08

CONTINUED FROM PREVIOUS PAGE

LEAD <5 PPM

SULFATE <0.25%

MEETS FCC-IV SPECIFICATIONS

QUALITY CONTROL
ACCEPTANCE DATE FEBRUARY 1999

ALDRICH CHEMICAL COMPANY
DAVID SWESSEL

~~JULY 18, 2000~~
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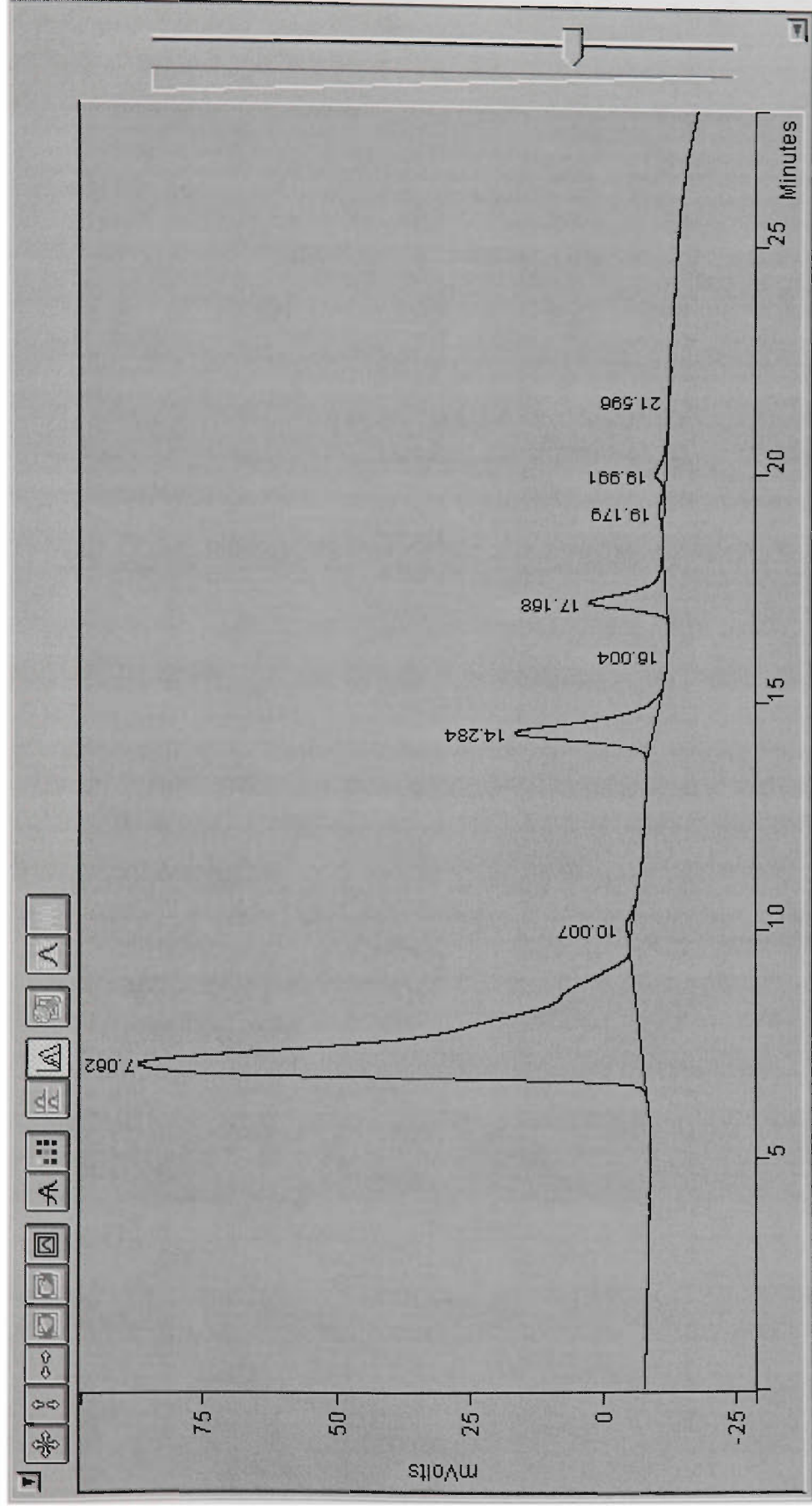


Figure Appendix 1. The peaks for oligosaccharides that were measured using HPLC for 0.25% M100.

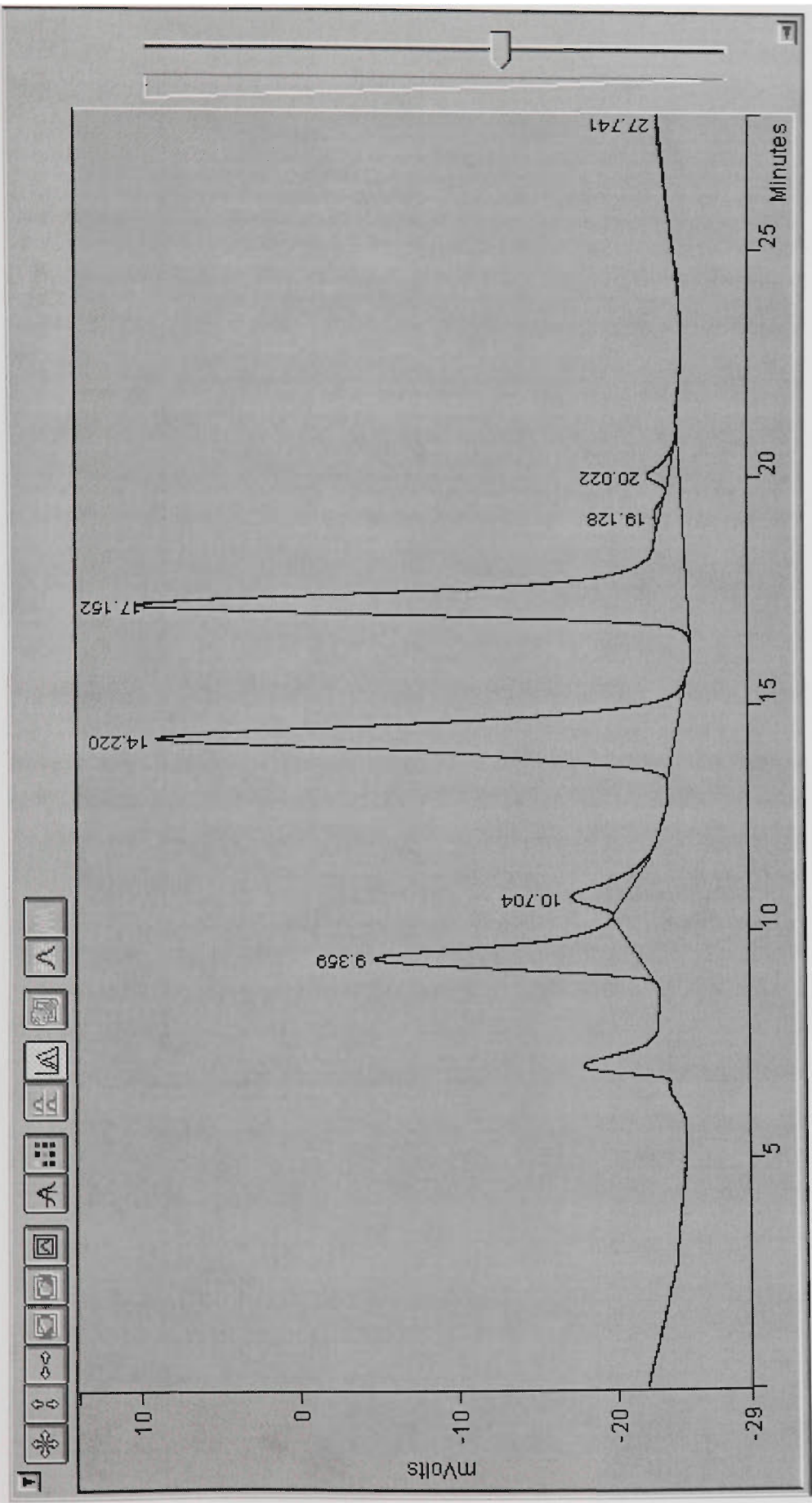


Figure Appendix 2. The typical peaks for oligosaccharides that were measured using HPLC for M100 based mozzarella cheese.

