THE IMPROVEMENT OF PHYSICAL PROPERTIES OF YOGHURTS BY VARYING CASEIN/WHEY PROTEIN RATIO AND EPS-PRODUCING STARTER CULTURES

A thesis submitted for the degree of Doctor of Philosophy

By

Thanut Amatayakul

B.SC., M.App.Sc. (Food Sci. Tech)

2005

School of Molecular Sciences

Victoria University, Werribee Campus, VIC, Australia

I. Abstract

The overall objective of this study was to investigate effects of variation in casein (CN) to whey protein (WP) ratios and types of exopolysaccharide (EPS)-producing starter cultures on physical properties of yoghurts made at 9% and 14% total solids during 28 days of storage at 4°C. There are three main parts of this research: a preliminary study, a comparative study of three methods for determination of syneresis and the studies on physical properties of yoghurts made with various CN to WP ratios using non-EPS- or EPS-producing starter cultures.

The preliminary study examined the physical properties of set yoghurts made at 12% total solids with reconstituted skim milk (RSM) or RSM supplemented with 0.5% (w/w) of whey protein concentrate (WPC, 80% w/w total proteins) using non-EPS, EPS (ropy)-producing starter cultures and co-culturing with non-EPS- and EPS-producing starter cultures. The use of EPS-producing starter cultures or co-culturing decreased the firmness, apparent viscosity and susceptibility to syneresis, but increased the yield stress of set yoghurts. The products supplemented with 0.5% WPC showed an increase in firmness and EPS concentration, but reduced syneresis as compared to those made with RSM. A further increase in EPS concentration was observed upon co-culturing of non-EPS- and EPS-producing starter cultures in RSM containing 0.5% WPC. The viable counts of *L. delbrueckii* ssp. *bulgaricus* were lower in products supplemented with WPC than those made using RSM only.

However, it was unclear as to whether the changes in physical properties of set yoghurts supplemented with WPC were due to the increase in total protein content or due to a decrease in the ratio of CN to WP.

The centrifugation-, drainage- and siphon methods (a new method developed based on Lucey *et al.*, 1998c) were used to compare the level of syneresis in set yoghurts produced at 9% and 14% solids contents using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures. The level of syneresis determined by each method was different ranging from 0 to 8% (w/w) for the siphon method, from 10 to 40% for the drainage method and from 40 to 80% for the centrifugation method. It is interesting to note that the results from the drainage method did not show any significant difference of the level of syneresis between products. Based on the results, the siphon method appeared to represent the level of spontaneous syneresis of products and, therefore, it was chosen for future studies.

For further studies on the physical properties of yoghurts as affected by the combined use of the alteration in CN to WP ratio and non-EPS- or EPS-producing starter cultures, SMP, WPC 80 and lactose monohydrate were blended at suitable quantities to vary the ratios of CN to WP to 3:1, 2:1 and 1:1 without affecting total solids or protein levels of the resultant milk blends. RSM was regarded as the milk at the CN to WP ratio of 4:1. The protein profiles of heated and unheated milk examined by using both native-polyacrylamide gel electrophoresis (PAGE) and

sodium dodecyl sulphate (SDS)-PAGE under reducing conditions suggested that the denaturation of WP was not affected by a decrease in CN to WP ratio. However, the level of soluble denatured whey protein decreased with reducing CN to WP ratios. This suggested an increasing trend of denatured whey protein associated on the surface of casein micelles as the CN to WP ratio was decreased. An increase in the proportion of WP did not affect the viable counts of *S. thermophilus,* however those of *L. delbrueckii* ssp. *bulgaricus* decreased. The fermentation time, storage modulus (G') at the end of fermentation (6 h), representing gel strength, and firmness of yoghurt gels stored overnight (4°C) decreased with a reduction in the CN to WP ratio. The storage modulus (G')-time curve also showed that milk blended to lower ratios of CN to WP started to form gel earlier than that blended to higher ratios. The decrease in G' and the firmness of products as the CN to WP ratios was reduced is likely to be due to the complex of WP with casein preventing casein cluster formation and resulting in weaker or softer yoghurt gels.

No decline in EPS concentration during 28 days of storage was observed in products made at 9% solids with capsular EPS-producing starter cultures. The increase in EPS concentration during storage was only observed in the product made with ropy EPS-producing starter cultures with the highest concentration observed at the CN to WP ratio of 3:1 at day 21 (~ 75 mg L^{-1}). The viable counts of both starter cultures decreased during storage. However, the total counts were still higher than 10^6 cfu g^{-1}. The lactic acid concentration decreased from ~0.90% to ~0.60% (w/w) as the ratios of CN to WP were reduced, regardless of the type of

starter cultures used. There was no increase in the concentration of lactic acid during storage. The firmness and syneresis of set yoghurt decreased when the CN to WP ratios were reduced from 4:1 to 1:1 as well as with the EPS-producing starter cultures. No change in these properties was observed during storage. Stirred yoghurts produced with the CN to WP ratio of 3:1 and ropy EPS-producing starter cultures had higher apparent viscosity and the area of hysteresis loop between upward and downward flow curves than those made using capsular EPS- and non-EPS-producing starter cultures. These two parameters increased during storage.

As the total solids of yoghurts were increased from 9% to 14%, there were similar patterns of results including a decrease in lactic acid concentration, firmness and syneresis in set yoghurts with a reduction in the CN to WP ratio, and an increase in apparent viscosity and the area of hysteresis loop between upward and downward curves in stirred yoghurts made with ropy EPS-producing starter cultures as compared to those with non-EPS- or capsular EPS-producing starter cultures. The viable counts of starter cultures also decreased during the storage period. However, there was a significant increase in firmness, apparent viscosity, hysteresis loop area, concentration of lactic acid and EPS in yoghurts made at 14% solids as compared to those at 9%. Only a significant decrease in the level of syneresis was observed in the product made at 14% with the CN to WP ratio of 4:1 using non-EPS-producing starter cultures as compared to that at 9%. The firmness in set yoghruts made at 14% solids level using EPS-producing starter cultures (both capsular and ropy) did not decrease as observed in those at 9% products.

The confocal scanning laser microscope (CSLM)-micrographs of set yoghurts made at 9 and 14% solids with various CN to WP ratios using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures revealed that the density of protein matrix increased with increasing the solids content and reduction in the CN to WP ratios. The micrographs of products made at 9% solids with ropy EPS-producing starter cultures showed irregular shape of pore with larger pore size as compared to those with non-EPS- or capsular EPS-producing starter cultures. As the total solids of yoghurts were increased to 14%, there was little variation in pore size among products made with different starter cultures. The cryo-scanning electron microscope (SEM)- micrographs appeared to show less artefacts of EPS than those with conventional SEM. The cryo-SEM micrographs showed that those pores were filled with sheets of EPS.

Based on results obtained, it is possible that the physical properties of yoghurts can be maintained at an acceptable level by optimizing CN to WP ratios and using EPS-producing starter cultures. Set yoghurts should be made at 14% or higher total solids at a CN to WP ratio of 3:1 with ropy EPS-producing starter cultures with regard to syneresis and firmness. The use of ropy EPS-producing starter cultures was found to be suitable in the production of stirred yoghurt.

II. Certificate

Dr. Nagendra P. Shah (M.Sc., Ph.D)

Professor – Food Technology

School of Molecular Sciences,

Victoria University

Werribee Campus, Visctoria, Australia

CERTIFICATE

This is to certify that the thesis entitled "THE IMPROVEMENT OF PHYSICAL PROPERTIES OF YOGHURTS BY VARYING CASEIN/WHEY PROTEIN RATIO AND EPS-PRODUCING STRARTER CULTURES" submitted by Thanut Amatayakul in partial fulfillment of the requirement for the award of the Doctor of Philosophy in Food Technology at 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: 29. 8. 2005

(Prof. N. P. Shah)

Thesis Supervisor

III. Declaration

I delare that this thesis consists of my original work. Information from published sources has been clearly acknowledged withih the thesis. None of the work contained in this thesis has been submitted to any other university. I confirm that this thesis does not exceed 100,000 words.

Werribee, Australia

Date: Thanut Amatayakul

IV. Ackowledgement

I would like to thank my principal supervisor, Professor Nagendra P. Shah, School of Molecular Sciences, Faculty of Science, Engineering and Technology, Victoria University of Technology, Werribee Campus, Werribee, and my co-supervisor, Dr. Frank Sherkat, School of Applied Sciences-Food Science, RMIT University, City Campus, Melbourne, for their guidance and valuable discussions and suggestions throughout the course of study and in preparation of manuscripts.

I express my sincere thanks to Professor Andrew L. Halmos, School of Applied Sciences-Food Science, RMIT University, City Campus, Melbourne, for the permission to use Food Science equipment and valuable discussions about textural and rheological measurements of yoghurts. I would also like to thank Mr. Michael Kakoullis, Laboratory Manager, and Miss Katrina O'Reilly, Laboratory Technician, at the Department of Food Science, RMIT University, City Campus, for the technical support. I am thankful to Mr. Stephen Firth, Manager of Centre for Cellular and Molecular Biology, School of Biological and Chemical Science, Deakin University, Burwood, Victoria, Australia for the assistance in the use of CSLM, and Mr. Phil Francis, Senior Science Officer, Department of Applied Physics, RMIT University, City Campus, Melbourne, Victoria, Australia, for the assistance in the use of conventional and cryo-SEM.

I also like to thank Mr. Dele Tomlinson, Laboratory Manager, and all laboratory technicians at the School of Molecular Sciences, Victoria University of Technology, for their assistance, although it took quite a while in ordering chemicals.

I appreciate the great friendship I have made during my research at Victoria University. Special thanks go to Mr. Bogdan Zisu for his assistance and discussion. I also like to thank Mr. Raman K. Bhaskaracharya, Mr. Ankur Desai, Mr. Dimitri Tsangalis, Mr. Frank Bruno, Mrs. Mintze Liong, Miss Lydia Ong, Miss Chutima Kongjaroon, Mr. Paul Capella, Mr. Goran Gambiroza, Miss. Belinda Davis, Mr. Idris Mohammed and others whose names may have escaped my memory.

I would like to express my profound gratitude to my family for their support. I deeply appreciate the financial sacrifices they have made for my Ph.D study in Australia. I owe to my father who initiated the thought of my Ph.D study.

Werribee, Australia

Date: Thanut Amatayakul

V. List of Publications

Journal Publications

Amatayakul, T., Halmos, A.L., Sherkat, F., & Shah, N.P. 2005. Physical properties of yoghurt made by varying casein to whey protein ratios and using exopolysaccharide-producing starter cultures. *International Dairy Journal.* (in press)

Amatayakul, T., Sherkat, F., & Shah, N.P. 2005. Microstructure and syneresis of yoghurt made by varying casein to whey protein ratios and using exopolysaccharide-producing starter cultures during storage. *Food Hydrocolloids* (under review).

Amatayakul, T., Sherkat, F., & Shah, N.P. 2005. Physical characteristics in set yoghurt made at 9% (w/w) and 14% (w/w) total solids with varying casein to whey protein ratios and types of EPS-producing starter cultures. *Food Hydrocolloids* (under review).

Amatayakul, T., Sherkat, F., & Shah, N.P. 2005. Physical characteristics of set yoghurts as affected by co-culturing with non-EPS- and EPS-producing starter cultures and supplementation with WPC. *The Australian Journal of Dairy Technology* (in press).

Amatayakul, T., Sherkat, F., & Shah, N.P. 2005. Syneresis in set yoghurt as affected by EPS-producing starter cultures and levels of solids. *International Journal of Dairy Technology* (under review).

Chaired Oral presentations

Amatayakul, T., Sherkat, F., & Shah, N.P. 2003. Improving physical properties of yoghurts by varying whey protein ratios and using exopolysaccharide starter cultures. Presented at **the 36th annual convention of the Australian Institute of Food Science and Technology, 27 August – 2 September, Melbourne, Australia**

Amatayakul, T., Sherkat, F., & Shah, N.P. 2004. Microsturacture of yoghurt made by varying casein to whey protein ratios and using exopolysaccharide-producing starter cultures. Presented at **the 37th annual meeting of the Australian Institute of Food Science and Technology, 25 – 28 July, Brisbane, Australia**

Amatayakul, T., Sherkat, F., & Shah, N.P. 2004. Effects of varying casein to whey protein ratios and types of starter cultures on physical properties of set yoghurt made at low and high total solid. Presented at **the 7th International Hydrocolloids Conference, 29 August – 1 September, Melbourne, Australia**

Chaired Poster presentations

Amatayakul, T., Sherkat, F., & Shah, N.P. 2003. Effects of co-culturing EPS and non-EPS-producing starter cultures and supplementation with WPC on syneresis, textural and rheological properties of set yoghurt. Presented at **the annual meeting of the American Dairy Science Association, June 22-26, Phoenix, USA**

Amatayakul, T., Sherkat, F., & Shah, N.P. 2004. Investigating physical properties of yoghurts made by varying casein to whey protein ratios and using EPS-producing starter cultures during storage periods. Presented at **the 44th annual meeting of Institue of Food Science and Technology, 12 – 16 July, Las Vegas, USA**.

Amatayakul, T., Sherkat, F., & Shah, N.P. 2004. Microstructure of yoghurt made by using varying casein to whey protein ratios and using exopolysaccharide-producing starter cultures during storage. Presented at **the 37th annual meeting of the Australian Institute of Food Science and Technology, 25 – 28 July, Brisbane, Australia**

Table of Contents

Chapters **Page**

List of Tables

Tables **Page**

List of Figures

Figures **Page**

List of Abbreviations

AOAC = Association of Official Analytical Chemists

ANOVA = analysis of variance

AUD = Australian dollar

BSA = bovine serum albumin

cfu = colony forming unit

CN = casein

CSLM = confocal scanning laser microscope

Da = Dalton

DWS = diffuse wave spectroscopy

EPS = exopolysaccharide

g = gram

G' = storage modulus

G" = loss modulus

G* = complex modulus

GDL = glucono-delta-lactone

h = hour

HCl = hydrochloric acid

H_2SO_4 = sulphuric acid

Ig = immunoglobulin

LB = *Lactobacillus delbrueckii* ssp. *bulgaricus*

L = litre

mL = millilitre

M17 agar = agar for enumeration of *Streptococcus thermophilus*

min = minute

mm = millimetre

MPa = megapascal

MPC = milk protein concentrate

MRS agar = DeMan Rogosa sharpe agar / for enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus*

MW = molecular weight

N = Newton

rpm = revolution per minute

RSM = reconstituted skim milk

Pa = pascal

PAGE = polyacrylamide gel electrophoresis

s = second

SDS = sodium dedocyl sulphate

SEM = scanning electron microscope

SMP = skim milk powder

ST = *Streptococcus thermophilus*

tan δ = loss tangent

TCA = trichloroacetic acid

TEM = transmission electron microscope

V = volts

v/v = volume per volume

w/w = weight per weight

WP = whey protein

WPC = whey protein concentrate

WPI = whey protein isolate

x g = times gravitational force

α-La = alpha-lactalbumun

β-Lg = beta-lactoglobulin

κ-CN = kappa-casein

μ = micro

μg = microgram

μL = microlitre

°C = degree Celsius

1.0 INTRODUCTION

A set yoghurt should be smooth, firm without syneresis or wheying-off and without a gel breakage on the surface and stirred yoghurt should be smooth and viscous (Lucey, 2004). These physical properties of yoghurts can be improved by supplementation with dairy ingredients to increase the solids level of milk and the use of exopolysaccharide (EPS)-producing starter cultures (Tamime and Deeth, 1986; Shah, 2003).

According to the statistics of export dairy product from Dairy Australia (2005) during 2003-04/June-April, the price of exported SMP is $AUD 2.4/kg, while the price of exported whey products, including WPC, is $AUD 1/kg. The supplementation of WHC should result in a significant reduction in production costs. It is inevitable that the supplementation will change the ratios of casein (CN) to whey protein (WP) depending on the type of ingredients used. Puvanenthiran *et al.* (2002) reported an improvement in gel strength and syneresis in set yoghurt made with decreasing CN to WP ratios. The alteration in CN to WP ratios may be the actual reason that leads to an improvement in the physical properties of yoghurts. The whey flavour is another major issue in supplementing WPC in yoghurt. A reduction in supplemented whey protein while maintaining physical properties of yoghurts at an acceptable level may be possible by optimizing the CN to WP ratios and use of EPS-producing starter cultures.

This study aimed at investigating the combined effects of varying CN to WP ratios (4:1, 3:1, 2:1 and 1:1) and use of EPS-producing starter cultures (capsular and ropy) on physical properties (firmness, syneresis, apparent viscosity) of yoghurts made with reconstituted nonfat milk blends with controlled total solids and protein contents. The textural and rheological characteristics as well as the microstructure of set and stirred yoghurts made at 9% and 14% solids contents were examined using a texture analyzer, a rheometer, a scanning electron microscope (SEM) and a confocal scanning laser microscope (CSLM).

This thesis starts with the literature review in Chapter 2. Only concise and relevant literatures have been provided. The review covers yoghurt production and factors affecting its physical properties, EPS (structure, production, purification and quantification), the texture and rheology of yoghurts, syneresis, microscopy and artefacts of SEM and CSLM and, protein-polysaccharide interaction and its effects on yoghurts. Chapter 3 gives details of materials and methods as well as the experimental design and statistical analysis for subsequent chapters.

Chapter 4 reports a preliminary study on physical properties of set yoghurts as affected by co-culturing with non-EPS- and EPS-producing starter cultures and supplementation with whey protein concentrate (WPC). The supplementation with WPC changed the ratio of CN to WP. Some improvements in physical properties of yoghurt were observed. However, there were some difficulties in the interpretation of results as to whether those phenomena occured due to the changes in the CN to WP ratio or due to an increase in the total protein of products supplemented with WPC. Chapter 5 deals with a comparative study of three methods for the determination of syneresis in set yoghurts including the drainage, centrifugation and siphon methods for their suitability in determination of spontaneous syneresis. The siphon method was chosen for further study because it represented the spontaneous syneresis of samples. The difficulty in the interpretation of the results in Chapter 4, which were due to the increase in protein contents as a result of changes in CN to WP ratio, raised a need for a method that can be used for varying the CN to WP ratios without changing the total protein or total solids contents of the milk. Chapter 6 examines a method for varying CN to WP ratios of milk blends without affecting total solids or protein contents and its effects on yoghurt production as well as gel characteristics. The CN to WP ratios were varied to 4:1, 3:1, 2:1 and 1:1. The yoghurts were made using non-EPS-producing starter cultures. The composition, bacterial counts and the fermentation pattern, protein profiles before and after heat treatment and gel characteristics of set yoghurt were examined.

Chapters 7 and 8 report the results of chemical, microbiological and physical properties of set and stirred yoghurts made with non-EPS, capsular and ropy EPS-producing starter cultures and various CN to WP ratios (4:1, 3:1, 2:1 and 1:1) at 9% and 14% solids levels during 28 days of storage period at 4°C. The parameters of interest include bacterial counts, concentrations of EPS and lactic acid, firmness and syneresis of set yogurt and the apparent viscosity, flow curve and the area of hysteresis loop of stirred yoghurts. These parameters were investigated at day 1, 7, 14, 21 and 28. A reduction in CN to WP ratio, the use of EPS-producing starter cultures, storage period as well as the increase in solids levels from 9 to 14% affected physical properties of yoghurts.

The microstructure of set yoghurts studied in Chapters 7 and 8 was investigated using CSLM at day 1, 14 and 28 of storage (4°C) and reported in Chapter 9. The SEM was also used to observe the microstructure at day 1 of storage. The micrographs showed that the protein matrix was altered as a result of decreasing the CN to WP ratios and using EPS-producing starter cultures.

Chapter 10 concludes on results reported in Chapters 4 to 9. Chapter 11 discusses future research direction, followed by bibliography in Chapter 12 and appendix section.

2.0 LITERATURE REVIEW

2.1 Yoghurt

Yoghurt is defined as a fermented milk product made with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Shah, 2003; Tamime and Deeth, 1980). There are several types of yoghurts available in the market which can be classified according to the physical properties of gels (set-, stirred- and drinking yoghurt), the application of probiotic bacteria or prebiotics (probiotic- or prebiotic yoghurt), fat content (full-fat-, reduced fat- and low-fat- yoghurt) and the addition of flavour (such as sweeteners, fruits or flavouring). Furthermore, there are other variations of products including frozen-, concentrated-, dried-, low-calorie-, low-lactose- and pasteurized yoghurt (Tamime and Deeth, 1980; Shah, 2003). This review will concentrate on set and stirred yoghurts only, as these products have been used in this study.

2.2 Quality of yoghurt

The quality of yoghurt can be assessed through its chemical, microbiological and physical properties. In general, the chemical and microbiological parameters are controlled under food legislation of each country. For example, the Australian Food Standard Code (2004, standard 2.5.3 Fermented milk products) requires that the viable counts of yoghurt starter cultures be no less than 10^6 cfu g^{-1} of a product throughout the storage period. The protein content must be no less than 30 g kg^{-1}

while the pH of product must be less than 4.50. In some European countries, the use of stabilizers is prohibited (De Vyust and Degeest, 1999). However, there is no legal requirement for the physical properties of a product. In general, set yoghurt should be firm, smooth in texture, free from lump or graininess and spoonable without any syneresis on the surface of the product. Stirred yoghurt should be viscous and smooth in texture (Tamime and Robinson, 1999). These characteristics can be improved by a number of ways; these have been reviewed in Section 2.4. However, the assessment of physical parameters is very subjective. To date, methods have been used for assessing physical properties of yoghurts include sensory and instrumental assessments.

2.3 Yoghurt manufacture

There are four main steps in yoghurt manufacture as given below:

1) milk preparation
2) treatment before fermentation (homogenization and heat treatment)
3) inoculation and incubation
4) treatment and handling after incubation (stirring, cooling and storage).

All these steps affect physical properties of yoghurts.

2.3.1 Milk preparation

In order to achieve thick yoghurts with firm texture without syneresis, supplementation of liquid milk with dried dairy ingredients such as skim milk

powder (SMP), sodium (Na)-caseinate, calcium (Ca)-caseinate, whey protein concentrate (WPC), whey protein isolate (WPI), milk protein concentrate (MPC) is a common practice to increase the level of total solids to above 15% (Tamime and Deeth, 1980; Shah, 2003). Each ingredient affects physical properties of yoghurt differently. The effects of these dairy ingredients on physical properties of yoghurt are discussed in Section 2.4.3. In addition, other methods can be used for the elevation of milk solids content before yoghurt production including ultrafiltration, reverse osmosis and evaporation (Robinson and Tamime, 1993). The solids content in yoghurt can also be increased after fermentation by ultrafiltration as carried out by Tamime *et al.* (1991). Stabilizers such as gelatine, vegetable gums, dextran or xanthan gum are used to improve physical properties of yoghurt, particularly for preventing syneresis. However, further review will focus only on the supplementation with dairy ingredients.

2.3.2 Treatments before fermentation

2.3.2.1 Homogenization

Homogenization is an important treatment in the manufacture of full-fat yoghurt as it prevents the formation of a cream layer on the surface of the product during incubation. Homogenization breaks large fat globules of milk ranging from 1 to 15 μm in diameter to a more homogeneous size of 1 or 2 μm (Shah, 2003). Homogenization prevents fat globules from coalescence and forming a cream layer. There are other benefits of homogenization including improved viscosity, whiteness and mouth-feel of the product as well as increased coagulum stability. The process

is usually carried out after pasteurization. In addition, homogenization also reduces syneresis and disintegrates particles of added materials (dairy ingredients or stabilisers). The process is usually carried out at 60°C at 15 to 18 MPa by passing the milk through a small orifice of the homogenizer.

2.3.2.2 Heat treatment

For yoghurt making, heat treatment is usually carried out at 85°C for 30 min or 90°C for 10 min. The level of heat treatment is more severe than that used for pasteurization of milk. There are several benefits of using such a high level of heat treatment including:

1) destruction of all pathogenic and most of spoilage bacteria
2) inactivation of most enzymes which may cause undesirable effects to the finished product
3) creation of micro-aerophilic condition suitable for the growth of starter cultures by removal of oxygen from milk
4) conversion of calcium into a soluble form leading to a decrease in time for milk coagulation
5) improvement in firmness and syneresis of yoghurt as a result of whey protein denaturation (mainly β-lactoglobulin and α-lactoglobulin) (Lucey, 2002 and 2004).

2.3.2.3 Inoculation and incubation

The steps following heat treatment are inoculation and incubation. The heat treated milk is cooled to 42°C prior to inoculation. The level of inoculum of starter cultures can range from 0.5% to 5% (v/v). Although yoghurt can be produced in a short time due to rapid acid production when inoculated with a high level of starter culture (5%), this leads to defects in aroma and syneresis (Biliaderis *et al.*, 1992; Lucey, 2004). The optimum level is 1% each of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*. The inoculation is usually carried out at 42°C. As the optimum temperature for the growth of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* are 37°C and 45°C, respectively, the incubation at 42°C would favour the growth of both microorganisms. During incubation, associative growth between the two bacteria provides suitable growth conditions for each other. For example, glycine, valine and hystidine amino acids produced as a result of casein hydrolysis by *L. delbrueckii* ssp. *bulgaricus* have been shown to promote the growth of *S. thermophilus* (Bautista *et al.*, 1966; Tamime and Robinson, 1985; Shah, 2003), whereas the latter produces formic acid, which stimulates the growth of the former. Moreover, *S. thermophilus* starts to grow in the initial phase of the incubation producing acid and depleting oxygen from milk, thus making oxidation-reduction potential more favorable for *L. delbrueckii* ssp. *bulgaricus*. As the pH of milk drops to 5.40, *L. delbrueckii* ssp. *bulgaricus*, which is acid-tolerant, continues to grow and produce lactic acid until a desired pH in the product is reached. For a set yoghurt, the incubation is carried out in tubs, while for a stirred yoghurt it is carried out in bulk and the gel is then broken to provide a smooth and homogeneous gel. The end point of incubation may depend on the food standard of each country. The

Australian Food Standard Code requires that the pH value of yoghurts be no more than 4.50, while the US standard requires a minimum tritratable acidity of 0.90% as lactic acid.

2.3.2.4 Treatment and handling after incubation

Once the desired level of acidity has been achieved, the yoghurt is cooled to 4°C. In a stirred yoghurt, the set gel in the incubation tank is broken, followed by cooling and mixing with flavourings, fruits or sweeteners and packaging. Both types of yoghurts should be stored at a temperature of less than 10°C. During the first 48 h of storage, a distinct change occurs in the set yoghurt. The level of syneresis decreases as a result of the hydration of casein micelles (Shah, 2003; Lucey, 2004). In addition, the gel strength also increases as the temperature of the set yoghurt is decreased (Guyomarc'h *et al.*, 2003). Therefore, it is very important to store the product overnight at 4°C and avoid rough mechanical handling to prevent excessive syneresis.

2.4 Factors influencing physical properties of yoghurts

Many factors influence physical properties of yoghurts including type of ingredients, total solids and protein contents, types and level of starter culture, improper heat treatment and incubation temperature as well as mechanical handling and storage (Lucey, 2004). By controlling these factors, the textural properties and appearance

of yoghurt can be achieved at an acceptable level. Some of these factors are reviewed in this chapter including the influence of total solids and protein contents, heat treatment, fortification with dairy ingredients and exopolysaccharide (EPS)-producing starter cultures.

2.4.1 Total solids and protein contents

It is difficult to differentiate between the effect of an increase in the level of solids from that of the protein content on physical properties of yoghurt as a result of fortification. However, the main objective of supplementation is to prevent syneresis or whey separation on the surface of set yoghurts. In addition, supplementation also increases the firmness and viscosity of set and stirred yoghurts (Rohm and Schmid, 1993; Jaros *et al.*, 2002; Krasarkoopt *et al.*, 2004). Schkoda *et al.* (2001) reported that increasing protein content of skim from 3.5 to 7.0% resulted in an increase in gel firmness. Similar results have been reported by Biliaderis *et al.* (1992). They observed higher gel strength in yoghurts made from ultrafiltrated milk which contained the highest protein content than those supplemented with SMP. The yoghurts were produced at the same level of solids content. The results reported by Biliaderis *et al.* (1992) appeared to depend mainly on the level of protein content, but not on solids content. As shown in the microstructure of yoghurt (Kalab *et al.*, 1983; Puvanenthiran *et al.*, 2002; Remuef *et al.*, 2003; Hassan *et al.*, 1995a, 1995b, 2002b, an 2003b), the gel structure of yoghurt is mainly composed of protein network that entraps other constituents. Kalab *et al.* (1983) showed an increase in the density of protein matrix as the levels of total solids and protein contents were

increased. Harwalkar and Kalab (1986) also reported a decrease in syneresis as the density of protein matrix increased. The change in the structure of protein matrix as a result of increased total protein may be the reason for the reduction in syneresis or the increase in gel strength of yoghurt (Harwalkar and Kalab, 1986; Biliaderis *et al.*, 1992; Rohm and Schmid, 1993; Schkoda *et al.*, 2001; Jaros *et al.*, 2002; Krasarkoopt *et al.*, 2004). However, it is not only the level of total protein that influences the texture and appearance of yoghurt; the type of dairy ingredients used for supplementation also has substantial effect. This is reviewed in Section 2.4.3.

2.4.2 Heat treatment

Heat treatment is essential in yoghurt manufacture. It contributes to improving the texture and appearance of the product (Sawyer, 1969). Savello and Dargan (1997) observed a reduction in syneresis with high heat treatment including UHT or vat heating. Guyomarc'h *et al.* (2003) compared the storage modulus (G', representing gel strength) and syneresis of heated (95°C for 10 min) and unheated reconstituted skim milk with various CN to WP ratios acidified using glucono-δ-lactone (GDL) and reported that heated milk showed higher value of G' than that of unheated milk. The syneresis of heated milk gels was lower than that of unheated products. In addition, they observed that heated milk started to gel at higher pH values than that of unheated milk. Similar results were reported by Lucey *et al.* (1998a) and Schorsch *et al.* (2001).

The changes in milk proteins and physical properties of yoghurt as a result of heat treatment can also be seen and explained by microstructure of yoghurt. After heat treatment, appendages appeared on the surface of casein micelles as compared to unheated milk (Davies *et al.*, 1978; Harwalkar and Kalab, 1980; Parnell-Clunies *et al.*, 1987; Mottar *et al.*, 1989). Several researchers have agreed that the appendages were denatured whey proteins, mainly β-lactoglobulin and α-lactalbumin, which when denatured exposed SH groups forming disulphide-bridge with κ-casein on the surface of casein micelles. In addition, they observed that yoghurt gels made from heated milk showed an increase in the density of protein matrix with finer protein strands than those made with unheated milk (Parnell-Clunies *et al.*, 1987; Lycey *et al.*, 1998a; Schorsch *et al.*, 2001). The appendage layer on the surface of casein micelles is believed to prevent the contact and aggregation between them. The increase in the density of protein matrix enhances water-holding ability of the gel as observed by Hashizume and Sato (1988).

The interaction between casein and whey proteins also depends on the pH of milk at heating. Vasbinder and de Kruif (2003) and Vasbinder *et al.* (2004) reported that heating milk at pH values greater than 6.55 promoted the formation of soluble denatured whey protein aggregates, while heating at pH values less than 6.55 promoted the formation of denatured whey protein-bound casein micelles. Similar results have been reported by Singh and Fox (1985a, 1985b, 1986) and Singh (2004). They reported that the majority of denatured whey proteins associated with casein micelles when milk was heated at pH values less than 6.80, but dissociated

when heated at pH values greater than 6.80. The difference between the results of Vesbinder and de Kruif (2003), Vesbinder *et al.* (2004) and that of Singh and Fox (1985a and 1985b) could be due to the condition of heat treatment. While Vesbinder and de Kruif (2003) and Vesbinder *et al.* (2004) heat treated milk at 90°C, Singh and Fox (1985a and 1985b) carried out the experiment at 120°C. Moreover, Vasbinder *et al.* (2004) reported that the G′ of acidified milk gels adjusted to pH values greater than 6.55 prior to heating were higher than that adjusted to pH values below 6.55. Schorsch *et al.* (2001) showed that the milk gels acidified with GDL made by pre-heating whey proteins and casein separately (80°C for 30 min) showed higher values of G′ than those made by co-heating of casein and whey protein. The former procedure promoted the formation of whey protein aggregates, while the latter promoted the association of casein-whey protein complex. These results suggested that types of denatured whey proteins contributed substantially to gel characteristic of yoghurt.

2.4.3 Fortification with dairy ingredients

As mentioned previously, each dairy ingredient affects physical properties of yoghurt differently. In general, dairy ingredients can be classified into two groups: 1) casein-based such as SMP, Na-caseinate, Ca-caseinate, MPC, and 2) whey-based such as WPC and WPI. However, there are variations in the composition of each ingredient. Morr and Foegeding (1990) studied the composition and functionality of commercial whey and milk protein concentrate and isolates and found that the composition and functionality of WPC and WPI varied from one batch to another.

The variation may be caused by the manufacturing processes as suggested by Karleskind *et al.* (1995a and b). The concentration of protein in WPC can also vary from 35% to 80% (Zadow, 1992); this may explain the contradicting reports on the effects of supplementation of milk with WPC on physical properties of yoghurt. Such variations exist in other ingredients, which may influence physical properties of yoghurt as well.

Yoghurts fortified with casein-based ingredients (SMP, Na-caseinate or Ca-caseinate) showed an increase in firmness (or viscosity) and a reduction in syneresis compared to unfortified yoghurts (Modler *et al.*, 1983; Guzmán-Gunzález *et al.*, 1999, 2000; Remuef *et al.*, 2003). On the other hand, there were no consistent trends between the physical properties of yoghurts and the addition of whey protein-based ingredients (WPI or WPC). Guzmán-Gunzález *et al.* (1999) found that yoghurt supplemented with WPC had lower apparent viscosity than the control yoghurt made without supplementation. The protein contents of those yoghurts were kept constant. Baig and Prasad (1996) and Bhullar *et al.* (2002) found that the supplementation with WPC in milk improved the apparent viscosity and textural properties of the resultant yoghurt. Dave and Shah (1998b) observed that supplementation with 2% WPC improved firmness of the product as compared to the control yoghurt. These differences may be due to the variation from the composition of whey protein-based ingredients used, processing conditions as well as determination methods. Remuef *et al.* (2003) showed that extending heating time of milk supplemented with WPC from 1 min to 5 min at 90°C increased the

apparent viscosity of stirred yoghurt. On the other hand, the apparent viscosity of stirred yoghurt fortified with Na- and Ca-caseinate was not affected by the increase in the heating time. Nonetheless, the fortification with WPC reduced syneresis dramatically. The improvement in physical properties of yoghurt as affected by fortification with dairy ingredients may be caused by several factors including heat treatment, interaction between casein and whey proteins and changes at the microstructural level of the product. These are reviewed in the following sections.

2.4.4 EPS-producing starter cultures

Appropriate use of starter cultures in yoghurt manufacture has a substantial impact on textural and rheological properties and appearance of yoghurts. Selection of an appropriate type of starter cultures in addition to the level of inoculum (Section 2.3.2.3) is very important. The starter cultures are either non-EPS- or EPS-producer (Cerning, 1990; Broadbent *et al.*, 2003; De Vuyst and Degeest, 1999). The EPS-producing bacteria can be capsular, in which EPS remains attached to the cell wall or ropy (slime), in which EPS is secreted into the surrounding environment (Cerning, 1990). In some cases, the microorganisms can produce both forms of EPS (Zisu and Shah, 2003; Hassan *et al.*, 2001).

EPS-producing starter cultures, particularly the ropy strains, have been used in fermented products such as yoghurt, viili and kefir. This is due to the thickening effect and water-binding ability of EPS produced from such starter cultures

(Wacher-Rodate *et al.*, 1993; Bouzar *et al.*, 1997; Hess *et al.*, 1997; Marshall and Rawson, 1999; De Vuyst *et al.*, 2003; Ruas-Madiedo and Zoon, 2003). Capsular EPS-producing strains have been used in cheese manufacture. Perry *et al.* (1997) and Low *et al.* (1998) reported that the use of capsular EPS-producing *S. thermophilus* MR-1C increased moisture content of low-fat mozzarella cheese (~1.3%) compared to that made with non-EPS-producing strain. Similar results have been reported by Zisu and Shah (2004).

It is interesting to note that set yoghurts made with EPS-producing starter cultures (both capsular and ropy) showed lower gel firmness than those made with non-EPS-producing starter cultures (Hassan *et al.*, 1995a; Hess *et al.*, 1997). However, EPS yoghurts showed a dramatic reduction in syneresis compared to those made with non-EPS-producing starter cultures. Hassan *et al.* (2002a) observed an increase in storage modulus (G') in yoghurts made with capsular EPS-producing strain compared to those made with non-EPS- or ropy EPS-producing starter cultures. Hassan *et al.* (1996a, 2002a) observed an increase in shear stress as well as the area of hysteresis loop between upward and downward flow curves, which represent the damage of the structure of stirred yoghurts during shearing, in batches made with ropy EPS-producing starter cultures. Similar results of increased apparent viscosity in stirred yoghurt made with ropy EPS-producing starter cultures have been reported by several workers (Wacher-Rodate *et al.*, 1993; Rawson and Marshall, 1997; Marshall and Rawson, 1999; Jaros *et al.*, 2002). In addition, yoghurts made with ropy EPS-producing starter cultures were reported to have better mouthfeel

than those made with non-EPS-producing starter cultures (Wacher-Rodate *et al.*, 1993). Marshall and Rawson (1999) proposed that an increase in apparent viscosity of the stirred yoghurt made using ropy EPS-producing starter cultures was due to the stretchability of EPS. The varying effect of EPS starter culture on physical properties of set and stirred yoghurts may be partly due to the nature of their gel structure. In set yoghurt, the structure of protein network is not disrupted and EPS may interfere with protein gel structure, which mainly contributes to physical properties of yoghurt gel (Lucey, 2002), leading to a decrease in gel strength or firmness. On the other hand, a disruption in protein network in stirred yoghurt is likely to reduce the effect of protein gel, but increase that of EPS on gel characteristics.

2.5 EPS

2.5.1 Structure and composition of EPS

EPS produced by lactic acid bacteria is either homopolysaccharide or heteropolysaccharide depending on the strain. Yoghurt starter cultures, *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus,* produce heteropolysaccharide EPS (De Vuyst and Degeest, 1999). In general, the structure of their EPS consists of branches of repeating units of α- and β-linkage of sugar moieties. The sugar monomers of EPS are mainly composed of galactose, glucose and rhamnose. Minor components may also be present such as fructose, mannose, arabinose, and N-acetyl-aminosugars. The molecular weight of EPS can range from 10^4 to 10^6 Da (De Vuyst and Degeest, 1999). The structure, composition, molecular weight and the

concentration of EPS appear to be dependent on microorganism, and environmental conditions. Furthermore, lactic acid bacteria could produce more than one type of EPS. Riccardi *et al.* (2002) reported that *S. thermophilus* SY produced two main fractions of EPS (high molecular weight (MW) of 2 x 10^6 and low MW of 5 x 10^4 Da) with same sugar composition. This was also observed in *L. casei* CRL 87 with MW of 8.0 x 10^5 and 5.0 x 10^5 Da (Mozzi *et al.*, 1996) and *S. thermophilus* OR 901 with MW of 9.0 x 10^6 and 1.1 x 10^6 Da (Ariga *et al.*, 1992) fermented in skim milk media. Grobben *et al.* (1997) observed two MW fractions of EPS produced from *L. delbrueckii* ssp. *bulgaricus* NCFB 2772 (1.7 x 10^6 and 4.0 x 10^4 Da) grown in a chemically defined media. However, these two fractions did not share the same ratios of sugar composition. Many techniques have been used for studying the structure and composition of these EPS such as acid hydrolysis, methylation analysis, Smith degradation, acid hydrolysis (methylation analysis) and 1D and 2D NMR spectroscopy (Faber *et al.*, 2001; Gruter *et al.*, 1992; Gruter *et al.*, 1993; Yang *et al.*, 2000; Robijn *et al.*, 1996; Marshall *et al.*, 2001; Marshall *et al.*, 1995).

2.5.2 Factors affecting EPS production

Several workers have observed a correlation between an increase in EPS concentration and the apparent viscosity of yoghurt (Cerning *et al.*, 1988; Bouzar *et al.*, 1997; De Vuyst *et al.*, 2003). Generally, EPS-producing starter cultures produce only small amount of EPS in yoghurt ranging from 40 to 400 mg L^{-1} (De Vuyst and Degeest, 1999). This could be due to short fermentation time as well as due to a decrease in pH during the yoghurt manufacture. An increase in EPS concentration

has been observed when a starter culture was grown under optimized conditions. Gassem *et al.* (1997) observed that the periodic pH adjustment had greater effects on EPS production than supplementation with nutrients and minerals. Glucose was the most efficient carbon source and its concentration in the range of 10 to 20 g L^{-1} had a marked effect on EPS production (Cerning *et al.*, 1994; Grobben *et al.*, 1996; van Geel-Schutten *et al.*, 1999). This is possibly due to the effect of carbon source on enzymes responsible for EPS production in biochemical pathway as shown by Mozzi *et al.* (2001) and Degeest and De Vuyst (2000). In addition, carbon source influenced the composition of EPS. Supplementation with nitrogen sources such as yeast extract (De Vuyst *et al.*, 1998) and WPC (Hassan *et al.*, 2001; Zisu and Shah, 2003) to the media increased EPS production. Degeest *et al.* (2001) reported that low temperature of 15°C as compared to 42°C, enhanced the concentration of EPS produced by *Lactobacillus sakei* 0-1. This trend was also observed in *L. delbrueckii* ssp. *bulgaricus* RR that produced a maximum concentration of EPS at 38°C instead of its optimum growth temperature of 45°C (Kimmel *et al.*, 1998). Limitation in manganese and phosphate ions has been reported to reduce the concentration of EPS produced by *L. delbrueckii* ssp. *bulgaricus* NCFB 2772 (Grobben *et al.*, 2000). Interestingly, Petry *et al.* (2000) reported that *L. delbrueckii* subsp. *bulgaricus* grown in a chemically defined media produced the maximum EPS during stationary phase. De Vuyst *et al.* (1998) and Zisu and Shah (2003) observed that EPS production by *S. thermophilus* was maximum during log phase. The fermentation process also has an impact on the production of EPS. Petit *et al.* (1991) reported that *S. thermophilus* fermented using fed-batch process converted nutrients into EPS more efficiently than the batch process. Optimization of these factors is required in

order to enhance EPS production resulting in improved texture and appearance of yoghurts.

2.5.3 Purification and quantification of EPS

The methods of purification and quantification of EPS from yoghurt are important in studying the effect of EPS on physical properties of yoghurts. The purification processes involve removing proteins and fat followed by precipitating carbohydrates from the supernatant and removing the lactose. Although there are only three main steps in purification, the procedures are tedious and take a significant amount of time. Figure 2.1 shows a typical flow chart of EPS purification process from a yoghurt sample. Milk proteins can be removed by several methods such as digestion with an enzyme, precipitation with trichloroacetic acid or by boiling. The combination of these methods has been used followed by centrifugal separation of the clear supernatant (Cerning *et al.*, 1988; Gancel and Novel, 1994; Bouzar *et al.*, 1996, 1997; Mozzi *et al.*, 1996; Zisu and Shah, 2003). The boiling process also inactivates the protease and other enzymes that are capable of degrading EPS (Cerning *et al.*, 1994). Ariga *et al.* (1992) used a sonicator to detach capsulated EPS from bacterial cell wall. The next step involves the precipitation of lactose and EPS using chilled absolute ethanol (a minimum of 1:1 volume ratio of chilled ethanol to supernatant) overnight at 4°C. This step is carried out to prevent contamination from other milk components in the supernatant. The precipitate of insoluble carbohydrates (mainly lactose and EPS) is collected by centrifugation (Zisu and Shah, 2003).

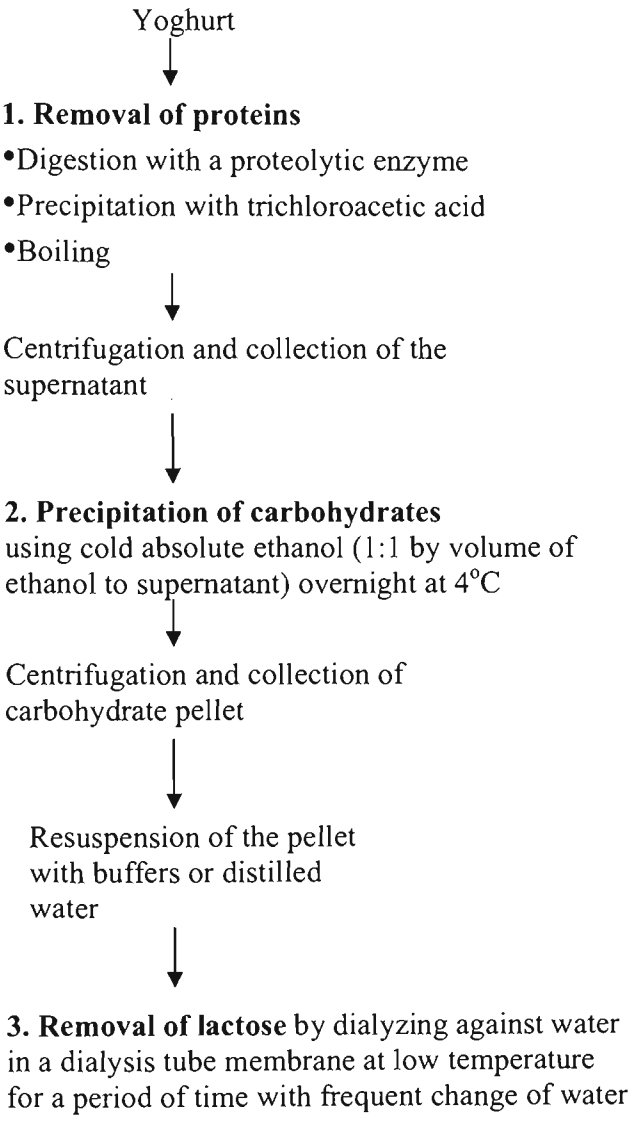

Figure 2.1: A typical flow chart showing EPS purification process from yoghurt

De Vuyst *et al.* (1998) used several organic solvents for this process including ethanol, acetone and isopropanol and observed that acetone gave the best result. The resultant pellet of carbohydrate is then re-suspended in buffer solution or distilled water and dialyzed against buffer solution or distilled water using a dialysis tube membrane with MW cut-off values ranging from 3000 to 13,000 Da at low temperature (4 to 10°C) over a period of time sufficient to remove remaining lactose (Zisu and Shah, 2003; Kimmel *et al.*, 1998; Petry *et al.*, 2000). The water needs to be changed frequently. The dialysed solution containing EPS inside the dialysis tube is then used in quantification.

There are two common methods for the quantification of EPS: phenol-sulphuric and polymer dry mass method. Because the phenol sulphuric method (Dubois *et al.*, 1956) is normally used for the quantification of total sugar, the contamination of lactose may influence the result. Quantification of EPS using polymer dry mass method (Toba *et al.*, 1992; De Vuyst *et al.*, 1998; De Vuyst *et al.*, 2003) by filtering the dialysed solution through an ultrafilter membrane is simple with less potential hazards (from phenol and concentrated sulphuric acid) than the former method. Recently, Macedo *et al.* (2002) used near-infrared spectroscopy (NIRS) to quantify EPS, lactic acid and lactose in a culture broth. They observed a good correlation (91% correlation coefficient) in EPS concentration between the NIRS method and the two reference methods; phenol-sulphuric and polymer dry mass methods, and suggested that NIRS could be a useful method for rapid determination of EPS.

2.6 Rheology, sensory evaluation and instrumental measurement

"Rheology is the science of the deformation and flow of materials" (Steffe, 1992). This is particularly important for foods because they have to be deformed before ingestion. Many phenomena occur during eating. One of those is mouthfeel that can be defined as the sensory experience derived from the sensation in the mouth and on the tongue after ingestion of food (McKenna, 2003). Mounthfeel is influenced by the textural and rheological characteristics of foods. Bourne (1982) gave the following definition of texture:

"The textural properties of a food are the group of physical properties that arise from the structural elements of the food, are sensed by the feeling of touch, are related to the deformation disintegration and flow of the food under a force, and are measured objectively by function of mass, time and distance".

The textural and rheological characteristics are interrelated. However, one should not be used for the other as rheological parameters do not cover all the factors that constitute to the texture of foods.

Applying rheology in food production is essential in order to understand its behaviour, and control and manipulate a desirable food texture for consumers.

However, texture is a subjective word. One taster may like thick soup while other may not. Although a suitable sensory evaluation can give the perception of consumer toward a product, there are several difficulties involved such as the recruitment of panelists (number and sensitivity), limitation in the number of products and tests, reproducibility of results and environmental factors. Moreover, this method can only be used for the assessment of the finished product. Modern testing instruments resolve most of these difficulties. However, the results from instrumental measurements do not relate to the preference of consumers unless a correlation between these two is established. For example, Carson *et al.* (2002) used spectral stress strain analysis and partial least square regression to predict sensory texture of yoghurt using compression/penetration method. They found good predictive models for "spoon impression" (R = 0.93), "visual thickness" (R = 0.89) and "slipperiness" (R = 0.81). Other sensory texture attributes (oral thickness (R = 0.78), cohesiveness (R = 0.73) and stickiness (R = 0.57) were less accurately predicted. Skriver *et al.* (1999) observed a good correlation between sensory texture analysis and some rheological properties of stirred yoghurt. They reported that non-oral viscosity (penetrating stirred products with a spoon) highly correlated with the complex modulus (G*) from the dynamic oscillatory measurements (R = 0.823) and the viscosity from a Brookfield viscosmeter operated at 5 rpm (R = 0.862). For the oral viscosity, they found a good correlation with the hysteresis loop area from the viscometry test (R = 0.867) and the viscosity obtained from the Posthumus funnel (R = 0.834). Presently, the textural and rheological properties of foods are assessed mostly by instruments. This is due to the reproducibility of results and efficiency of the test.

2.7 Textural and rheological properties of yoghurt

Yoghurt is a semi-solid fermented milk gel product with a firm (set) and viscous (stirred) texture, and possesses a viscoelastic property (Lucey, 2002; Puvanenthiran *et al.*, 2002). The term "viscoelastic" describes materials that exhibit both the viscous property of a liquid and the elastic property of a solid simultaneously under applied stress. It also behaves as non-Newtonian fluid with a yield value, and exhibits thixotropy (decreasing in viscosity with time during shearing at specified shear rate) with partial recovery and shear-thinning behaviour (decreasing in viscosity with the shear rate) when measured under non-linear viscoelastic region (Ramaswamy and Basak, 1991; Steffe, 1992). From the processing point of view, it means that an inappropriate use of processing and mechanical handling can cause breakage of a set gel, which will never fully recover or may decrease the apparent viscosity of a stirred product influencing the quality of the product. Similarly, distribution of stirred yoghurt as soon as it is packed can result in a product of low viscosity.

2.7.1 Assessment of the textural and rheological characteristics of yoghurts

There are several testing procedures and instruments available for assessing textural and rheological characteristics of yoghurts. The testing procedures can be divided into two categories: 1) small deformation or nondestructive and 2) large deformation or destructive testing procedures. It is important to note that the nondestructive measurement cannot be used to observe mechanical characteristics

of yoghurt such as fracture and yield stress (Lucey, 2002; van Vliet and Luyten, 1995). Modern instruments can perform both type of measurements within the same machine. Two commonly used equipments are compression type (Instron Universal Testing machine, Texture Analyser) and rotational type (Brookfield viscometer and Bohlin, Haake or Physica rheometer).

2.7.2 Oscillatory measurements (non-destructive)

Many workers have used oscillatory or dynamic testing procedures to characterize the rheological properties of milk gels (Hassan *et al.*, 2002a, 2003a; Lucey *et al.*, 1997, 1998a, b, c; Salvador and Fiszman, 1998; Anema *et al.*, 2004; Haque *et al.*, 2001; Guinee *et al.*, 2002). It has also been applied for stirred yoghurt. The dynamic test is regarded as a nondestructive measurement, and can be used to characterize the viscoelastic properties of milk gels (both acid and rennet type gels) (Lucey, 2002). Some of the main parameters that are usually determined include the elastic or storage modulus (G', which is a measure of the energy stored per oscillatory cycle and usually interpreted as the strength of gels), the viscous or loss modulus (G", which is a measure if the energy dissipated per cycle), and tan δ (which is the ratio of the viscous to elastic properties; G"/G'.

The dynamic tests can also be sub-divided into three most common operating modes. Firstly, a stress sweep mode (Figure 2.2a), which is used to determine the

limits of linear viscoelastic behaviour of a substance (Figure 2.2b).

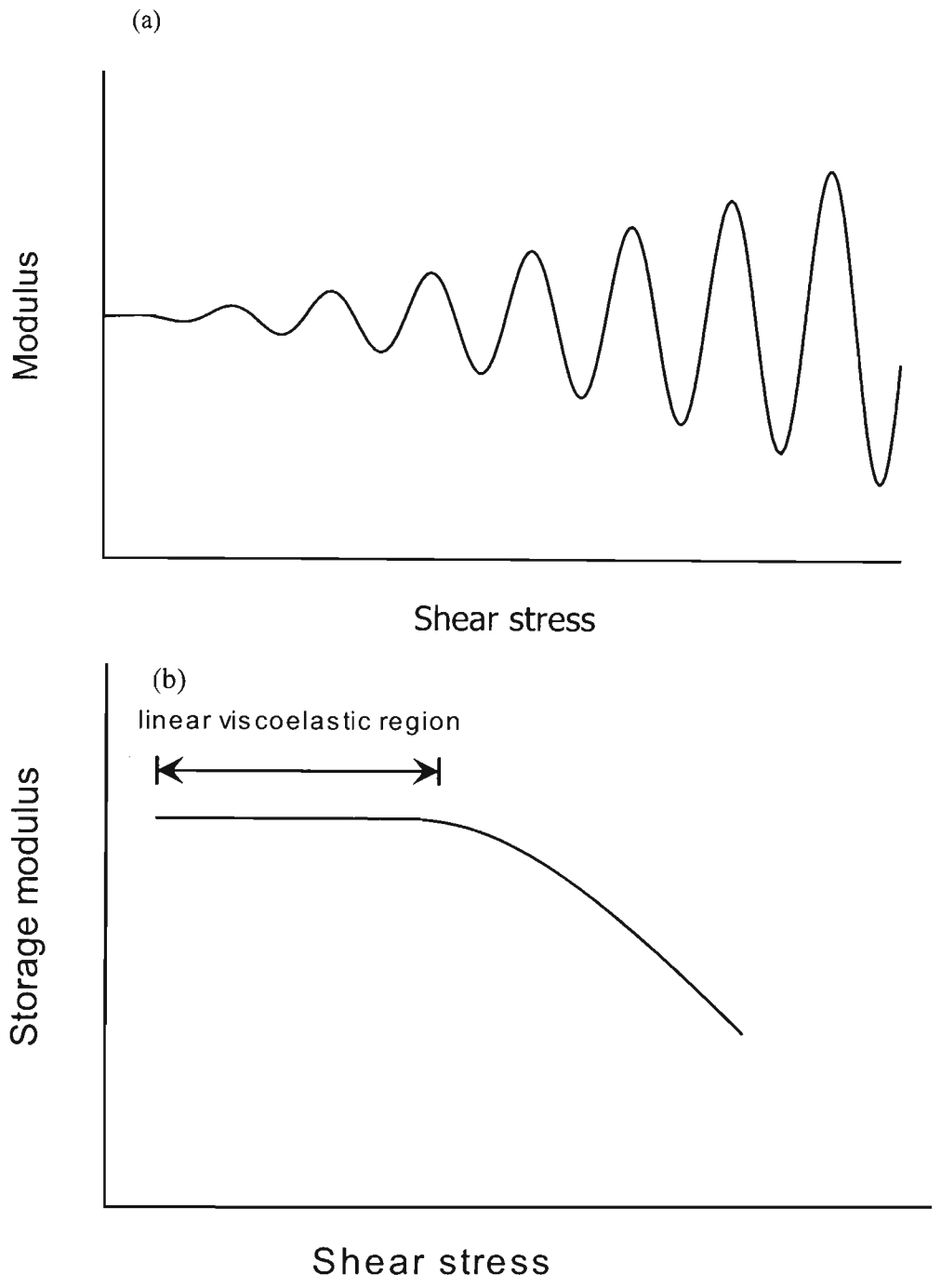

Figure 2.2: Stress sweep mode shows linear viscoelastic region (a) and the increase in amplitude of stress (b) during testing.

In the linear viscoelastic region, rheological properties are not strain- or stress dependent. Secondly, a frequency sweep (Figure 2.3), which shows how the rheological properties change with the rate of application of stress or strain.

Thirdly, a time sweep mode (Figure 2.4), which is carried out by observing the changes in rheological parameters, whereas the amplitude (stress or strain) and frequency are constant. The time sweep test has been widely used in monitoring milk gel development during fermentation (Lucey *et al.*, 1997, 1998a and 1998b; Guinee *et al.*, 2002).

However, one drawback of using these tests for the characterization of set yoghurt gel is that the sample needs to be intact meaning that the set yoghurt needs to be manufactured within the rheometer system. This means that only one or two sample can be produced per day causing difficulties for observation during storage. Another issue is slippage in oscillatory measurement. This causes a reduction in the value of modulus. Haque *et al.* (2001) used a time sweep mode to monitor the development of yoghurt gel using both cup and bob, and cone and plate sensor geometry. They observed higher slippage effect in the measurement using the cone and plate geometry than that measured by the cup and bob. They believed that the slippage occurred due to the sedimentation of casein aggregates, leading to the formation of protein-depleted layer at the contact surface (horizontal upper surface in cone and plate and the vertical surface in the case of cub and bob) between the milk and the

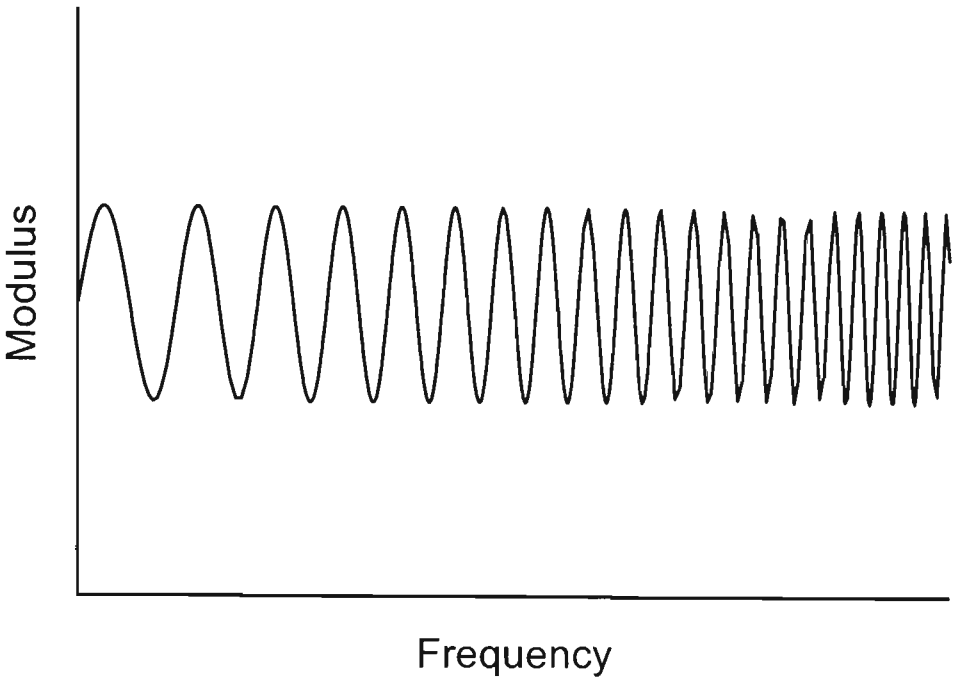

Figure 2.3: Frequency sweep mode

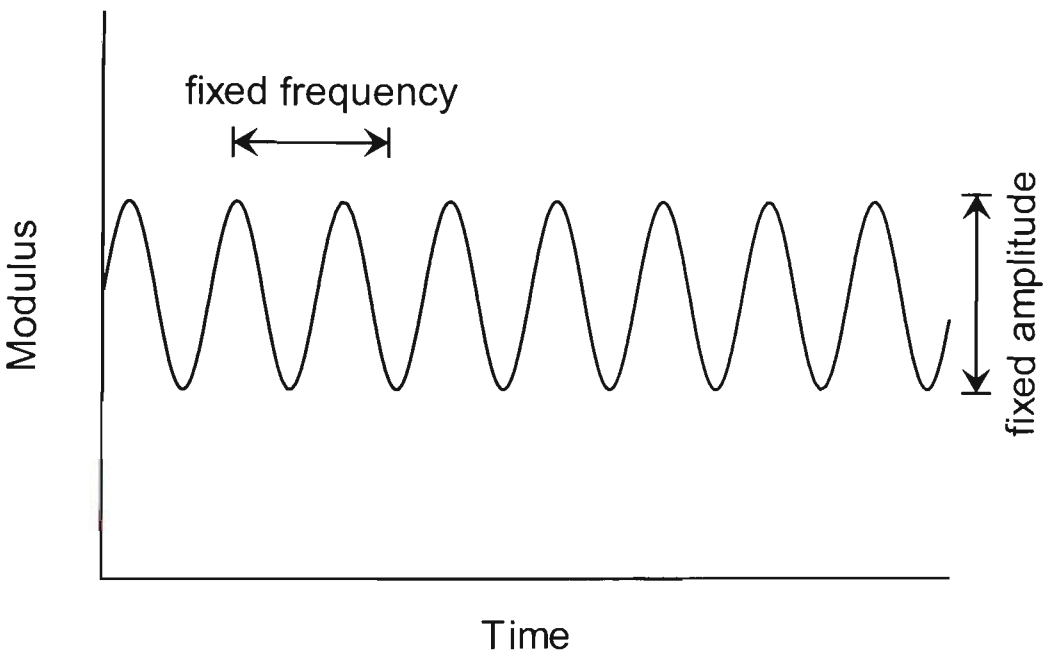

Figure 2.4: Time sweep mode

sensor, and suggested that the slippage effect would be more severe in the horizontal protein-depleted layer (cone and plate) than in the vertical one (cup and bob). Furthermore, ultrasound and diffusing wave spectroscopy (DWS) are relatively new techniques that can be used to monitor the development of milk gels (Horne and Davidson, 1990; Benguigui *et al.*, 1994; Nassar *et al.*, 2001; Vasbinder and de Kruif, 2003).

2.7.3 Texture Profile Analysis (destructive)

The textural characteristics of yoghurts can also be studied using destructive measurements. A compression test coupling with texture profile analysis (TPA) testing procedure is commonly used with set yoghurt. However, Marshall and Rawson (1999) used back extrusion-TPA method with stirred yoghurt. Haque *et al.* (2001) characterized stirred yoghurt by monitoring time taken for a fixed volume of the sample passing through a narrow orifice as a result of compression. Generally, the TPA testing procedure is carried out by compressing samples with an appropriate probe for two cycles. It imitates the masticating action of the human mouth (Pons and Fiszman, 1996; Tunick, 2000). Although the testing conditions are not the same as those in the mouth due to the lack of saliva and mixing action, the results have been shown to correlate well with sensory evaluation (Szczesniak, 1963). Figure 2.5 represents a typical TPA curve (force-time). Several parameters can be extracted from the curve as defined below (Bourne, 1978; Tunick, 2000).

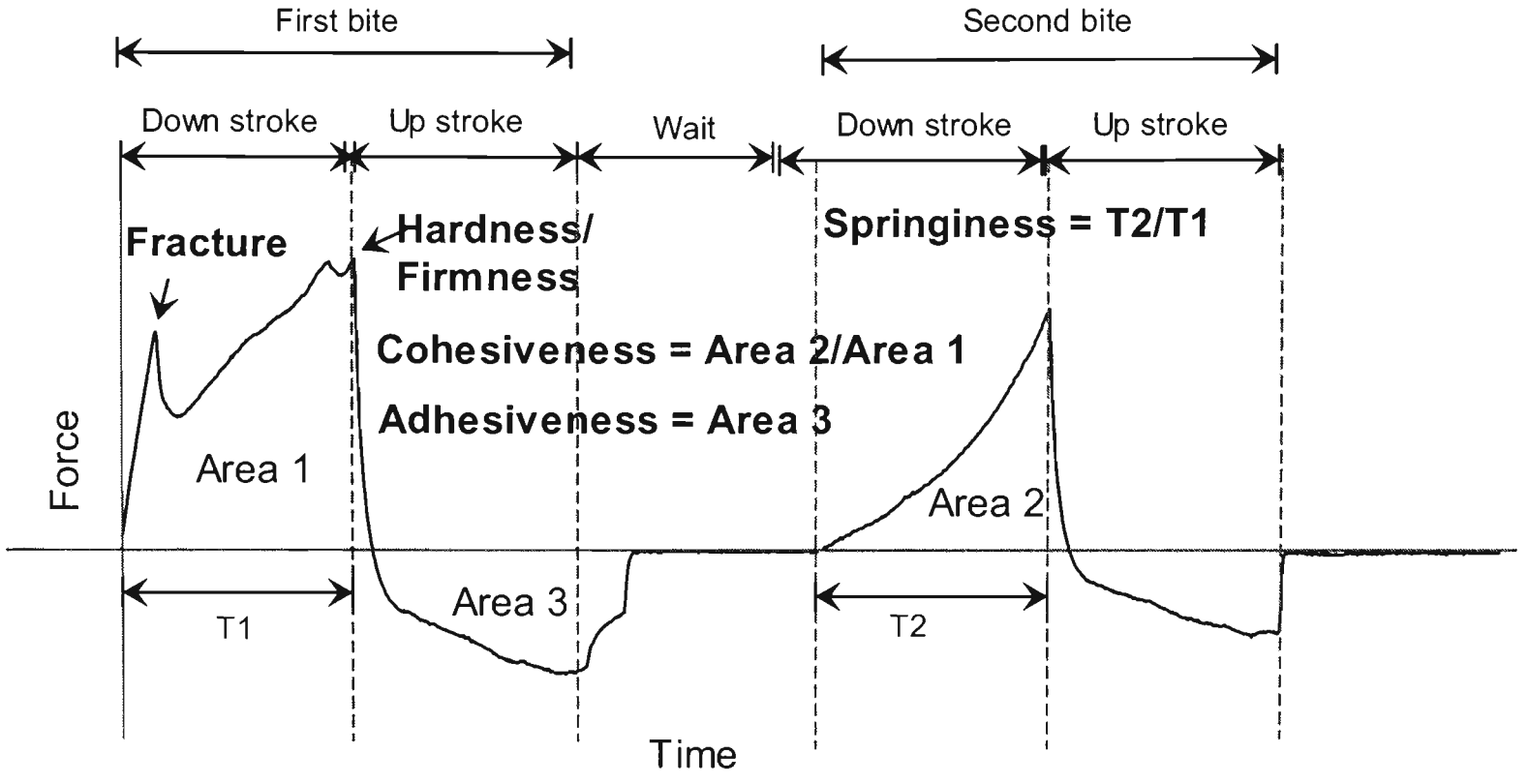

Figure 2.5: A typical texture profile analysis (TPA) curve

(1) Fracturability or brittleness is defined as the force at the first significant break in the curve. It represents the force with which a food fractures. The phenomenon is related to the structure of the sample. According to van Vliet and Luyten (1995), fracture occurs at weak points as a result of the discontinuity in food structure. Nevertheless, the mechanisms of fracture in foods are complex. Further information can be found in reviews by Lillford (2002) and van Vliet and Luyten (1995).

(2) Hardness or firmness is defined as the maximum force required to attain a given deformation during the first compression (first bite).

(3) Cohesiveness is defined as the ratio of the positive force area during the second compression to that of the first compression (Area 2/Area 1). This parameter represents the strength of a food's internal bonds.

(4) Adhesiveness is defined as the negative force area of the first compression representing the work necessary to pull the probe out from the food sample. Theoretically, the adhesiveness means the attractive force between surfaces of the two materials. The negative force resulting from pulling the probe out from food sample may also be influenced by the normal stress.

(5) Springiness or elasticity is defined as the height that deformed foods recover during the time that elapses between the end of the first bite and the start of the second bite.

(6) Gumminess is defined as the product of hardness x cohesiveness representing the force needed to disintegrate a semisolid food to a state ready for swallowing

(7) Chewiness is defined as the product of gumminess x springiness (which is equivalent to hardness x cohesiveness x springiness) representing the work required to masticate a solid food to a state ready for swallowing.

Some textural characteristics do not occur within the same food product. For example, liquid foods such as milk does not exhibit fracturability, while brittle foods such as crackers do not exhibit adhesiveness.

All parameters can be used to test texture of yoghurt, except chewiness. Furthermore, the results from the compression test can be influenced by several factors including the ratio of the diameter of a sample to that of the probe, the degree of deformation, the speed of compression, the time elapsed between two compressions and lubricating effects. The ratio of the diameter of the sample or containers to that of the probe (smaller diameter of probe to the sample is commonly used in yoghurt testing) is very important, particularly for testing a set yoghurt in a container. When the ratio is close to 1 to 1, the results tend to be more influenced by boundary or due to wall effect. According to Bourne (2002), the ratio should be at least 3 to 1. Jones *et al.* (2002) observed a dramatic increase in hardness of polymer gels composed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone) in association with the reduction of the ratio of the diameter of sample to probe from 2 to 1 onward. They also reported a linear relationship between the increase in hardness of gels and speed of probe displacement. The friction between the probe and the sample also influences results.

Lubricating the probe before the measurement can eliminate this problem (if it exists) (Bourne, 2002).

2.7.4 Yield stress (destructive)

Yield stress is another rheological characteristics observed in many foods including yoghurt (Harte *et al.*, 2002). Yield stress is defined as a maximum stress required to initiate the deformation or flow of materials (Steffe, 1992). The value of yield stress can be quantified using a rheometer. Vane sensor is preferred for preventing slippage effect compared to other types of sensors. In theory, the shear rate or shear strain is slowly increased and the yield stress is determined as the interception of two slopes of shear stress vs. shear rate or shear strain. Interestingly, Bhattacharya (1999) used several methods to quantify yield stress in mango pulp using a cup and bob sensor. Those methods included 1) stress relaxation method, 2) control stress experiment, 3) stress-strain experiment and 4) multiple plot experiment. The author observed that the yield stress was best determined by control stress, stress-strain, or stress relaxation techniques.

2.7.5 Flow curve (destructive)

A flow curve can be used to characterize rheological characteristics of yoghurt, particularly stirred-type product. The flow curve is constructed by shearing (varying shear rate or shear stress) the sample and observing the response (shear stress or shear rate depending on the type of the rheometer). The flow curve should cover the whole range (e.g. from shear rate as low as 0.1 s^{-1} to 1000 s^{-1}) as carried out by Hess

et al. (1997) and Teggatz and Morris (1990). Further, due to the partial recovery characteristics of yoghurt samples, one should be cautious about shear history of samples during sample preparation. A slight deviation from the routine sample preparation procedure can yield varying results.

A most crucial point in textural and rheological measurement that has not been mentioned is the suitability of a testing condition. In this context, it means that the rheological measurements should be carried out under similar conditions as they occur in a real system. For example, the apparent viscosity of a yoghurt after shearing overnight at room temperature will not represent that of a chilled product before consuming. Furthermore, the temperature of a sample is one of the most important factors. For instance, the apparent viscosity of cream measured at 5°C would be higher than that at 20°C. Therefore, controlling temperature of samples during the measurement is mandatory.

2.8 Syneresis and the methods of determination

Syneresis or wheying-off, which is regarded as a defect in a set yoghurt, occurs spontaneously and naturally. The cause is believed to be due to the shrinkage of the set yoghurt gel (Lucey, 2002, 2004). Lucey *et al.* (1998c) observed a relationship between whey separation and the rearrangement of protein network of a set yoghurt. Choosing a correct method for investigating the level of syneresis of a product is essential. This helps decide the best strategy that can be used to reduce syneresis in

set yoghurts. It is important that the selected method represents the actual level of spontaneous syneresis of a product. The drainage and centrifugation methods are used traditionally. Other methods have also been applied including the measurement of the gel permeability (Lucey *et al.*, 1998c; Ruas-Madiedo and Zoon, 2003) and the use of NMR (Hinrichs and Weisser, 2003) for the observation of water-holding capacity and the structure of yoghurt gels.

Whey separation measured using the drainage and centrifugation methods do not represent the spontaneous syneresis in products. The results can be influenced by gel breakage as well as external forces (gravitational force in the case of the drainage method and centrifugation force in the centrifugation method). Lucey (2002, 2004) suggests that those two methods were not relevant to the spontaneous whey separation that occurs in a set yoghurt. Lucey *et al.* (1998c) and Lucey (2001) developed a method to investigate the level of spontaneous syneresis in the yoghurt. They fermented milk to make a set yoghurt in a volumetric flask. The spontaneous whey separation on the surface of the yoghurt was collected by tilting the volumetric flask. The objective of their method was not to damage the gel of yoghurt. However, the difference in the container's geometry between a yoghurt cup and that of a volumetric flask may affect heat transfer and the activity of starter cultures of the product. A better method that can measure the level of spontaneous syneresis in an actual yoghurt container is therefore required.

2.9 Microscopy and yoghurts

The choice of ingredients, milk solids and protein content, heat treatment, starter cultures and handling procedures affect the textural and rheological characteristics of yoghurts, as they affect the microstructure of the product (Heertje *et al.*, 1985). For instance, Ozer *et al.* (1999) observed the microstructure of labneh (a concentrated yoghurt) manufactured by the traditional cloth bag method, ultrafiltration, reverse osmosis or direct reconstitution. They reported that the compactness of protein structure depended on the protein content of the sample and the difference in manufacturing procedures affected the microstructure of yoghurt. Similarly, an increase in milk solids increased the compactness of the protein network and reduced syneresis of a set yoghurt (Harwalkar and Kalab, 1986). The microstructure of set yoghurt fortified with Na-caseinate was more open than that with other ingredients (Tamime *et al.*, 1984; Remuef *et al.*, 2003). The application of starch based fat substitutes and thickening agents has been shown to influence the microstructure of yoghurts (Tamime *et al.*, 1996; Kalab *et al.*, 1975). The changes in the rheology and microstructure of ropy yoghurt during shearing have been studied by Teggatz and Morris (1990). Heat treatment altered the microsturcture of the yoghurt as well as the surface structure on casein aggregates (Parnell-Clunies *et al.*, 1987).

2.10 Scanning electron microscopy and confocal scanning laser microscopy

Scanning electron microscope (SEM) and confocal scanning laser microscope (CSLM) are most commonly used for studying the microstructure of yoghurts. Each instrument has its advantages and disadvantages. Greater details of microstructure can be observed with SEM due to its higher resolution than that of CSLM. The resolution in the latter is between that of a light microscope and an electron microscope (Herman, 1998). However, the specimens for SEM have to go through chemical fixation and dehydration processes. The SEM micrographs do not represent the microstructure in hydrated state as those taken using CSLM (Hoppert, 2003; Herman, 1998). Other benefits of using CSLM are that each component such as fat, protein and carbohydrate can be observed separately, but simultaneously. Micrographs can be observed in thin sections without mechanical sectioning processes. The microstructure can also be observed in 3-dimensional images by combining and constructing the volume of specimens from several thin sections (Herman, 1998; Vodovotz *et al.*, 1996; Takeuchi and Frank, 2001).

2.11 Artefacts in micrographs

Artefact is an important issue in the microstrucural study as micrographs should represent as close to the real specimens as possible without shrinkage, distortion, or other changes. The artefacts mostly occur due to sample preparation and imaging processes. Glauert and Lewis (1998) stated that

"every electron micrograph is in a sense, an artefact. At each stage, from the initial fixation onwards, changes in ultrastructure are inevitable: material is extracted; dimensions are altered; and molecular rearrangement occurs. The best that can be done is to keep these changes to a minimum."

This statement can be applied to CSLM and it is important to understand the principle of sample preparation in order to minimize artefacts with each method.

2.11.1 SEM: Artefacts and sample preparation

The sample preparation for scanning electron microscopy can be categorized into two main groups: conventional sample preparation and cryo-sample preparation. The conventional sample preparation procedures involve chemical fixation and dehydration prior to gold-coating and imaging. Biological and food specimens are first fixed with aldehyde-buffer solution (formaldehyde or glutaraldehyde) and osmium tetroxide in order to preserve the protein and lipid structure in the specimens, respectively (Glauert and Lewis; 1998; Hoppert, 2003; Crang, 1990). The use of aldehyde solution causes the cross-linking of proteins. The chemical and physical dehydration are the next steps. Typically, series of ethanol solution (30 to 100%) are used as a dehydrant (Glauert and Lewis; 1998; Hoppert, 2003; Crang, 1990). The water-replaced specimens are then dried using a critical point dryer or a freeze dryer. Improper fixative procedures (time allowed for the diffusion of the solutions, thickness of specimen as well as concentration of the solvents) can cause changes (e.g. collapsing, distortion, shrinkage) of specimens during critical point

drying. The use of ethanol solution in the conventional sample preparation has been reported to affect the structure of carbohydrates (Serp *et al.*, 2002a, 2002b). Kchlany *et al.* (2001) observed that the EPS produced by *Pseudomonas putida* G7 appeared as strands of fibrils using conventional SEM micrographs. However, it appeared as a continuous layer of EPS in SEM micrographs of the samples prepared with the cryo-fixation method.

For cryo-sample preparation, the procedures can be further sub-divided into freezing in liquid nitrogen, plunge freezing, freezing on the cold surface (cold block freezing), high-pressure freezing and freeze-fracturing and freeze-etching (Hoppert, 2003). Freeze-fracturing followed by freeze-etching is commonly used for preparing delicate biological as well as food specimens that contains a significant amount of water. The specimens are frozen at high freezing rates in order to prevent the formation of large ice crystals, which can damage the structure of specimens. The main factor that controls artefacts (created by large ice crystals formation) is the freezing rate; theoretically, it should be between 1 and 1000 Kelvin s^{-1} (Hoppert, 2003). This condition can be created by freezing specimens under liquid nitrogen slush or using high-pressure freezing (Walther, 2003). The thickness of specimens affects this process. The fracturing process is carried out immediately in a cryo-preparation chamber under vacuum. The specimens are carefully fractured using a cold blade without sweeping action, otherwise this could leave blade marks on the surface. The fractured specimens are then etched or sublimed to partially remove ice crystals covering the structure of interest. The final process is gold-coating

before imaging. Further information about electron microscopy can be found in Hoopert (2003), Glauert and Lewis (1998) and Kuo and Yao (1991).

2.11.2 CSLM: Artefacts and sample preparation

The CSLM is a type of fluorescence microscope. The CSLM-micrographs are constructed from the scanned signals emitted from fluorophores which are excited by projecting laser beams selected at a specific wavelength. Due to the principles of specificity between the excitation wavelengths of laser beams, fluorophores and the target components in the structure of specimens, several target components can be distinguished and observed simultaneously.

The sample preparing procedures for CSLM are much simpler than those for SEM. The selected fluorophore is used to stain the target components. This can be done through two procedures. The first and the most common procedure involves the adsorption of fluorophores into specimens. The fluorophores are then randomly bound to target components. The principle of this method depends mainly on the specificity and affinity between fluorophores and target components. For instance, Fast Green FCF and Nile Blue or Nile Red have been used to stain proteins and lipids in milk gels (Lucey *et al.*, 1998a; Auty *et al.*, 2001). Rhodamine B and lectin conjugated with fluorophores (wheat germ agglutinin and concanavalin A) have been used to stain starch granule and EPS (van de Velde *et al.*, 2002; Hassan *et al.*, 2002b, 2003a). The second procedure is called the covalent labeling technique. van

de Velde *et al.* (2003) covalently labeled proteins and polysaccharide prior to preparation of protein-polysaccharide mixtures. Their CSLM-micrographs showed a good separation between the protein and polysaccharide phases. Nonetheless, according to this method, the target component is needed to be covalently labeled prior to observation, which may not be suitable for bacteria or live specimens.

The downside of using CSLM is the limitation on the resolution and magnification, limitation on the choice of laser light and fluorophores, stability of fluorophores to laser beams (photo bleaching), specificity, accessibility and affinity of fluorophores to target components, alteration of hydrated specimens due to mechanical preparation (Takeuchi and Frank, 2001; Vodovotz *et al.*, 1996). The most crucial point is that the microscope interprets the signal emitted from fluorophores that are attached to specific target components. If the target components have not been stained, this would cause serious artefacts and mislead the interpretation of data. van de Velde *et al.* (2002) showed that rhodamine B, which was used to stain starch granules, accumulated in protein phases when the concentration of whey protein in the mixed starch granules-whey protein solution was increased. There are other problems associated with the imaging technique. Further information about CSLM can be found in Hoppert (2003), Herman (1998), Takeuchi and Frank (2001) and Vodovotz *et al.* (1996).

2.12 Protein-polysaccharide interactions

Foods are heterogeneous in nature. Proteins and polysaccharides are the two major macromolecules in foods. How they co-exist and interact play a significant role in the structure, stability, and the textural and rheological properties of food products. The next two subsections elaborate on the basic principle of protein-polysaccharide interactions as well as their effects on yoghurt gel characteristics.

2.12.1 Basic principles of protein-polysaccharide interactions

Mixing a solution of protein and that of a polysaccharide can possibly yield several systems resulting from their interactions as shown in Figure 2.6 (Tolstoguzov, 1997, 1998, 2000). Those systems include two types of a single phase system (1 and 2) and two types of two phases or phase-separated systems (3 and 4).

2.12.1.1 Complexes or compatible systems

Systems 1 (single phase) and 3 (two phases) can be classified into the same category according to their protein-polysaccharide complex formation. However, they differ in a sense that one system (system 1) contains soluble complexes in a single phase system. Another system (system 3) contains a concentrated insoluble complex in one phase that separated from a depleted biopolymer phase. The complex formation occurs because of the attractive charges of ions in the molecules of proteins and polysaccharides (Tolstoguzov, 1997, 1998, 2000).

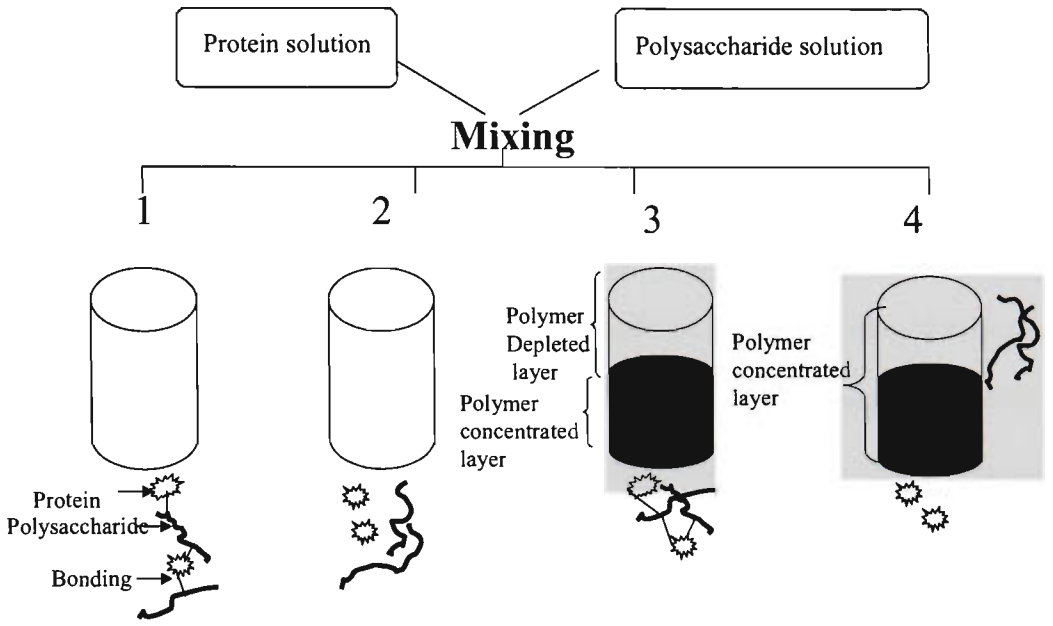

Figure 2.6: A schematic presentation of four possible systems obtained by mixing solutions of a protein and a polysaccharide

The environmental condition, concentration as well as the nature of the individual protein and polysaccharide govern the complex formation (Lundin *et al.*, 2003). At pH values below the isoelectric point of proteins and at low ionic strength, protein molecules have a net positive charge. Usually, the isoelectric point of most food proteins is between pH 4 and 7; at this pH range, some polysaccharides, which contain carboxyl or phosphate groups, have a net negative charge.

2.12.1.2 Limited compatible or incompatible systems

Systems 2 (single phase) and 4 (double phases) are in the same group according to the repulsive interaction between protein and polysaccharide molecules. These systems are called incompatible as they do not form a complex. In system 2, the protein and polysaccharide are co-soluble but no complex formation occurs. System 4 is a result of the system 2 when a critical co-solubility threshold is reached. In system 4, two biopolymers separate into two separate phases. Each phase contains a high concentration of one type of biopolymer. In contrast to complex systems, incompatibility systems usually take place at a high ionic strength and pH values above isoelectric point of proteins. These conditions create like charges (negative charge) and repulsive force between the molecules of proteins and polysaccharides (Tolstoguzov, 1997, 1998, 2000).

The depletion interaction of proteins in a solution can occur due to protein-polysaccharide incompatibility. This phenomenon usually takes place when a system contains two types of biopolymer; the dispersed type and the dissolved type. The incompatibility between the two biopolymers causes a depleted layer of the dissolved biopolymer between the two dispersed biopolymers creating a solvent chemical gradient. These conditions facilitate two dispersed biopolymers to overcome their repulsive barrier causing an aggregation via Brownian motion (Tolstoguzov, 1997).

The factors such as concentration, ratio and type of protein and polysaccharide in solution determine their phase behavior. In the compatible system, the complex between protein and polysaccharide can be thought as a neutralization process. As a result of the process, if the net charges of protein or polysaccharide become zero, the macromolecules lose their hydrophilic properties and solubility, which can then lead to precipitation. In the incompatible system, these factors determine a co-solubility threshold, which controls the change from a single phase co-soluble system (system 2) to a two phase system (system 4). Further details on the topics can be found in Tolstoguzov (1997, 1998, 2000) and Lundin *et al.* (2003).

2.12.2 Yoghurt gel characteristics and protein-polysaccharide interactions

Polysaccharides such as xanthan gum, alginate and vegetable gums have been used for reducing syneresis in set yoghurts and as thickening agents in stirred yoghurts (Tamime and Robinson, 1985). These polysaccharides have been shown to exhibit incompatibility with casein micelles (Schorsch *et al.*, 1999; Tolstoguzov, 1997; Thaiudom and Goff, 2003). Incompatibility of EPS and casein micelles has also been reported (Tuinier and de Kurif, 1999). Schorsch *et al.* (1999) observed that phase separation of locust bean gum-skim milk solution also depended on the intrinsic viscosity of the system. In yoghurt, which is a gel-type product, the phase separation can also occur at macroscopic and microscopic level. As suggested by Tolstoguzov (2000), compatibility of proteins and polysaccharides increases as a result of gelation. Nonetheless, incompatibility still affects gel characteristics, textural, rheological and microstructural properties of yoghurt gels.

EPS produced by yoghurt starter culture behaves like a filler in the protein gel structure of yoghurt (Hassan *et al.*, 2002b, 2003a). Typically, the elastic modulus (G') of such system decreases with increasing volume fraction of the filler (Tolstoguzov, 1997). This is supported by a reduction in the firmness of set yoghurt made using EPS-producing starter cultures as reported by several researchers (Hess *et al.*, 1997; Hassan *et al.*, 1996b). At a microstructural level, Hassan *et al.* (2002b) showed that EPS produced from *S. thermophilus* segregated from milk protein network in yoghurt gel after stirring. This showed an incompatibility between milk proteins and EPS.

Many factors influence physical properties of yoghurts. Understanding interactions between each ingredient and influence of each step during yoghurt manufacture help better control the finished product.

3.0 Materials and Methods

3.1 Starter cultures and maintenance

Non-EPS-producing *S. thermophilus* ASCC 1342 and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466, capsular EPS-producing *S. thermophilus* ASCC 285, and ropy EPS-producing *S. thermophilus* ASCC 1275 were obtained from the Australian Starter Culture Research Centre (Werribee, Australia) and were characterized by Zisu and Shah (2003). The stock cultures were maintained at -80°C in 12% (w/w) RSM and 40% (v/v) sterile glycerol. The microorganisms were activated by growing in 12% (w/w) sterile RSM for 18 h consequently 3 times prior to yoghurt manufacture.

3.2 Enumeration of starter cultures

The counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were determined using the pour plate technique. *S. thermophilus* was incubated aerobically using M17 agar (Amyl Media Pty. Ltd., Dandenong, Australia) at 37°C for 48 h, and *L. delbrueckii* ssp. *bulgaricus* was incubated anaerobically using MRS agar (Merck, Darmstadt, Germany) at 42°C for 48 h (Dave and Shah, 1998).

3.3 General procedure for yoghurt manufacture

Dry ingredients were blended and hydrated with distilled water overnight at 4°C, followed by heat treatment (85°C for 30 min) at natural pH, cooling (42°C), and inoculation with selected starter cultures. The inoculated mix was aseptically poured into separate plastic containers (69 mm top diameter, 60 mm bottom

diameter and 46 mm height) in 100 mL quantities and the incubation was carried out in an airflow oven at 42°C until a pH of 4.70 was reached. The set yoghurt was then transferred into a walk-in-cooler (4°C). For making stirred yoghurt, the set yoghurts after overnight storage were passed through a sieve (pore size ~ 1 mm^2) using a spoon and stored at 4°C for further analysis.

3.4 Analysis of milk blend composition

The composition of milk blend was determined according to the AOAC methods (1995) including total solids (method 925.23), fat (Babcock; method 989.04), ash (method 945.46) and protein contents (Kjeldahl method, % N x 6.38). The concentration of casein protein was determined as the difference between the level of total nitrogen (method 991.20) and non-casein nitrogen (method 998.05). The level of whey protein was determined as the difference between non-casein nitrogen and non-protein nitrogen (method 991.21). The concentration of lactose in milk was quantified enzymatically using a sucrose/lactose/glucose kit (Megazyme, Wicklow, Ireland). The proteins in milk were precipitated by 12% (w/v) trichloroacetic acid (Sigma-Aldrich Co., St. Louis, MO, USA) and filtered out using a Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England). The pH of the clear filtrate was adjusted to 4.50 using 2 M NaOH, and diluted 20 times with 1M acetate buffer pH 4.50.

3.5 Protein profiles, whey protein denaturation and soluble denatured whey protein

The protein profiles of unheated and heated milk samples were observed using discontinuous native-polyacrylamide gel electrophoresis (PAGE) gel system in a mini-Protein II electrophoresis unit (Bio-Rad Laboratories, Richmond, CA, USA). The procedure for preparing native-PAGE gel adapted from Andrews (1983) was used. Separating gel was prepared at 12% (w/v) monomer concentration using 4.0 mL of 30% (w/v) acrylamide/bis-acrylamide (3.3% C) monomer solution (Bio-Rad Laboratories), 2.5 mL of 1.5M Tris/HCl buffer (pH 8.80) and 4.4 mL of distilled water. Stacking gel was prepared at 4% (w/v) monomer concentration using 0.44 mL of the monomer solution, 0.83 mL of 0.5M Tris/HCl (pH 6.80) and 2 mL of distilled water. The separating and stacking gel solutions were degassed. Ammonium persulphate (10% w/w) and N,N,N',N-tetramethylethylenediamine (TEMED) (Bio-Rad Laboratories) were then added to the separating gel solution at 50 μL and 3.3 μL, and to the stacking gel solution at 16.7 μL and 1.7 μL, respectively. Milk samples at 9 and 14% solids were diluted 2 and 5 times, respectively, with distilled water and further diluted 2 times with sample buffer (0.125 M Tris-chloride, 20% (v/v) glycerol, 0.02% bromophenol blue, pH 6.80) prior to loading at 7 μL. The bands in native gel was identified by comparing with previous works of Andrews (1993) and Özer *et al.* (2003) shown in Appendix A.

The level of denatured and soluble denatured whey protein in milk was also investigated using a discontinuous sodium dodecyl sulphate (SDS)-PAGE under reducing conditions. The procedure for separating casein micelles and denatured whey protein bound casein micelles from milk serum (containing soluble denatured

whey protein) described by Anema *et al.* (2004) was followed. Heated milk with or without adjusting to pH 4.60 was centrifuged at 25,000 x g at room temperature for 2 h using an Eppendorf centrifuge model 5471C. Heated milk or its clear supernatant with or without adjusting to pH 4.60 (10μL) was mixed with 10% (w/v) SDS (10 μL) (Sigma-Aldrich) and 2-mercaptoethanol (10 μL) (Bio-Rad Laboratories). The mixtures were heated at 95°C for 10 min, cooled and kept in an ice bath. All samples were diluted 2 times with sample buffer containing 4% SDS as previously described and then loaded at 7 μL. The SDS-PAGE was prepared as described above, except that a portion of water (0.1 mL for separating gel and 33 μL for stacking gel) was replaced with 10% (w/v) SDS solution. Both native- and SDS gels were run at 200 V and stained with Coomassie Blue R-250 dissolved in 7% (v/v) acetic acid and 40% (v/v) methanol solution. Gels were de-stained with several changes of 7% (v/v) acetic acid and 40% (v/v) methanol solution. Images were recorded and quantified using a Fuji Film Intelligent Dark Box II with Fuji Film LAS-1000 Lite V1.3 software. The bands in SDS-PAGE gel were identified by comparing with the previous work of Malin *et al.* (1994) as shown in Appendix B. The density of β-lactoglobulin band (SDS-PAGE) in centrifuged samples (no pH adjustment) was compared against that of heated milk samples.

3.6 Quantification of lactic acid in yoghurt

The concentration of lactic acid in yoghurt was determined using a high performance liquid chromatography (HPLC). The HPLC (Varian, Varian Associates, Walnut Creek, CA, USA) comprised of a solvent delivery system

(model 9100) connecting with an autosampler (model 9012), a UV light detector (model 9050) and an organic acid analysis column (Aminex HPX-87H, 300 x 7.8 mm, Bio-Rad Lab, Richmond, CA, USA). The method of Shin *et al.* (2000) was followed. The mobile phase was 0.009 N H_2SO_4 with a flow rate set at 0.6 mL min^{-1} and the temperature of the column was set at 65°C. Lactic acid was detected at 220 nm. Five grams of the sample were mixed with 100 µL of 15.8 N HNO_3 and 5.9 mL of 0.009 N H_2SO_4 before subjecting a 1.5 mL aliquot of the mixture to centrifugation at 11,600 x g for 15 min at room temperature (~20°C). The supernatant was filtered through a 0.45 µm membrane filter (Schleicher & Schvell, Dassel, Germany) and 50 µL of the filtrate was injected into the HPLC system. Lactic acid standard was purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA).

3.7 EPS isolation, purification and quantification from yoghurt

The EPS in yoghurt was isolated, purified and quantified according to the method of Zisu and Shah (2003). The proteins in 50 mL of diluted yoghurt sample (1:1 ratio of yoghurt:Mili-Q water; Millipore Corp, Bedford, MA, USA) were precipitated with 4 mL of 20% (w/v) trichloroacetic acid (Sigma Chemical Co., St. Louis, MO, USA) and separated by centrifugation (Sorvall RT 7, Kendro Instruments Australia Pty. Ltd., NSW, Australia) at 3,313 x g for 30 min at 4°C. The pH of supernatant was adjusted to 6.80 with 40% (w/v) NaOH followed by boiling at 100°C for 30 min in a sealed container to denature whey proteins, which were separated by centrifugation at 3,313 x g for 30 min at 4°C. An equal volume of cold absolute

ethanol was mixed with the supernatant and left overnight at 4°C to precipitate the carbohydrates (EPS and lactose) followed by separation by centrifugation (3,313 x g, 30 min, 4°C). The resultant pellet of carbohydrates was resuspended by adding 10 mL of Milli-Q water and the suspension was sonicated for 1 h at room temperature using a sonicator (FX 14PH sonication bath; Unisonics Pty Ltd., Sydney, Australia). The suspension was dialysed at 4°C in a dialysis membrane tube of molecular weight cut-off value of 13,000 Da (Carolina Biological Supply Company, NC, USA) against tap water over a 2 weeks period. Water was changed twice a day. The EPS concentration of the suspension in the dialysis tube was quantified using the phenol-sulphuric method of Dubois *et al.* (1956) and was expressed as glucose equivalent.

3.8 Determination of spontaneous syneresis by siphon method

The level of spontaneous syneresis in undisturbed set yoghurt was determined using the siphon method. The method was adapted from that of Lucey *et al.* (1998c) in which the amount of spontaneous whey separated from an acidified milk gel made in a volumetric flask was measured. In our study, a cup of set yoghurt after immediately removing from the walk-in-cooler (4°C) was weighed and kept at approximately 45° angle to allow fast whey collection at the side of the cup with minimum curd damage. A needle connected to a 25 mL syringe was used to siphon the whey from the surface of the product immediately after tilting the cup followed by reweighing the cup. Siphoning was carried out within 10 s to prevent further

leakage of the whey from the gel. The syneresis was expressed as the percent weight of the collected whey over the initial weight of the yoghurt sample.

3.9 Determination of syneresis by drainage method

A cup of set yoghurt was removed from the walk-in-cooler (4°C) and the surface whey was immediately siphoned off as mentioned in Section 3.8. Approximately, thirty grams of the gel was cut in a single action with a stainless steel ladle, weighed and drained on a 1 mm^2 pore size sieve for 15 min at room temperature (20°C). The drained whey was weighed and the syneresis was expressed as the percent weight of the whey separated from the gel over the initial weight of the gel.

3.10 Determination of syneresis by centrifugation method

A cup of set yoghurt was removed from the walk-in-cooler (4°C), and was homogeneously mixed using a glass rod. Approximately thirty grams of the stirred sample were transferred into a 50 mL Blue max™ polypropylene conical tube (Becton Dickinson Laboratory, NJ, USA) and left at 4°C for 2 h for stabilization. The stirred samples were then centrifuged (Sorvall RT 7) at 3,313 x g for 15 min at 10°C. The separated whey was weighed and syneresis was expressed as per cent of the weight of whey separated from the gel over the initial weight of the yoghurt sample.

3.11 Monitoring of yoghurt gel development during fermentation

The storage modulus (G')-time curve of yoghurt was constructed in an oscillatory mode using a RS 50 RheoStress (Haake Rheometer, Karlsruhe, Germany) and a cup and bob, Z20 DIN sensor. Approximately, ten milliliters of inoculated milk (9% and 14% solids) at CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 were loaded into a measuring cup of rheometer. A small amount of vegetable oil was added to cover the surface in order to prevent from moisture loss during fermentation. The yoghurt was fermented for 6 h at 43°C. The values of frequency and stress were set at 0.1 Hz and 0.1 Pa, respectively. The storage modulus (G') was monitored as an index of yoghurt gel development during 6 h of fermentation.

3.12 Gel characteristics of set yoghurt during storage (penetration test)

The characteristics of the set yoghurt gel were examined by a uniaxial compression with a TA-XT.2 Texture Analyzer (Stable Micro System, Goaldming, UK) with a P20 probe (diameter 20 mm) and 25 kg load cell. The speed of the probe was set at 1 mm s^{-1}. The ratio of the diameter of the yoghurt cup to probe was 3.5:1.0. The yoghurt samples were penetrated to 75% of their original height. The test was carried out immediately after removing the samples from the walk-in-cooler (4°C). The fracture was defined as the force at each peak while the firmness was defined as the maximum force in a TPA curve during penetration.

3.13 Determination of yield stress in set yoghurt

A RheoStress 50 (Haake Rheometer, Karlsruhe, Germany) was used to determine the yield stress of the set yoghurt. The yield stress of undisturbed yoghurts was determined using a vane sensor (FL16, d = 16 mm) according to the procedure described by Bhattacharya (1999). The vane sensor was allowed to penetrate into the set yoghurts immediately after removing from the walk-in-cooler (4°C). The shear stress was increased linearly until a breaking point (yield stress) was reached.

3.14 Flow curves and the area of hysteresis loop between upward and downward curves

The flow curve of stirred yoghurts was constructed using a RS 50 RheoStress (Haake Rheometer, Karlsruhe, Germany) equipped with a coaxial measuring cell, Z20 DIN sensor (gap = 0.85 mm). The samples (~ 10 mL) were loaded into the measuring cell using a spoon and the temperature of the sample was allowed to drop to 10°C prior to commencing the measurement. A flow curve was constructed using the method of Halmos and Tiu (1981), Ramaswamy and Basak (1991) and Hassan *et al.* (1996a, 2003a) by increasing the shear rate from 10 to 50 s^{-1} in 200 s (upward curve) followed by decreasing the shear rate from 50 to 10 s^{-1} in 200 s (downward curve). The shear rate in this range was chosen to prevent the slippage effect as discussed by Haque *et al.* (2001). The use of high shear rate (1000 s^{-1}), as used by other researchers (Teggatz and Morris, 1990; Bhattacharya, 1999; Hassan *et al.*, 1996a), would have severely destroyed the structure of the stirred yoghurt resulting in an inability to differentiate the flow behaviour between samples. Therefore,

constructing the flow curve at low shear rate (0 to 50 s^{-1}) would be a suitable for observing flow curve of yoghurt produced with low total solids.

In addition, the area of hysteresis loop between the upward and the downward flow curves was determined. This hysteresis loop represents the breakdown in structure of stirred yoghurt during shearing, as described by Halmos and Tiu (1981) and Ramaswamy and Basak (1991).

3.15 Apparent viscosity

The apparent viscosity of yoghurts was measured using a RS 50 RheoStress (Haake Rheometer, Karlsruhe, Germany) equipped with a coaxial measuring cell, Z20 DIN sensor (gap = 0.85 mm). A small portion of gel (set yoghurt) cut with a teaspoon in a single action and was loaded into the measuring cup of rheometer. The sensor was lowered resulting in the formation of an annular ring of broken gel. For stirred yoghurt, approximately ten millilitre of sample was loaded into the measuring cup using a teaspoon. The cutting and loading procedures were carried out in similar manners for all samples to prevent errors. The temperature of the measuring cup was allowed to drop to 10°C before commencing the measurement. The apparent viscosity was monitored during shearing of the product at a constant shear rate of 10 s^{-1} for 1 min. The value of apparent viscosity at the end of shearing (1 min) was also used for comparison.

3.16 Microstructural study

3.16.1 Confocal Scanning Laser Microscopy

A Leica TCS-SP2 confocal scanning laser microscope (CSLM) equipped with inverted microscope (model Leica DMIRE2, Leica Microsystems, Heidelberg, Germany) was used to examine the microstructure of set yoghurts in the confocal mode using Ar/Kr and He/Ne laser. The magnification of objective lens (HCX PL APO CS, 1.40 NA) was 100 times. Concanavalin A conjugated with Alexa Fluor 488 (Molecular Probes Inc., Eugene, OR, USA) and Fast Green FCF (Sigma Chemical Co., St. Louis, MO, USA) were used to specifically stain EPS and proteins, respectively. The procedure of Hassan *et al.* (2002b) was followed with some modification. A stock solution of concanavalin A at 1 g L^{-1} was prepared by dissolving the dye with 0.1 M sodium bicarbonate at pH 8.30 and stored at –20°C. The working solution was prepared by diluting the stock solution 20 times with whey separated from RSM acidified to a pH value of 4.70 with 1 M HCl by centrifugation at 3,313 x g. The concentration of working solution of Fast Green FCF was 100 mg L^{-1}. A few drops (~400 µL) of both dyes were added on to a piece of yoghurt sample (5 x 5 x 5 mm) on a glass slide and left at 4°C for 1 h. The excess dye was absorbed with a paper towel before covering with a coverslip with the help of UHU® adhesive rubber tac (Saunders Mfg. Co., ME, USA). The rubber tac sealed the gap between the slide and the coverslip preventing any leakage of liquid. The excitation wavelengths were set at 488 nm for concanavalin A conjugated with Alexa Fluor 488 and at 633 nm for Fast Green FCF. The emission wavelength of Alexa Fluor 488 and Fast Green FCF were set between 459 to 559 nm and 668 to

710 nm, respectively. Digital images were acquired in .tiff file format, 8 bits with 512 x 512 resolution.

3.16.2 Conventional SEM

A scanning electron microscope (FEI Philips XL 30, Hillsboro, OR, USA) was used to study the microstructure of set yoghurts made using EPS-producing starter cultures at day 1 using a conventional sample preparation technique. The procedure described by Puvanenthiran *et al.* (2002) was followed, except for the use of critical point drying. Specimens of 2 x 2 x 2 mm were fixed in 2.5% (v/v) glutaraldehyde in 0.03 M sodium cacodylate buffer (pH 7.20) for 1 h followed by dehydration in a series of ethanol solutions (for 10 min each in 20, 40, 60, 70, and 90% v/v) finishing with three changes of 100% (v/v) ethanol. Dehydrated specimens were frozen using liquid nitrogen and kept at a -80°C freezer overnight. Frozen specimens were dried using a Dynavac freeze drier (Model FD 300 freeze drier; Knoxfield, Victoria, Australia) overnight before storing in dry plastic containers at room temperature (~20°C). Before imaging, dry specimens were fractured, mounted on an aluminium stub using a double stick carbon tape and coated with gold/palladium. Images were taken at 20 kV.

3.16.3 Cryo-SEM

A scanning electron microscope (Quanta 200; FEI, Hillsboro, Oregon, USA) attached to a cryo-preparation chamber (Alto 2000; Gatan, Inc., Pleasanton, CA,

USA) was used. Specimens were prepared according to the method described by Hassan *et al.* (2003b), except for etching procedure. A tiny piece of set yoghurt was cut with a scalpel blade and mounted on an aluminum sample holder before plunging into liquid nitrogen slush at -196°C. Frozen specimens were transferred and kept under vacuum in the cryo-preparation chamber followed by fracturing with a cold scalpel blade. The fractured specimen was etched at -95°C for 40 min. The specimens were coated with sputtered gold and transferred onto the cold stage, which was kept at -140°C. Images were taken at 20kV.

3.17 Statistical analysis

All quantitative data in each chapter was analysed with one-way analysis of variance at 95% confidence level. The comparison between means of data (3 replications) was carried out using Tukey's test. The statistical software used was SPSS version 10.0 for Window® (SPSS Inc., NY, USA).

4.0 A PRELIMINARY STUDY ON PHYSICAL PROPERTIES OF SET YOGHURTS AS AFFECTED BY CO-CULTURING WITH NON-EPS- AND EPS-PRODUCING STARTER CULTURES AND SUPPLEMENTATION WITH WHEY PROTEIN CONCENTRATE

4.1 Introduction

Some strains of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* produce EPS. These EPS are heteropolysaccharides and can be classified as capsular EPS (which is tightly bound to the cell surface) and ropy EPS (which is excreted into the surrounding environment) (Broadbent *et al.*, 2003; Zisu and Shah, 2003; Cerning, 1990; De Vuyst and Degeest, 1999). The EPS-producing starter cultures are of interest in yoghurt manufacture due to their water-binding ability and texture promoting property (Broadbent *et al.*, 2003; Cerning, 1990; De Vuyst and Degeest, 1999). Hassan *et al.* (2002a) have reported that set yoghurts made using capsular EPS-producing starter cultures formed gel earlier than that made with non-EPS- or ropy EPS-producing starter cultures. Wacher-Rodarte *et al.* (1993) observed that the use of ropy EPS-producing starter cultures reduced syneresis but increased viscosity considerably. The results of sensory evaluation from their study also suggested that ropy yoghurts had better mouthfeel and were most acceptable compared to the

control yoghurt. Marshall and Rawson (1999) reported that the use of ropy EPS-producing starter cultures increased the apparent viscosity of stirred yoghurts substantially. Hassan *et al.* (1996b) observed a reduction in firmness and level of syneresis in set yoghurts made using EPS-producing starter cultures. Cerning *et al.* (1988) observed that the amount of EPS strongly affected the viscosity of fermented milks. However, EPS-producing starter cultures generally produce EPS at small amounts ranging from 40 to 400 mg L^{-1} (De Vuyst *et al.*, 2003; Broadbent *et al.*, 2003; Cerning, 1990; De Vuyst and Degeest, 1999). Several factors including pH, temperature and composition of medium such as the level of carbon and nitrogen source are reported to affect the production of EPS by starter cultures (Cerning *et al.*, 1988; Degeest and De Vuyst, 1999; Cerning *et al.*, 1994; Hassan *et al.*, 2001; Mozzi *et al.*, 1996). Zisu and Shah (2003) reported that the EPS concentration produced by a ropy EPS-producing *S. thermophilus* ASCC 1275 fermented in RSM increased from 406 mg L^{-1} to 1029 mg L^{-1} when the RSM was supplemented with 0.5% WPC, and to 832 mg L^{-1} by the use of co-culturing of non-EPS- and EPS-producing starter cultures in RSM without supplementation with WPC. The fermentation was carried out for 24 h maintaining a constant pH of 5.50 throughout the process. Both the supplementation with WPC and co-culturing appear to increase the EPS concentration in yoghurt. In addition, supplementation with WPC influences physical properties of set yoghurt. Guzmán-González *et al.* (1999) observed lower apparent viscosity and firmness in yoghurts supplemented with WPC than the control yoghurt without supplementation. However, the level of syneresis decreased substantially. Baig and Prasad (1996) and Bhullar *et al.* (2002)

found that the supplementation with WPC improved the apparent viscosity and textural properties of yoghurts.

The combined use of WPC and co-culturing of non-EPS- and EPS-producing starter cultures might further increase EPS production as compared to that of EPS-producing strain alone, which in turn may help improve physical properties of set yoghurts.

4.2 Aim

This study investigated the changes in physical properties of nonfat set yoghurts made with non-EPS- or EPS-producing starter cultures separately or in combination (co-culturing), with or without supplementation with 0.5% WPC.

4.3 Materials and Methods

4.3.1 Experimental design

This study examined the physical properties of set yoghurts made with or without WPC supplementation and use of non-EPS-, ropy EPS-producing starter cultures or co-culturing of non-EPS- and ropy EPS-producing starter cultures. Six batches of set yoghurts were produced as shown in Table 4.1.

Table 4.1: Summary of types of milk and starter cultures used for the production of six batches of yoghurts

Batches	Types of milk	Starter cultures
1	12% (w/w) SMP	**Non-EPS**: 1% (v/v) of *S. thermophilus* ASCC 1342 (non-EPS) + 1% of *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
2	12% (w/w) SMP	**Ropy EPS**: 1% of *S. thermophilus* ASCC 1275 (ropy EPS) + 1% of *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
3	12% (w/w) SMP	**Co-culturing**: 0.75% of *S. thermophilus* ASCC 1275 (ropy EPS) + 0.25% of *S. thermophilus* ASCC 1342 (non-EPS) + 1% of *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
4	11.5% SMP + 0.5% WPC	**Non-EPS**: 1% (v/v) of *S. thermophilus* ASCC 1342 (non-EPS) + 1% of *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
5	11.5% SMP + 0.5% WPC	**Ropy EPS**: 1% of *S. thermophilus* ASCC 1275 (ropy EPS) + 1% of *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
6	11.5% SMP + 0.5% WPC	**Co-culturing**: 0.75% of *S. thermophilus* ASCC 1275 (ropy EPS) + 0.25% of *S. thermophilus* ASCC 1342 (non-EPS) + 1% of *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)

The parameters that were evaluated included the concentration of EPS, firmness, susceptibility to syneresis, yield stress and apparent viscosity of set yoghurts after overnight storage. In addition, the viable counts of starter cultures at inoculation and after overnight storage (4°C) were also carried out. The experiment was carried out in triplicate.

4.3.2 Starter culture

Non-EPS producing *S. thermophilus* ASCC 1342 and non-EPS-producing *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 were used in this study. The maintenance

and activation of these bacteria were carried out as described in Chapter 3, Section 3.1.

4.3.3 Yoghurt manufacture

Six batches of reconstituted low-fat yoghurts were prepared as described in Section 4.3.1 and Table 4.1. Two types of reconstituted milk were prepared at 12% (w/w) total solids using low heat SMP (34% protein; Murray Goulbourn Co-operative Co. Ltd., Brunswick, Australia) or 11.5% (w/w) SMP and 0.5 % (w/w) WPC 80 (80.4% protein; New Zealand Dairy Board, Wellington, New Zealand). The procedure for yoghurt manufacture as described in Chapter 3, Section 3.3 was followed. Table 4.2 represents the codes of these yoghurts. All measurements were carried out in triplicate on the following day.

4.3.4 Enumeration of starter cultures

The counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* at inoculation and after overnight storage (4°C) were determined as described in Chapter 3, Section 3.2.

4.3.5 EPS isolation, purification and quantification

The EPS in yoghurt was isolated, purified and quantified according to the procedures described in Chapter 3, Section 3.7.

4.3.6 Susceptibility of yoghurt to syneresis

The susceptibility of yoghurt to syneresis was determined by the centrifugation method as described in Chapter 3, Section 3.10.

4.3.7 Firmness

The firmness of set yoghurts was determined using TA-XT.2 Texture Analyzer. The measurement was carried out according to procedure described in Chapter 3, Section 3.12 immediately after the sample was removed from the walk-in-cooler (4°C).

4.3.8 Yield stress and apparent viscosity

The yield stress and apparent viscosity of set yoghurts were determined by a RheoStress 50 (Haake Rheometer, Karlsruhe, Germany) equipped with a vane sensor (FL16, d = 16 mm) according to the procedure described in Chapter 3, Sections 3.13 and 3.14, respectively.

4.3.9 Statistical analysis

The data were analysed using one-way analysis of variance at 95% confidence level with SPSS version 10 for Windows (SPSS Inc., NY, USA). The means of data were compared using Tukey's test.

4.4 Results and Discussions

4.4.1 Viable counts

Table 4.3 shows the viable counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* after inoculation and 1 day after storage (4°C) in set yoghurts made with non-EPS- and EPS-producing starter cultures as well as by co-culturing of non-EPS- and EPS-producing starter cultures with or without 0.5% WPC. The enumeration technique used was not able to distinguish between the colonies of non-EPS- and EPS-producing *S. thermophilus* in products made using the co-culture. However, the overall counts were similar in all products. The supplementation of milk with 0.5% WPC decreased the counts of *L. delbrueckii* ssp. *bulgaricus*. Baig and Prasad (1996) observed that the addition of fresh cottage cheese whey to the yoghurt mix increased the growth of both *S. thermophilus* and *Bifidobacterium bifidum*. Zisu and Shah (2003) reported that the supplementation of RSM with 0.5% WPC increased EPS production by *S. thermophilus*. Bury *et al.* (1998) reported that supplementation with WPC to yoghurt enhanced lactic acid production by starter cultures. This may explain why there was a reduction in the fermentation time in our study. Reduction in fermentation time limits the time allowed for the growth of *L. delbrueckii* ssp. *bulgaricus*.

4.4.2 EPS concentration

The concentration of EPS in yoghurts made with non-EPS- and EPS-producing starter cultures and with co-culturing of non-EPS- and EPS-producing starter

cultures with or without 0.5% WPC is shown in Table 4.4. The EPS concentration in products made with or without 0.5% WPC supplementation using non-EPS-producing starter cultures (S-non-EPS and SW-non-EPS), ~20 mg L^{-1}, was not significantly different ($P > 0.05$). The *S. thermophillus* ASCC 1342 was later confirmed by CSLM as EPS producer. The concentration of EPS in products made without supplementation with WPC using EPS (S-EPS) was not significantly different ($P > 0.05$) from those made with co-culturing of non-EPS- and EPS-producing starter cultures (S-non-EPS+EPS). Although the EPS concentration in products made using EPS-producing starter cultures and supplemented with WPC (SW-EPS) was higher (100 mg L^{-1}) than that with RSM (S-EPS), the difference was not statistical significant ($P > 0.05$). The use of co-culture and WPC (SW-non-EPS+EPS) further increased EPS concentration to around 120 mg L^{-1}. However, the increase in EPS concentration did not correlate with the increase in the viable counts of EPS-producing *S. thermophilus* strain as compared to that of non-EPS-producing strain (Table 4.3). Although Zisu and Shah (2003) reported that *S. thermophilus* ASCC 1275, also used in this study, produced EPS at 1,029 mg L^{-1} when RSM was supplemented with 0.5% of WPC and subjected to fermentation in a control pH fermentor (pH 5.50) over a 24 h period, the maximum concentration of EPS detected in yoghurts in our study was only 126 mg L^{-1}. This might be due to shorter fermentation time (6 h) as well as a drop in pH during yoghurt fermentation. Generally, the activity of *S. thermophilus* slows down when the pH of milk decreases to ~5.4 (Shah, 2003). The decrease in pH during incubation may slow down the activities and EPS production by *S. thermophilus*.

4.4.3 Firmness of set yoghurts

Figure 4.1 shows the firmness of set yoghurts made with non-EPS- and EPS-producing starter cultures and with co-culturing of non-EPS- and EPS-producing starter cultures with or without 0.5% WPC. The yoghurts made using EPS (S-EPS and SW-EPS) and co-culture of non-EPS- and EPS (S-non-EPS+EPS and SW-non-EPS+EPS) starter cultures were significantly ($P < 0.05$) softer than those made using non-EPS-producing starter cultures (S-non-EPS and SW-non-EPS). These results are in agreement with those of other researchers (Hassan *et al.* 1996b; Marshall and Rawson, 1999; Hess *et al.*, 1997). Incompatibility between EPS and milk proteins may cause the depletion interaction of milk proteins (de Kruif and Tuinier, 2001), affecting the aggregation and gelation of milk proteins. The presence of EPS may also interfere with the continuity and integrity of protein gel network. As a result, there was a reduction in the firmness of yoghurts. There was no significant difference in firmness of products made using non-EPS-producing starter cultures with or without supplementation with 0.5% WPC ($P > 0.05$).

4.4.4 Susceptibility of set yoghurt to syneresis

The susceptibility of set yoghurts made with different type of starter cultures with or without supplementation with WPC to syneresis is shown in Figure 4.2. Theoretically, the results (from centrifugation method as carried out in this study) did not represent the actual level of syneresis or spontaneous whey separation on the surface of the yoghurt. It represents the tendency of yoghurt gels to synerese.

However, the term syneresis has been traditionally used and will be used in this chapter. The yoghurts supplemented with WPC (SW-non-EPS, SW-EPS and SW-non-EPS+EPS) showed a significant decrease in the level of syneresis as compared to those made with RSM (S-non-EPS, S-EPS and S-non-EPS+EPS). Both products made using EPS-producing starter cultures or co-culture and supplementation with WPC (SW-EPS and SW-non-EPS+EPS) gave the lowest level of syneresis. The syneresis levels were not significantly different, but were significantly lower by approximately 10% and 5%, respectively than the products made with RSM using non-EPS-starter cultures (S-non-EPS) and that with WPC supplementation (SW-non-EPS). This could be due to the combined effects of water-binding ability of EPS and WPC (Walzem *et al.* 2003; Broadbent *et al.* 2003; Cerning, 1990; De Vuyst and Degeest, 1999). The supplementation with WPC may have both direct and indirect effects on syneresis. The supplementation increases the protein content of yoghurts that may cause an increase in the density of protein matrix (Bhullar *et al.* 2002; Puvanenthiran *et al.* 2002) leading to a reduction in the susceptibility to syneresis. Supplementation with WPC also increased the EPS concentration (Table 4.3) in yoghurts thus further preventing syneresis.

4.4.5 Yield stress

The yield stress of set yoghurts made with various types of starter cultures with or without 0.5% of WPC is shown in Figure 4.3. This value represents the minimum stress value required to initiate the deformation of yoghurt gels. The yield stress increased as a result of the use of EPS-producing starter cultures, co-culture with non-EPS- and EPS-producing starter cultures as well as with WPC supplementation

(0.5%). However, the product made using EPS-producing starter cultures (SW-EPS) supplemented with WPC had yield stress values similar to non-supplemented yoghurts made with EPS-producing starter cultures (S-EPS) or co-culture (S-non-EPS+EPS). The supplementation with WPC showed a greater effect on the yield stress as compared to the use of EPS-producing starter cultures or co-culture of non-EPS- and EPS-producing starter cultures. This could be due to an increase in total protein content (4.08% in products containing RSM vs. 4.31% in those containing WPC, based on calculation) as well as a decrease in CN to WP ratio in products supplemented with WPC.

4.4.6 Apparent viscosity

The apparent viscosity of yoghurts made with different types of starter cultures with or without WPC supplementation (0.5%) is shown in Figure 4.4. The apparent viscosity of yoghurts made using non-EPS-producing starter cultures (S-non-EPS and SW-non-EPS) was higher than other products (S-EPS, S-non-EPS+EPS, SW-EPS, and SW-non-EPS+EPS). Marshall and Rawson (1999) observed higher apparent viscosity in stirred yoghurts made with ropy EPS-producing starter cultures compared to those made with non-EPS-producing starter cultures. Bhullar *et al.* (2002) and Guzmán-González *et al.* (1999) reported that stirred yoghurts fortified with WPC had higher apparent viscosity than the unfortified yoghurts. The difference between our results and those of others may be caused by sample preparation procedures as well as the types of instruments (Brookfield, Helipath with T spindle vs. Haaka Rheometer, a cup and bob measuring cell). For instance,

Bhullar *et al.* (2002) pre-stirred their samples prior to the measurement with a Brookfield viscometer (Helipath drive with a T spindle). In our study, a portion of set yoghurt was cut and loaded into the measuring cup of the rheometer using a teaspoon. Then, the probe was lowered breaking the yoghurt gel creating an annular ring of broken gel between the probe and the cup. After the temperature was allowed to equilibrate at 10°C, the change in apparent viscosity was followed immediately during 1 min of shearing at the shear rate of 10 s^{-1}. This condition may only break some protein gel structure of set yoghurts unlike the stirred yoghurt, which already has higher degrees of gel deformation before commencing the measurement. Therefore, the higher value of viscosity in the control product (S-non-EPS) may be explained by less structural damage of the product during 1 min of shearing. This may suggest that the effects of ropy EPS on the apparent viscosity would be predominant in stirred yoghurts because the protein gel structure is severely broken as compared to set yoghurt.

Furthermore, the products made with supplementation of WPC using non-EPS-producing starter cultures (SW-non-EPS) had lower apparent viscosity than those without WPC supplementation and non-EPS-producing starter cultures (S-non-EPS). This may suggest higher structural deformation during shearing of the former.

4.5 Conclusions

The use of EPS-producing starter cultures or co-culture with non-EPS and EPS-producing starter cultures as well as supplementation with 0.5% WPC significantly

affected physical properties of set yoghurts. The firmness, apparent viscosity and syneresis decreased, but the yield stress increased in set yoghurts made using EPS or co-culture. The products supplemented with 0.5% WPC had firmer texture. The EPS concentration increased in products supplemented with WPC. A further increase in the EPS concentration was observed only in the product made using co-culture of non-EPS- and EPS-producing starter cultures. The supplementation with WPC decreased the bacterial count of *L. delbrueckii* ssp. *bulgaricus*.

It is interesting to note that there was a significant reduction in the syneresis on supplementation with 0.5% WPC as well as the use of EPS producing starter cultures. However, the level of syneresis is not an actual level of spontaneous syneresis in set yoghurts. The results determined using the centrifugation method may have been influenced by other factors such as centrifugation force or the damage to gels. Hence, a suitable method for the determination of spontaneous syneresis is needed. In addition, this preliminary study did not focus on the effects caused by the variation in the quantity of WPC used for supplementation and that of total protein. Supplementation as in our study would inevitably increase the total protein content as well as change the ratios of CN to WP of milk. In order to study the specific effect of varying the ratios of CN to WP on physical properties of yoghurts, it is therefore necessary to keep both solids and protein contents constant.

Table 4.2: Codes and descriptions of types of yoghurts

Codes	Descriptions
S-non-EPS	Set yoghurt made with 12% RSM using non-EPS-producing starter cultures
S-EPS	Set yoghurt made with 12% RSM using EPS-producing starter cultures
S-non-EPS+EPS	Set yoghurt made with 12% RSM using co-culturing of non-EPS- and EPS-producing starter cultures
SW-non-EPS	Set yoghurt supplemented with 0.5% WPC using non-EPS-producing starter cultures
SW-EPS	Set yoghurt supplemented with 0.5% WPC using EPS-producing starter cultures
SW-non-EPS+EPS	Set yoghurt supplemented with 0.5% WPC using co-culturing of non-EPS- and EPS-producing starter cultures

Table 4.3: Viable counts of *S. thermophilus* (ST) and *L. delbrueckii* ssp. *bulgaricus* (LB) in set yoghurts made using non-EPS-, EPS- and co-culture of non-EPS- and EPS-producing starter cultures with or without supplementation with 0.5% WPC, after inoculation and 1 day of storage at 4°C

Treatments	Bacterial count (log_{10}(cfu g^{-1}))[1]					
	ST			LB		
	Inoculation	Overnight storage	Growth	Inoculation	Overnight storage	Growth
S-non-EPS	6.66	8.39	1.73	5.43	7.83	2.40
S-EPS	6.72	8.59	1.87	5.42	7.87	2.45
S-non-EPS+EPS	6.57	8.59	2.02	5.35	7.84	2.49
SW-non-EPS	6.55	8.59	2.04	5.87	7.40	1.53
SW-EPS	6.63	8.58	1.95	5.78	7.87	2.09
SW-non-EPS+EPS	6.71	8.75	2.04	6.10	7.91	1.81

[1] n = 3

Table 4.4: EPS concentration in set yoghurts made using non-EPS-, EPS- and co-culture of non-EPS- and EPS-producing starter cultures with or without supplementation with 0.5% WPC

Treatments	EPS concentration (mg L^{-1})[1, 2]
S-non-EPS	20.54 ± 0.74^{c}
S-EPS	92.67 ± 1.12^{b}
S-non-EPS+EPS	91.89 ± 3.34^{b}
SW-non-EPS	19.35 ± 2.05^{c}
SW-EPS	$106.10 \pm 5.95^{a,b}$
SW-non-EPS+EPS	126.38 ± 11.01^{a}

[1] n = 3
[2] Presented value show mean ± standard deviation
[3] Values within the same column not sharing the same superscript differ significantly ($P < 0.05$)

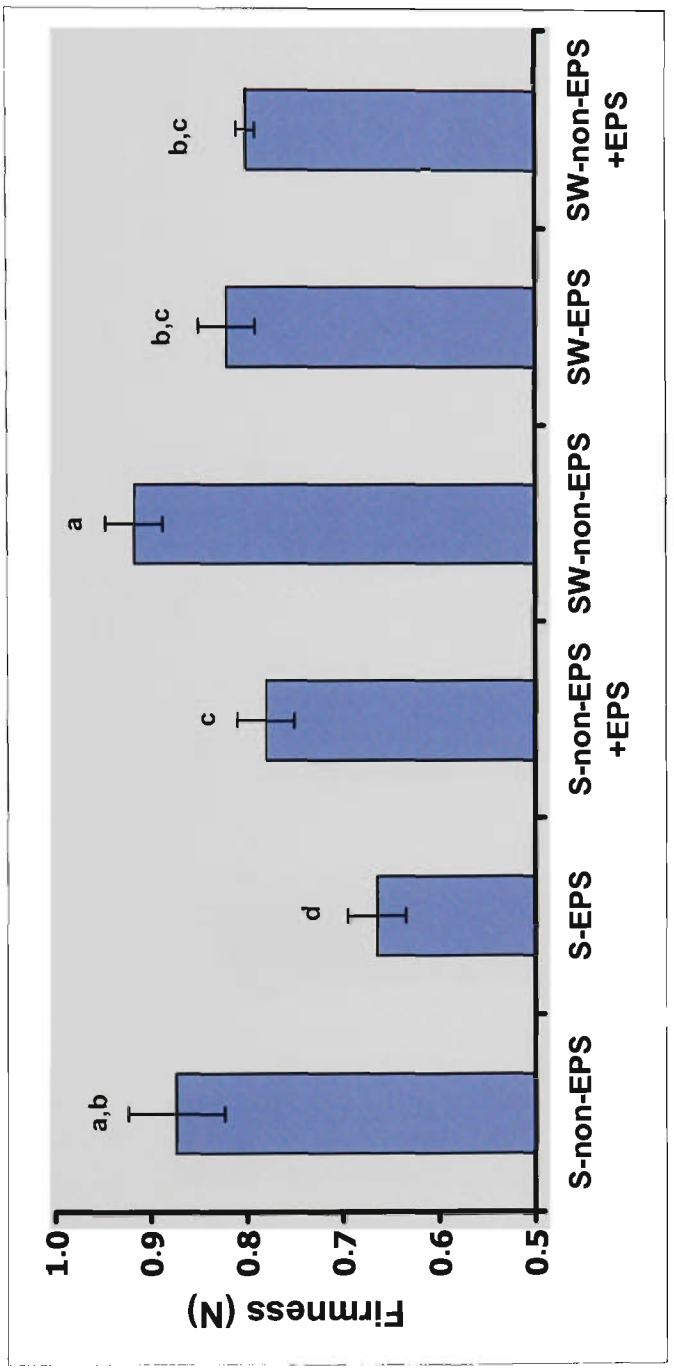

Figure 4.1: Firmness in set yoghurts made using different types of starter cultures and with or without supplementation with 0.5% WPC. Error bars represent standard deviation (n = 3). Bars that do not share same letters (a, b, c, d) are significantly different (P < 0.05).

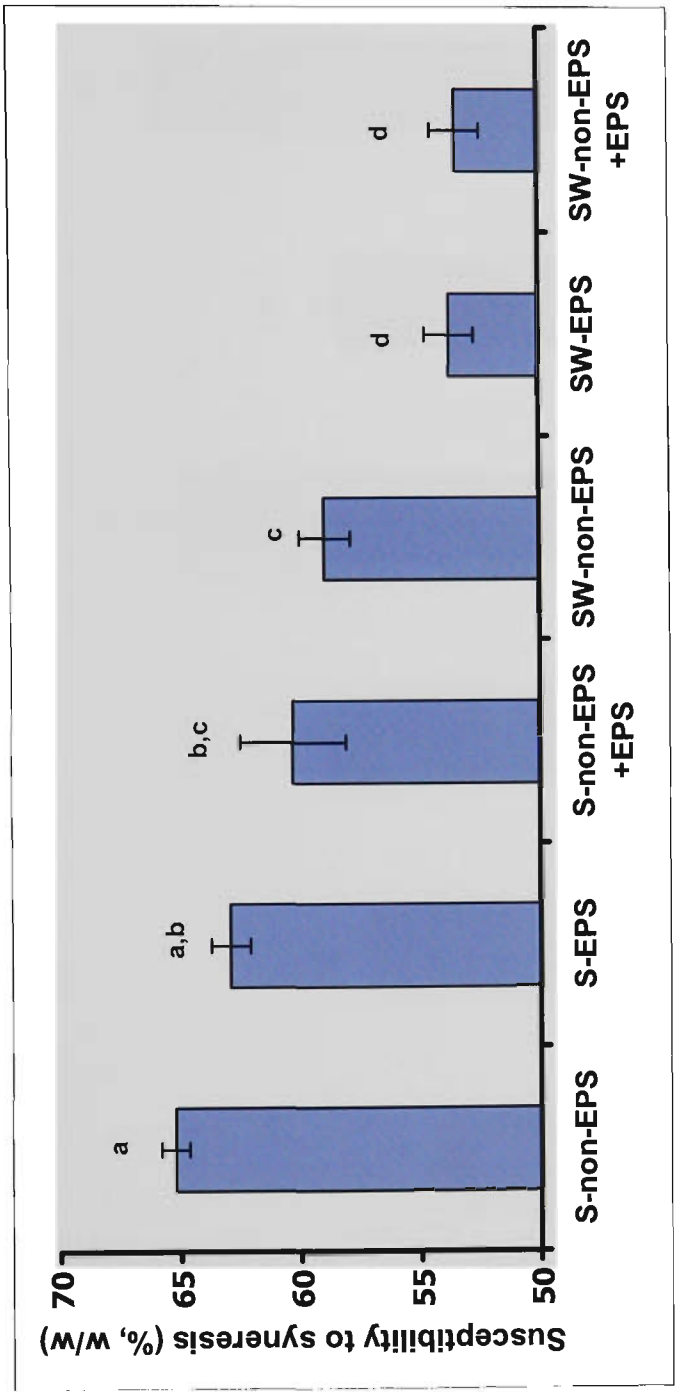

Figure 4.2: Susceptibility to syneresis of set yoghurts made using various types of starter cultures and with or without supplementation with 0.5% WPC. Error bars represent standard deviation (n = 3). Bars that do not share same letters (a, b, c, d) are significantly different ($P < 0.05$).

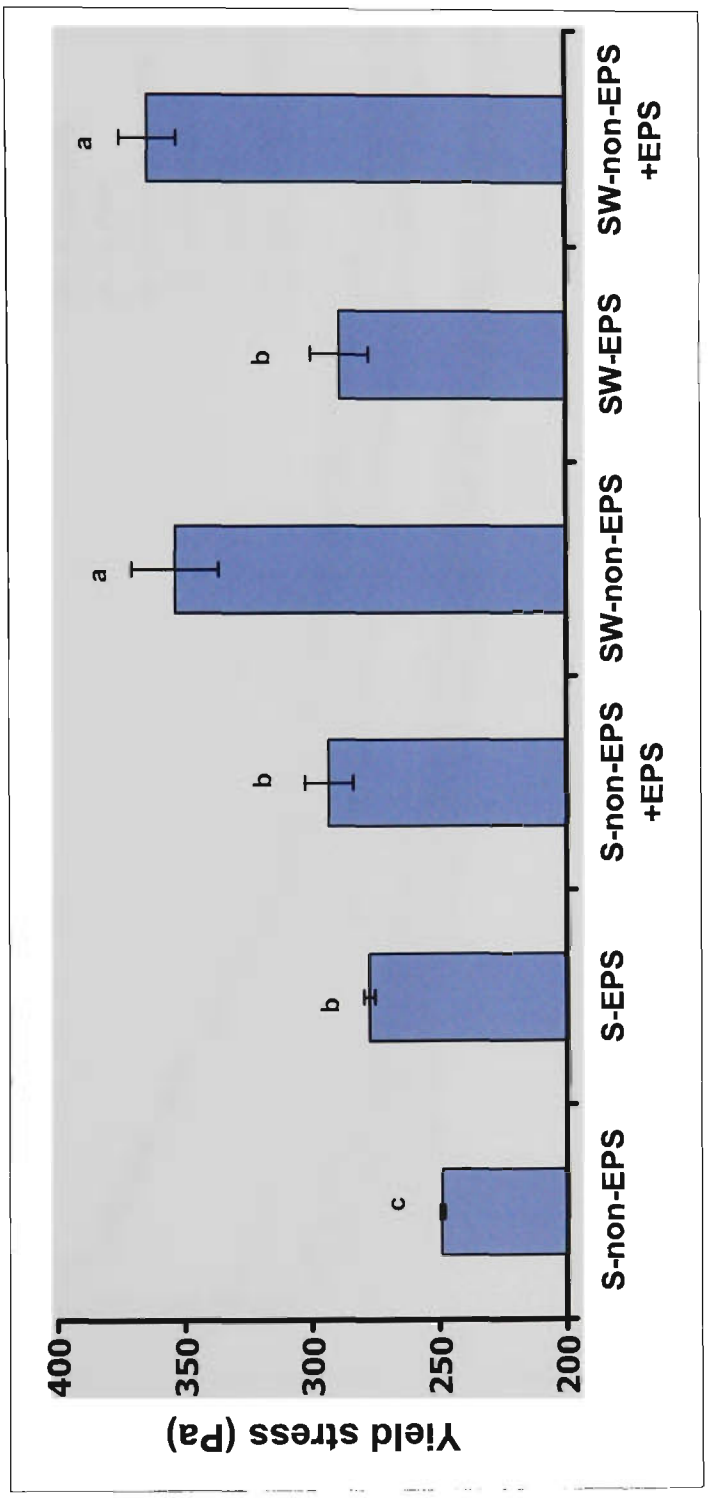

Figure 4.3: Yield stress of set yoghurts made using various types of starter cultures and with or without supplementation with 0.5% WPC. Error bars represent standard deviation (n = 3). Bars that do not share same letters (a, b, c, d) are significantly different ($P < 0.05$).

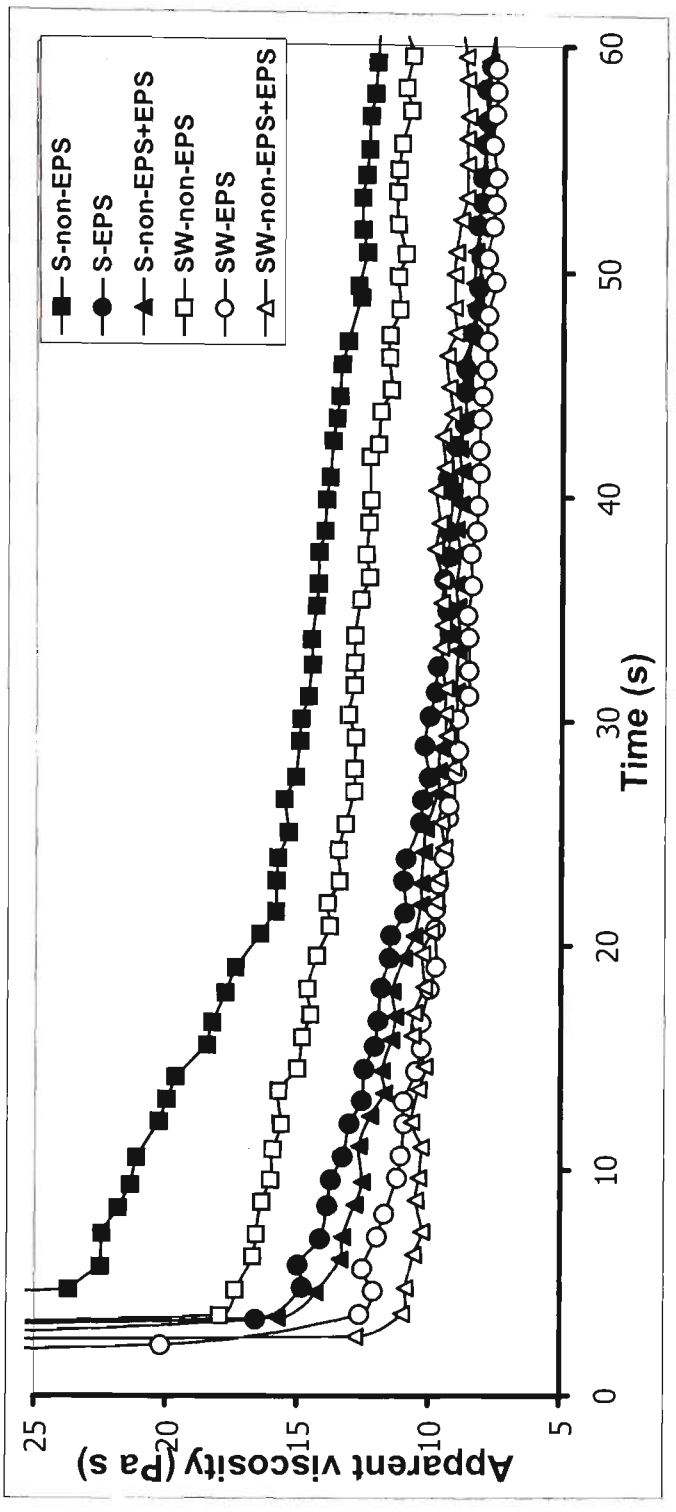

Figure 4.4: Apparent viscosity of set yoghurts made using various types of starter cultures and with or without supplementation with 0.5% WPC (n = 3).

5.0 A COMPARATIVE STUDY OF THREE METHODS USED FOR THE DETERMINATION OF SYNERESIS IN SET YOGHURTS MADE AT 9% AND 14% TOTAL SOLIDS WITH NON-EPS- AND EPS-PRODUCING STARTER CULTURES

5.1 Introduction

A spontaneous whey separation on the surface of a set yoghurt also known as "syneresis" is regarded as a defect. This problem can be reduced or eliminated by increasing the level of milk solids to ~ 15% (w/w) (Shah 2003; Tamime and Deeth, 1986). Other alternatives to reduce syneresis include the use of stabilizers such as starch, gelatine, vegetable gum or exopolysaccharide (EPS) - producing starter cultures.

To determine the best strategy for preventing syneresis (spontaneous whey separation) in set yoghurt, a correct method for its determination is essential. The drainage and centrifugation methods are commonly used for the determination of syneresis (Bhullar *et al.*, 2002; Guzmán-González *et al.*, 1999, 2000; Harwalkar and Kalab, 1986; Jaros *et al.*, 2002). For the drainage method, a certain quantity of set yoghurt (disturbed or undisturbed gel) is placed on a sieve over a certain period at a fixed temperature and the whey is allowed to drain under the gravitational force.

Syneresis is calculated as the percent weight of the drained whey over the initial weight of the gel. The centrifugation method is generally used for studying the water holding capacity of yoghurt. A certain quantity of set yoghurt (disturbed or undisturbed gel) is centrifuged at a specified speed over a certain period of time at a fixed temperature. Syneresis is calculated as the percent weight of the separated whey over the initial weight of the gel. Although these methods give results with high precision, they do not represent the actual value of spontaneous syneresis in a set yoghurt. Breakage of the yoghurt gel as well as the presence of EPS may influence the result. Lucey *et al.* (1998c) developed a method for the measurement of spontaneous syneresis of acidified milk gels made in a volumetric flask. The spontaneous whey separated on the surface of the gel was collected by tilting the volumetric flask. The objective of their method was to collect the whey on the surface without damaging the gel. However, the method cannot be used for the measurement of the spontaneous syneresis of set yoghurt in the actual container. In addition, the difference in geometry of the yoghurt cup and that of volumetric flask may affect the heat transfer and fermentation of the product. Hence, a new method that can measure the level of spontaneous syneresis in an actual yoghurt container is required.

5.2 Aim

This study compared three methods used for the determination of syneresis in set yoghurts made at 9% and 14% (w/w) total solids using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures. These methods included the drainage-,

centrifugation- and siphon method. The siphon method was developed based on Lucey *et al.* (1998c) with some modifications.

5.3 Materials and Methods

5.3.1 Experimental design

Three methods used for the determination of syneresis including the drainage, centrifugation and siphon were compared for their suitability in determination of spontaneous syneresis in six batches of set yoghurts made at 9% or 14% total solids using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures as shown in Table 5.1. The concentration of lactic acid and EPS in set yoghurt was also quantified. The experiment was carried out in triplicate.

Table 5.1: Summary of types of milk and starter cultures used for the production of six batches of yoghurts

Batches	Types of milk	Starter cultures
1	9% SMP	**Non-EPS**: 1% (v/v) each of *S. thermophilus* ASCC 1342 (non-EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
2	9% SMP	**Capsular EPS**: 1% (v/v) each of *S. thermophilus* ASCC 285 (capsular EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
3	9% SMP	**Ropy EPS**: 1% (v/v) each of *S. thermophilus* ASCC 1275 (ropy EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
4	14% SMP	**Non-EPS**: 1% (v/v) each of *S. thermophilus* ASCC 1342 (non-EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
5	14% SMP	**Capsular EPS**: 1% (v/v) each of *S. thermophilus* ASCC 285 (capsular EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
6	14% SMP	**Ropy EPS**: 1% (v/v) each of *S. thermophilus* ASCC 1275 (ropy EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)

5.3.2 Starter culture

Non-EPS-producing *S. thermophilus* ASCC 1342 and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466, capsular EPS-producing *S. thermophilus* ASCC 285, and ropy EPS-producing *S. thermophilus* ASCC 1275 were used in this study. The maintenance and activation of these bacteria were carried out as described in Chapter 3, Section 3.1.

5.3.3 Yoghurt manufacture

Six batches of low-fat yoghurts were made at 9% and 14% total solids with reconstituted SMP and using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures. The yoghurt samples were made in triplicate. The procedure for yoghurt manufacture as described in Chapter 3, Section 3.3 was followed.

5.3.4 Quantification of lactic acid

The concentration of lactic acid in yoghurt was determined using a high performance liquid chromatography (HPLC) according to the procedures described in Chapter 3, Section 3.6.

5.3.5 EPS isolation, purification and quantification

EPS in yoghurts was isolated, purified and quantified according to the procedures described in Chapter 3, Section 3.7.

5.3.6 Determination of spontaneous syneresis by siphon method

The level of spontaneous syneresis in undisturbed set yoghurt was determined using a siphon method as described in Chapter 3, Section 3.8.

5.3.7 Determination of syneresis by drainage method

The level of syneresis in yoghurts was determined by the drainage method as described in Chapter 3, Section 3.9.

5.3.8 Determination of syneresis by centrifugation method

The level of syneresis in yoghurts was determined by the drainage method as described in Chapter 3, Section 3.10.

5.3.9 Statistical analysis

The data were analysed using one-way analysis of variance at 95% confidence level with SPSS version 10 for Windows (SPSS inc., NY, USA). The Tukey's test was used in multiple comparison tests.

5.4 Results and Discussions

5.4.1 Lactic acid concentration

The concentration of lactic acid in set yoghurts made at 9% and 14% (w/w) solids using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures is shown in

Table 5.2. In general, the lactic acid concentration was higher in products made at 14% solids than that at 9%. The capsular EPS-producing starter cultures produced the lowest lactic acid concentration in both 9% and 14% yoghurts. The reason why capsular EPS producing starter cultures produced the lowest lactic acid concentration is unknown. The increase in the concentration of lactic acid in yoghurts at 14% solids suggested the influence of increased nutrients as a result of increasing milk solids content on the activities of bacteria. Other workers have also shown that the increase in the concentration of available nutrients (supplementation of media with yeast extract and WPC) affected the EPS and lactic acid production by lactic acid bacteria (Amrane and Prigent, 1998; Zisu and Shah, 2003; Hassan *et al.*, 2001).

5.4.2 EPS concentration

The EPS concentration in set yoghurts made at 9% and 14% total solids using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures is shown in Table 5.3. Yoghurts made at 14% solids content produced more EPS than those at 9% solids (~100 vs. ~40 mg L^{-1}). The level of EPS was detected at 9.10 and 20.77 mg L^{-1} in yoghurts made at 9% and 14% solids level using non-EPS-producing starter cultures. The EPS concentration was not significantly different ($P > 0.05$) between products made using capsular EPS and ropy EPS-producing starter cultures. This was found at both 9% and 14% solids levels. These results suggest that EPS production was enhanced by the increase in milk solids content. This phenomenon was also observed in the previous experiment (Chapter 4) where supplementation

with 0.5% WPC enhanced the production of EPS. This agreed with the results reported by Zisu and Shah (2003) who observed that the supplementation of 0.5% WPC to 10% RSM enhanced the production of EPS during fermentation.

5.4.3 Syneresis as determined by the siphon method

Figure 5.1 shows the levels of spontaneous syneresis determined by the siphon method in yoghurts made at 9 and 14% solids using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures. At 9% solids, the yoghurt made using EPS-producing starter cultures showed a significant decrease in the level of syneresis ($P < 0.05$) as compared to the products made with non-EPS-producing starter cultures. The products made using capsular EPS-producing starter cultures showed the lowest values. Only a small decrease in syneresis was observed in both capsular and ropy yoghurts as a result of raising solids content from 9 to 14%, but this decrease was not statistically significant. However, a significant decrease in the level of syneresis ($P < 0.05$) was observed in the product made with non-EPS-producing starter cultures.

5.4.4 Syneresis as determined by the drainage method

The level of syneresis determined by the drainage method in yoghurts made at 9% and 14% solids using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures is shown in Figure 5.2. This method could not differentiate the level of syneresis among products made using non-EPS-, capsular EPS- or ropy EPS-

producing starter cultures, at both 9% and 14% solids levels. As the yoghurt gels were cut prior commencing the drainage, an excessive leakage of whey at the cut surface of gels may explain this result. However, the level of syneresis decreased by around 25% when the total solids were increased to 14%. This was in agreement with the results of Jaros *et al.* (2002) and Harwalkar and Kalab (1986), who observed a reduction in syneresis in set yoghurts when the total solids were increased. It is interesting to note that the influence of EPS on the reduction of syneresis as determined by this method diminished.

5.4.5 Syneresis as determined by the centrifugation method

The level of syneresis determined by the centrifugation method in yoghurts made at 9% and 14% solids using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures is shown in Figure 5.3. The yoghurts made at 9% solids using both capsular and ropy EPS-producing starter cultures had significantly higher level of syneresis than those with non-EPS-producing starter cultures. The highest level of syneresis was detected in the product made using ropy EPS-producing starter cultures. The results for yoghurts made at 14% solids showed the opposite trend. The products made with ropy EPS-producing starter cultures had the lowest level of syneresis, while those made using non-EPS-producing starter cultures showed the highest level of syneresis.

These results may be explained by microstructure and protein-polysaccharide incompatibility theory. Hassan *et al.* (1995a, 1995b) observed larger pore sizes in the microstructure of set yoghurts made using EPS-producing starter cultures compared to those made using non-EPS-producing starter cultures. In general, when mixing milk proteins and polysaccharide together at a certain concentration, the solution may show a phase separation into milk protein-rich phase and polysaccharide-rich phase if they are incompatible (de Kruif and Tuinier, 2001). Hassan *et al.* (2002b) observed that the EPS separated from the protein matrix and collected together formed large pools of EPS after set yoghurt was stirred. In our study, the centrifugation is likely to have enhanced phase separation between milk proteins and EPS in stirred products made at 9% solids. This may explain why there was an increase in syneresis in products made using EPS-producing starter cultures. At the 14% level, the products made using EPS-producing starter cultures showed lower level of syneresis than those made using non-EPS-producing starter cultures. Many factors may be responsible for such results including a decrease in phase separation of milk protein-EPS, an increase in EPS concentration leading to increased viscosity as well as adsorption of water as the total solids were elevated to 14%.

The results from each method of the determination of syneresis showed different pattern. The siphon method determines the level of spontaneous whey separated on the surface of gels. As predicted, the results from this method showed that the level of syneresis decreased with increasing total solids content from 9 to 14% with the

use of EPS-producing starter cultures. The drainage method measures the level of whey separated from cut gels under the influence of gravity. The centrifugation method measures the level of whey separated from the collapsed gels as a result of centrifugal force. Lucey and Singh (1997) reviewed that whey collected from the drainage method is more relevant to the products such as cottage cheese than yoghurt gels. Although there were high deviations of result determined using the siphon method, the procedure was easy, quick and inexpensive compared to other methods. Importantly, the siphon method represented the spontaneous syneresis of set yoghurts.

5.5 Conclusions

The pattern of syneresis as determined by the siphon-, drainage- and centrifugation method was different. The level of syneresis could be influenced by the methods of determination as well as by the level of solids content and the type of starter cultures. When comparing the three methods, the siphon method would be more appropriate in the determination of the level of spontaneous syneresis on the surface of a set yoghurt. Therefore, the siphon method has been chosen for subsequent studies.

Table 5.2: The concentration of lactic acid in set yoghurts made at 9% and 14% total solids made using non-EPS, capsular EPS and ropy EPS-producing starter cultures

Total solids (%, w/w)	Lactic acid concentration (% w/w)[1,2,3]		
	Non-EPS	Capsular EPS	Ropy EPS
9	0.93 ± 0.03^{a}	0.85 ± 0.03^{b}	$0.89 \pm 0.02^{a,b}$
14	1.25 ± 0.06^{b}	$1.20 \pm 0.07^{a,b}$	1.32 ± 0.06^{a}

[1] n = 3
[2] Presented values are the mean values ± standard deviation.
[3] The value in the same row that does not share the same superscript differs significantly ($P < 0.05$).

Table 5.3: The concentration of EPS in set yoghurt made at 9% and 14% (w/w) total solids made using non-EPS, capsular EPS and ropy EPS-producing starter cultures

Total solids (%, w/w)	EPS concentration (mg L^{-1})[1,2,3]		
	Non-EPS	Capsular EPS	Ropy EPS
9	9.10 ± 2.11^{b}	32.41 ± 0.73^{a}	41.57 ± 8.19^{a}
14	20.77 ± 3.05^{b}	114.63 ± 10.34^{a}	109.80 ± 9.25^{a}

[1] n = 3
[2] Presented values are the mean values ± standard deviations.
[3] The value in the same row that does not share the same superscript differs significantly ($P < 0.05$).

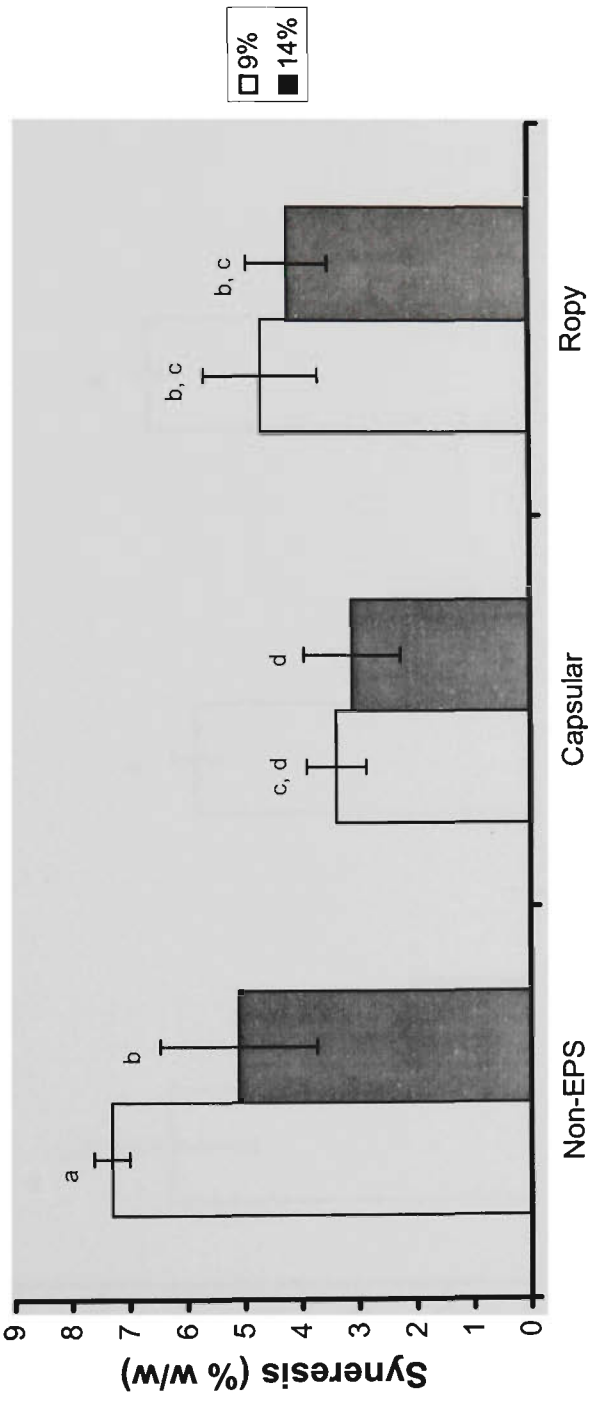

Figure 5.1: The level of spontaneous syneresis as determined by the siphon method in set yoghurts made at 9% and 14% (w/w) total solids using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures. Error bars represent standard deviation (n = 3). The bar that does not share the same letter differs significantly ($P < 0.05$).

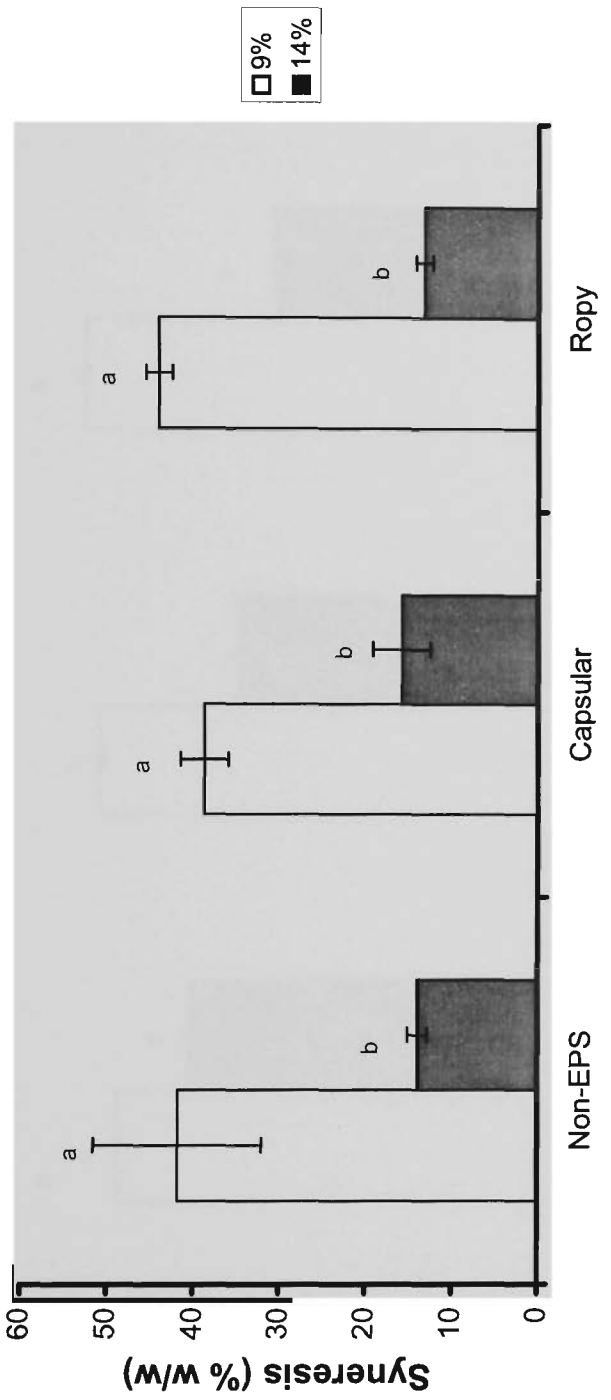

Figure 5.2: The level of syneresis as determined by the drainage method in set yoghurts made at 9% and 14% (w/w) total solids using non-EPS-, capsular EPS- or ropy EPS-producing starters. Error bars represent standard deviation (n = 3). The bar that does not share the same letter differs significantly ($P < 0.05$).

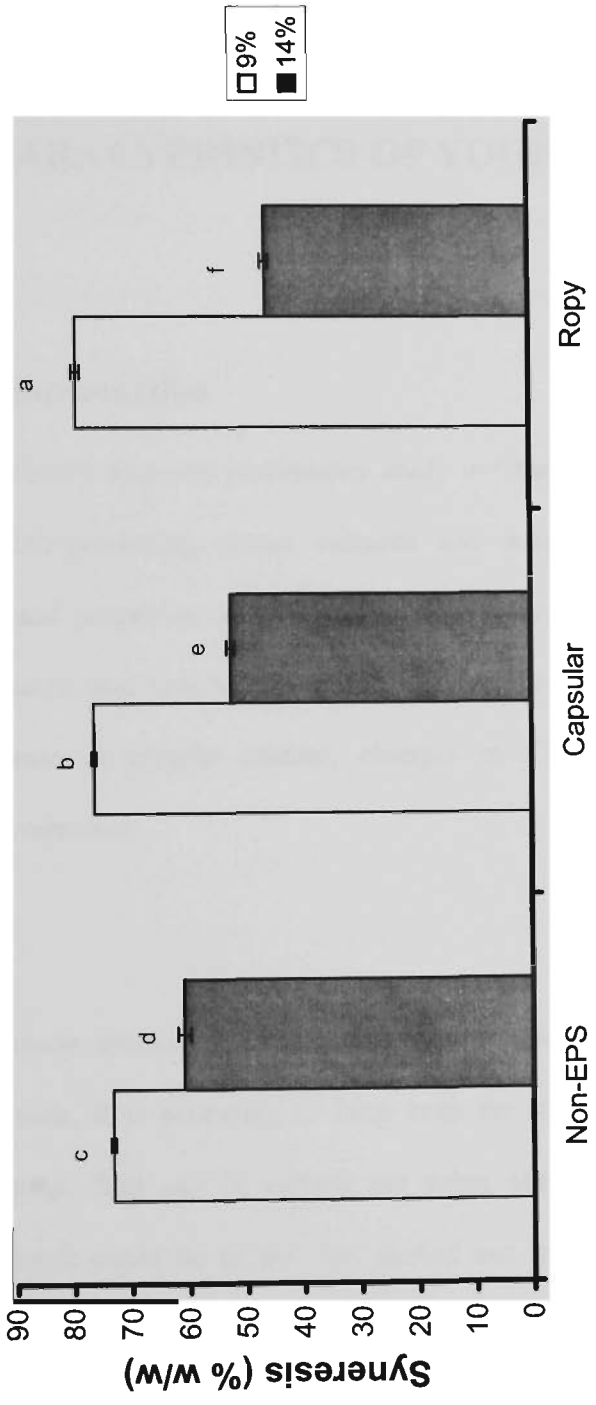

Figure 5.3: The level of syneresis as determined by the centrifugation method in set yoghurts made at 9% and 14% (w/w) total solids using non-EPS-, capsular EPS- or ropy EPS-producing starters. Error bars represent standard deviation (n = 3). The bar that does not share the same letter differs significantly ($P < 0.05$).

6.0 VARYING CASEIN TO WHEY PROTEIN RATIOS: EFFECTS ON COMPOSITION, BACTERIAL GROWTH, FERMENTATION PATTERN AND GEL CHARACTERISTICS OF YOGHURT

6.1 Introduction

The results from the preliminary study in Chapter 4 showed that the combined use of EPS-producing starter cultures and supplementation with WPC improved physical properties of set yoghurts (e.g. increased firmness, yield stress, apparent viscosity, and reduced syneresis). The improvements could have been due to an increase in protein content, changes in CN to WP ratios as well as EPS concentration.

To study effects of altering CN to WP ratio of milk on physical properties of yoghurts, it is necessary to keep both the total protein and total solids contents constant. This can be carried out using one of the three approaches. The first approach could be as per that carried out by Puvanenthiran *et al.* (2002). They blended SMP and WPC; the latter was produced by ultrafiltration to contain a similar level of total protein as SMP to achieve a desirable CN to WP ratio upon blending. Guyomarc'h *et al.* (2003) used a different approach to adjust the CN to WP ratio. A batch of RSM was subjected to centrifugation at 14,750 x g (20°C for 2

h) to separate casein. About two-third of the clear serum was then mixed with another batch of RSM to produce whey protein-enriched skim milk solution. The resultant casein pellet and the rest of the whey were mixed and homogenized to produce casein-enriched skim milk. Both casein- and whey protein-enriched milk were then freeze-dried to produce casein- and whey protein-enriched milk powders. The CN to WP ratios of reconstituted milk were varied by blending these powders at suitable quantities. However, the production of these powders in large quantity may be limited by the use of high speed centrifugation and a commercial freeze-dryer. The third approach is to blend SMP and WPC to a desirable CN to WP ratio and at a constant protein content. Lactose powder is then added to adjust the total solids level of milk blends. This method does not require ultrafiltration and freeze dryer units and the milk blends can be produced in large quantities.

Puvanenthiran *et al.* (2002) and Guyomarc'h *et al.* (2003) studied the effects of various CN to WP ratios on physical properties of yoghurt and acidified milk gels, respectively. They reported an increase in gel strength and a reduction in syneresis in set yoghurt gel or milk gels acidified with glucono-δ-lactone as the CN to WP ratio was reduced. From these studies, it might be possible to reduce costs associated with the supplementation of dairy ingredients in yoghurt-making processes, if the physical properties of set and stirred yoghurts made at low solids level can be maintained similar to those made at high solids content by the combination of altered CN to WP ratio and the use of EPS-producing starter cultures.

6.2 Aims

This chapter investigated the effects of varying CN to WP ratios of milk from 4:1 to 3:1, 2:1 and 1:1 without affecting the total solids and protein content on composition, growth of starter cultures (non-EPS) as well as the development of yoghurt gel during fermentation. The gel characteristics of yoghurt after 1 day of storage were investigated. The CN to WP ratios were varied by blending SMP, WPC and lactose monohydrate. The yoghurts were made at 9% and 14% total solids. The effect of heat treatment on denaturation of whey protein was also investigated.

6.3 Materials and Methods

6.3.1 Experimental design

This study reported a method for varying the ratio of CN to WP in reconstituted milk blends to 4:1, 3:1, 2:1 and 1:1 at 9% and 14% solids using SMP, WPC and lactose monohydrate and assess the effects of varying CN to WP ratios on the composition, protein profiles of unheated- and heated milk. Bacterial growth (only non-EPS-producing starter cultures), fermentation patterns and the development of yoghurt gels during yoghurt fermentation were monitored. The characteristics of yoghurt gels after 1 day of storage (4°C) were also evaluated. The study was carried out in triplicate.

6.3.2 Starter culture

Non-EPS-producing *S. thermophilus* ASCC 1342 and non-EPS-producing *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 were used in this study. These bacteria were activated as described in Chapter 3, Section 3.1.

6.3.3 Milk preparation

The CN to WP ratios in milk made at 9% and 14% (w/w) total solids were varied from 4:1 to 3:1, 2:1 and 1:1 by blending low heat SMP (34% total protein; Murray Goulbourn Co-operative Co. Ltd., Brunswick, Australia, see Appendix C for composition), WPC 80 (76% total proteins; United Milk Tasmania Ltd., Spreyton, Tasmania, Australia, see Appendix D for composition) and lactose monohydrate (Merck, Darmstadt, Germany). The WPC 392 as used in Chapter 4 was replaced with WPC 80 due to the availability and quantity of the ingredient. This ingredient was used in subsequent chapters. The RSM was considered to represent the CN to WP ratio of 4:1. Lactose was added to standardized the total solids content as well as lactose content of milk blends (the CN to WP ratios of 3:1, 2:1 and 1:1) to that of RSM (the CN to WP ratio of 4:1).

The amount of each ingredient used was obtained based on calculation. The first step of the calculation was to create two polynomial equations for 1) the total proteins of reconstituted milk blends, and 2) the ratios of CN to WP in reconstituted milk blends from 2 variables: X and Y representing the quantity of SMP and WPC

80 used. The amount of CN and WP in each ingredient was estimated based on literatures (Walstra *et al.,* 1999; Morr and Foegeding, 1990). The required amounts of both ingredients were obtained by solving the two equations. As the protein content of WPC 80 was higher than SMP, blending these ingredients to a desired total protein level would decrease solids and lactose content of milk blends. Therefore, the additional lactose is required to standardize the level of solids and lactose content to that of RSM. The second step involved the adjustment of solids content of milk blends to that of RSM by adding lactose monohydrate. The amount of lactose monohydrate was calculated by subtracting the quantity of both SMP and WPC 80 used for the preparation of the reconstituted milk blends containing the CN to WP ratio of 3:1, 2:1 and 1:1 from that of SMP used for preparing a reconstituted milk blend at the CN to WP ratio of 4:1. The actual composition of milk blends was measured in Section 3.4. Table 6.1 shows the quantity of each ingredient used for the preparation of 500 mL of milk blends made at 9% solids content to desired CN to WP ratios. Table 6.2 shows the calculated amounts of total solids, total protein and CN to WP ratios of milk blends in Table 6.1. The actual composition of milk blends was also determined (Section 6.3.5).

6.3.4 Yoghurt manufacture

Low-heat SMP, WPC 80 and lactose monohydrate were blended at the quantities shown in Table 6.1. Dry ingredients were reconstituted at 9 and 14% solids with distilled water and hydrated overnight at 4°C. Set and stirred yoghurts were

manufactured using non-EPS-producing starter cultures according to procedures described in Chapter 3, Section 3.3.

6.3.5 Compositional analysis of liquid milk

The composition of milk blends including total solids, fat, ash and protein contents and CN to WP ratio were determined as described in Chapter 3, Section 3.4.

6.3.6 Enumeration of starter cultures during yoghurt fermentation

The viable counts of starter cultures during yoghurt fermentation were determined every 1 h until the pH of 4.70 was reached according to the procedures described in Chapter 3, Section 3.2.

6.3.7 Protein profiles and whey protein denaturation

The protein profiles of heated and unheated milk blends and the pattern of soluble denatured whey protein in heated milk blends were investigated using a discontinuous native- and SDS-PAGE according to the procedures described by Chapter 3, Section 3.5.

6.3.8 Monitoring of yoghurt gel development during fermentation

The monitoring of yoghurt gels development during fermentation (the oscillatory test) was carried out according to the procedure described in Chapter 3, Section 3.11.

6.3.9 Gel characteristics of set yoghurt after 1 day of storage (penetration test)

The characteristics of the set yoghurt gel were examined by a uniaxial penetration test using a TA-XT.2 Texture Analyzer (Stable Micro System, Goaldming, UK) according to the procedure described in Chapter 3, Section 3.12.

6.3.10 Statistical analysis

The data were analysed using one-way analysis of variance at 95% confidence level with SPSS version 10 for Windows (SPSS inc., NY, USA). The mean values were compared using Tukey's test.

6.4 Results and Discussions

6.4.1 The calculated and actual composition of milk blends

Table 6.1 shows the amount of SMP, WPC 80 and lactose monohydrate required to prepare 500 mL of milk blends at 9% (w/w) total solids with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 based on the calculation shown earlier (Section 6.3.3). Table 6.2 shows the calculated values of total solids, total proteins and ratios of CN to WP in milk blends based on the amount of ingredients shown in Table 6.1. The total

solids and total protein contents were kept constant, while the calculated ratios of CN to WP were 4.44, 3.00, 1.99 and 1.00. The actual composition of milk blends at 9% solids with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 determined using AOAC methods (AOAC, 1995) is shown in Table 6.3. The results confirmed that the composition of milk blends was similar to what was calculated (Table 6.2). This trend was also observed in milk blends made at 14% solids (Table 6.4).

6.4.2 Protein profiles of heated and unheated milk blends

The protein profiles of unheated (a) and heated (b) milk blends made at 9% (Figure 6.1) and 14% (Figure 6.2) solids with CN to WP ratios of 4:1 (lanes 1 and 2), 3:1 (lanes 3 and 4), 2:1 (lanes 5 and 6) and 1:1 (lanes 7 and 8) were investigated using native-PAGE. The bands of κ-, β-, αs-casein, α-lactalbumin and β-lactoglobulin A and B were present on the gel of unheated samples (Figures 6.1a and 6.2a). The α-lactalbumin and β-lactoglobulin A and B bands became thicker and darker as the ratios of CN to WP were decreased from 4:1 to 1:1. The gel of heated milk (Figures 6.1b and 6.2b) showed that the α-lactalbumin and β-lactoglobulin A, B bands in all samples disappeared. This suggested that all whey proteins denatured. Many researchers have agreed that denatured whey protein denatured and associated with casein micelles when milk was heated (Singh and Creamer, 1991; Singh, 2001; Vasbindar and de Kruif, 2003). Furthermore, the increase in the proportion of WP did not affect the level of WP denaturation. These phenomena could also be seen in SDS-PAGE patterns of the supernatant of heated milk adjusted to pH 4.60, followed by centrifugation. The β-lactoglobulin band disappeared from the serum of heated

milk (Figures 6.3b and 6.4b). Theoretically, denatured whey protein and casein micelles in heated milk adjusted to pH 4.60 would sediment during centrifugation. Interestingly, our results were in contrast with Cho *et al.* (2003). They used alkaline- PAGE or native-PAGE and SDS-PAGE (with or without treatment with reducing agents) to study heat-induced interaction (80°C for 5, 10, 15 and 30 min) between β-lactoglobulin A and κ-casein B in a phosphate buffer (pH 6.70) reporting a greater loss of native β-lactoglobulin in both native- and SDS-PAGE (without treatment with reducing agents) as the ratio of κ-casein to β-lactoglobulin was increased from 0.5:1 to 2:1. The difference between the results of Cho *et al.* (2003) and our study could be due to types of solution used in the study (β-lactoglobulin-κ-casein mix in phosphate buffer solution vs. milk blends). The presence of other proteins might also cause the difference. This may be due to the presence of αs_2-casein which contains cystine residues. It could interact with β-lactoglobulin during heat treatment.

It is interesting to note that the variation in pH of milk at heating has been reported to affect the whey protein aggregation and physical properties of yoghurts. Vasbindar and de Kruif (2003) and Singh (2004) studied the effect of adjusting the pH of milk ranging from 6.35 to 6.90 prior to heat treatment on whey protein aggregation. They reported that the pH values of milk greater than 6.55 promoted the formation of soluble denatured whey protein aggregates. Heating milk at pH values below 6.55 resulted in the formation of casein-whey protein complex. Anema *et al.* (2004) studied the rheological properties of glucono-δ-lactone

acidified RSM and reported that skim milk adjusted to pH 7.10 prior to heat treatment had higher level of soluble denatured whey protein aggregates than that at pH 6.50. In addition, they observed a correlation between the increase in storage modulus (G') and the level of soluble denatured whey protein aggregates.

In our study, the percentages of soluble denatured whey protein aggregates decreased significantly ($P < 0.05$) as the CN to WP ratio was reduced (Table 6.5). This trend was observed in milk made at both 9 and 14% total solids. The percentage of soluble denatured whey protein aggregates in milk at 14% total solids was less than those at 9% at the same CN to WP ratio. This may be explained by the influence of solids content. Increasing solids content might promote the creation of large whey protein aggregates. The large aggregates could sediment with casein during centrifugation. Based on the correlation between soluble denatured whey protein aggregates and storage modulus of milk gel (Anema *et al.*, 2004), the firmness as well as storage modulus of yoghurts should decrease with decreased CN to WP ratios.

6.4.3 Effects of varying CN to WP ratios on the growth of starter cultures and yoghurt fermentation

The effect of varying CN to WP ratios on the growth of *S. thermophilus*, and *L. delbrueckii* ssp. *bulgaricus* and the fermentation of yoghurts was also investigated. The time-pH curves of set yoghurts made at 9% and 14% solids with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 are shown in Figures 6.5 and 6.6. The fermentation was terminated at pH 4.70. In general, the product made at the CN to WP ratio of

1:1 was the first to reach the pH 4.70 followed by those at ratios 2:1, 3:1 and 4:1. According to Walstra and Jenness (1984), WP generally has lower buffering capacity than CN. Therefore, lowering the proportion of CN would result in a reduction in buffering capacity. The batches made at 14% solids showed similar patterns as those at 9%, although the fermentation was completed earlier. The phenomenon was similar to that reported by Biliaderis *et al.* (1992) who observed faster fermentation in yoghurts made at 16% than that at 12%. This phenomenon might be due the increased available nutrients from the increased solids and WPC contents.

The growth patterns of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* during fermentation of milk blends at 9% (Figures 5.7 and 5.8) and 14% (Figures 6.9 and 6.10) with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 were investigated. In general, *S. thermophilus* grew actively at the beginning of fermentation. *L. delbrueckii* ssp. *bulgaricus* started growing towards the end of fermentation. The latter is acid tolerant and required essential growth factors released by the former (Shah, 2003). Furthermore, *S. thermophilus* started growing earlier in the product at a CN to WP ratio of 1:1 followed by 2:1, 3:1 and 4:1 (Figures 6.7 and 6.9). However, the counts at the end of fermentation were similar. *L. delbrueckii* ssp. *bulgaricus* (Figures 6.8 and 6.10), on the other hand, had similar growth patterns between each batch, but the counts at the end of fermentation were slightly different. The shorter fermentation time in products with lower CN to WP ratios would limit the growth of *L. delbrueckii* ssp. *bulgaricus*. This could partly be due to the decrease in milk's

buffering capacity as mentioned previously. In addition, other researchers have also shown that an increase in the concentration of available nutrients (supplementation of media with yeast extract and WPC) affected the EPS and lactic acid production by lactic acid bacteria (Amrane and Prigent, 1998; Hassan *et al.*, 2001; Zisu and Shah, 2003). The supplementation with WPC could promote the lactic acid production by *S. thermophilus* resulting in the reduction of fermentation time. This limits the time for the growth of *L. delbrueckii* ssp. *bulgaricus.* As there was no difference in lactose concentration between milk blends, the reduction in fermentation time as well as the growth pattern of starter cultures should not have been caused by the added lactose.

6.4.4 Effects of varying CN to WP ratios on yoghurt gel development (storage modulus, G')

The storage modulus (G') of set yoghurts during fermentation made at 9% and 14% solids with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 is shown in Figures 6.11 and 6.12, respectively. The batch with CN to WP ratio of 1:1 was the first to show an increase in G' followed by those at ratios 2:1, 3:1 and 4:1. The order of the increase in storage modulus was similar to that of the decrease in pH during fermentation in the time-pH curves as previously discussed (Figures 6.5 and 6.6). Therefore, the early development of yoghurt gels (increase in G') made at low CN to WP ratio (ratio 1:1) might be partly due to the faster change in pH of milk influenced by the decrease in buffering capacity of milks blended at low CN to WP ratio (e.g. ratio 1:1). However, the G' at the end of fermentation of both 9% and 14% yoghurt gels

decreased with a reduction in the CN to WP ratios from 4:1 to 1:1. This can be interpreted as the yoghurt made at the CN to WP ratio of 4:1 as having more gel strength than those at ratios 3:1, 2:1 or 1:1. However, Guyomarc'h *et al.* (2003) reported the increase in G' of reconstituted milk gels acidified with glucono-δ-lactone as the CN to WP ratio was reduced. The contradicting results between the current study and that of Guyomarc'h *et al.* (2003) could be due to the difference in rate and amount of acid produced by starter cultures compared to that of GDL. Theoretically, the rate of gluconic acid released from GDL depends on temperature. However, lactic acid produced by starter cultures varies with solids level as well as available nutrients. Because the concentration of gluconic acid released should be similar in every batches, rapid change in G' in batches made at low CN to WP ratios (low buffering capacity) would be expected. This would also lead to higher G' at the end of incubation compared to those made at high CN to WP ratio. The CN to WP ratio of milk blends may also play a vital role. In the study of Guyomarc'h *et al.* (2003), the ratios of CN to WP range from 3.0:1 to 6.6:1, while the current study controlled the CN to WP ratios between 1:1 and 4:1.

6.4.5 Effects of varying CN to WP ratios on gel characteristics of set yoghurt (penetration test)

The gel characteristics determined using a penetration test of set yoghurts made at 9% and 14% solids with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 after 1 day of storage (4°C) are shown in Figures 6.13 and 6.14, respectively. The values of fracture and firmness in set yoghurts decreased as the CN to WP ratios were

reduced from 4:1 to 1:1 in both products at 9% and 14% solids. The yoghurts made at 14% solids were firmer than those at 9%. A correlation between the reduction in firmness with a decrease in CN to WP ratio was observed.

Tamime *et al.* (1984) reported that yoghurts made from milk blended at a CN to WP ratio of 4.62 were firmer than those at the CN to WP ratios of 3.20 to 3.40. Interestingly, the reduction in firmness as the CN to WP ratio was reduced contradicted the results reported by Puvanenthiran *et al.* (2002). They reported that the gel strength (representing yield point or the first fracture during the penetration test) increased as the CN to WP ratio was reduced. The contradicting results of firmness or gel strength between our study and that of Puvanenthiran *et al.* (2002) could be due to the differing level of whey protein denaturation and aggregation as a result of heat treatment of milk blends at different pH values. Puvanenthiran *et al.* (2002) heat treated milks at pH 7.00, and reported a relationship between an increase in the particle size of protein aggregates and gel strength of yoghurt. They explained that the heat treatment of milk at pH 7.00 promoted the formation of dissociated κ-casein-whey protein aggregates, whey protein-whey protein aggregates and agglomeration of aggregates as the CN to WP ratio was decreased. Vasbinder and de Kruif (2003) observed that heating milk at pH 6.90 resulted in higher degree of soluble denatured whey protein aggregates than at pH 6.35, at which WP tended to form complex with CN. Importantly, Anema *et al.* (2004) studied the rheological properties of GDL acidified milk gel the pH was adjusted prior to heat treatment. They reported that heated skim milk adjusted to pH 7.10

contained higher level of soluble denatured whey protein aggregates than that at pH 6.50. In addition, they observed a correlation between the increase in storage modulus (G') and the level of soluble denatured whey protein aggregates heated milk.

In our study, the casein-whey protein complex could form as a result of the heat treatment of milk blends at natural pH (~ 6.50). Heat treatment of milks at pH 7.00, as carried out in the study of Puvanenthiran *et al.* (2002), promoted the formation of soluble denatured whey protein aggregates and an increase in gel strength of yoghurt according to the relationship observed by Anema *et al.* (2004). Our results (Section 6.4.2) showed that most of denatured whey protein associated with casein micelles depending on the CN to WP ratio. Higher amount of denatured whey protein associated with casein micelles as the CN to WP ratios were reduced. The interaction between WP and CN as the ratio of CN to WP was reduced or the increase in proportion of WP could result in the prevention of casein cluster formation as suggested by Fox (2001). This could also result in a decrease in the firmness of yoghurt. Similar results were observed by Mottar *et al.* (1989). They reported that yoghurt made by heat treatment at 90°C was firmer than those by UHT. The casein micelles of the former had less appendage (denatured whey protein) on the surface of casein than the latter.

Interestingly, yoghurt made with low CN to WP ratios showed several fractures during penetration. The product made with the CN to WP ratio of 1:1 had the highest number of fractures as compared to that at 4:1, which had the lowest number of fractures. According to van Vliet and Luyten (1995), the fracture phenomenon occurs due to weak points or small cracks in the structure of solid or solid-like foods. As the force was applied, these small cracks grow spontaneously and cause fracture. The fracture phenomenon confirmed the results that the increasing proportion of WP increased the amount of denatured whey protein on the surface of casein micelles. This could prevent the formation of continuous casein network during the development of yoghurt gel, and may explain the decrease in gel strength as well as firmness of yoghurt as the ratios of CN to WP were decreased.

For further storage study in Chapters 7 and 8, the penetration test was chosen to assess the firmness of yoghurt gel, which was the main parameter of interest. For oscillatory test, yoghurt needed to be made in the measuring cup and tested. This meant that only one or two batches at the most could be made and measured per day. The measurement during storage is not practical with regards to the number of samples as well as replications.

6.5 Conclusion

The CN to WP ratios of milks can be adjusted to desirable values (3:1, 2:1 and 1:1) by blending low-heat SMP, WPC 80 and lactose at suitable quantities without

altering total solids, total protein and lactose contents of milk blends. The protein profiles of heated and unheated milks examined by native- and SDS-PAGE (under reducing condition) showed that the denaturation of WP was not affected by a decrease in CN to WP ratio. The increase in the proportion of WP decreased fermentation time, which is likely to be due to an increase in available nutrients from WP, but not from lactose used in the standardisation. The decrease in buffer system of milk was also suspected to affect the fermentation time. Varying CN to WP ratios did not affect the viable count of *S. thermophilus,* but the counts of *L. delbrueckii* ssp. *bulgaricus* decreased. The firmness and storage modulus (G') representing gel strength of yoghurt decreased as the CN to WP ratio was reduced. This is due to the interaction of CN and WP resulting from the heat treatment. Yoghurt made at a CN to WP ratio of 4:1 had the firmest texture.

Table 6.1: The amount of SMP, WPC 80 and lactose monohydrate required to prepare 500 mL of milk blends at 9% (w/w) total solids with various CN to WP ratios based on calculation

CN to WP	SMP (g)	WPC 80 (g)	Lactose monohydrate (g)	Distilled water (g)
4:1	46.88	0.00	0.00	453.12
3:1	43.07	1.74	2.18	453.12
2:1	38.13	3.91	5.09	453.12
1:1	28.48	8.23	10.71	453.12

Table 6.2: Total solids, total proteins and CN to WP ratios of milk blends at 9% total solids with various CN to WP ratios based on calculation as shown in Section 6.3.2

CN to WP ratios	Total solids (% w/w)	Total proteins (% w/w)	Calculated CN to WP ratios
4:1	9.00	3.06	4.44
3:1	9.00	3.06	3.00
2:1	9.00	3.06	1.99
1:1	9.00	3.06	1.00

Table 6.3: The actual composition of milk blends with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 9% (w/w) total solids

Compositional parameters	Casein to whey protein ratios[1, 2, 3]			
	4:1	3:1	2:1	1:1
Total solids (%, w/w)	8.93 ± 0.01^{a}	8.91 ± 0.04^{a}	8.87 ± 0.13^{a}	8.88 ± 0.11^{a}
Total protein (%, w/w)	3.05 ± 0.02^{a}	3.05 ± 0.01^{a}	3.05 ± 0.01^{a}	3.06 ± 0.03^{a}
Fat (%, w/w)	$< 0.1^{a}$	$< 0.1^{a}$	$< 0.1^{a}$	$< 0.1^{a}$
Lactose (%, w/w)	4.96 ± 0.08^{a}	4.96 ± 0.04^{a}	5.05 ± 0.04^{a}	5.08 ± 0.05^{a}
Ash (%, w/w)	0.68 ± 0.01^{a}	0.68 ± 0.03^{a}	0.69 ± 0.01^{a}	0.68 ± 0.01^{a}
CN to WP ratio	4.36 ± 0.25^{a}	2.98 ± 0.03^{b}	2.13 ± 0.01^{c}	1.21 ± 0.02^{d}

[1] $n = 3$,
[2] Values represent mean ± standard deviation
[3] Value in the same row not sharing the same superscript differ significantly ($P < 0.05$).

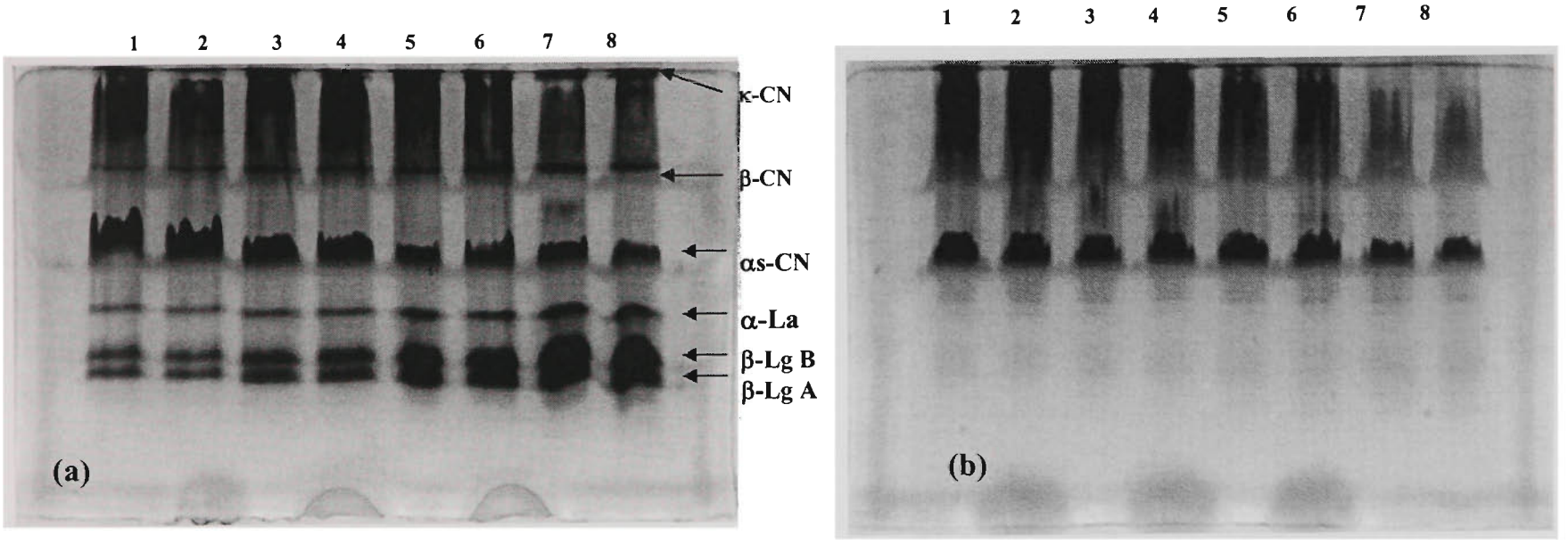

Figure 6.1: Protein profiles of unheated (a) and heated (b) milk blends made at 9% total solids with CN to WP ratios of 4:1 (lanes 1 and 2), 3:1 (lanes 3 and 4), 2:1 (lanes 5 and 6) and 1:1 (lanes 7 and 8) in native-PAGE. κ-CN = κ-casein, β-CN = β-casein, αs-CN = αs-casein, α-La = α-Lactalbumin, β-Lg A, B = β-Lactoglobulin A, B

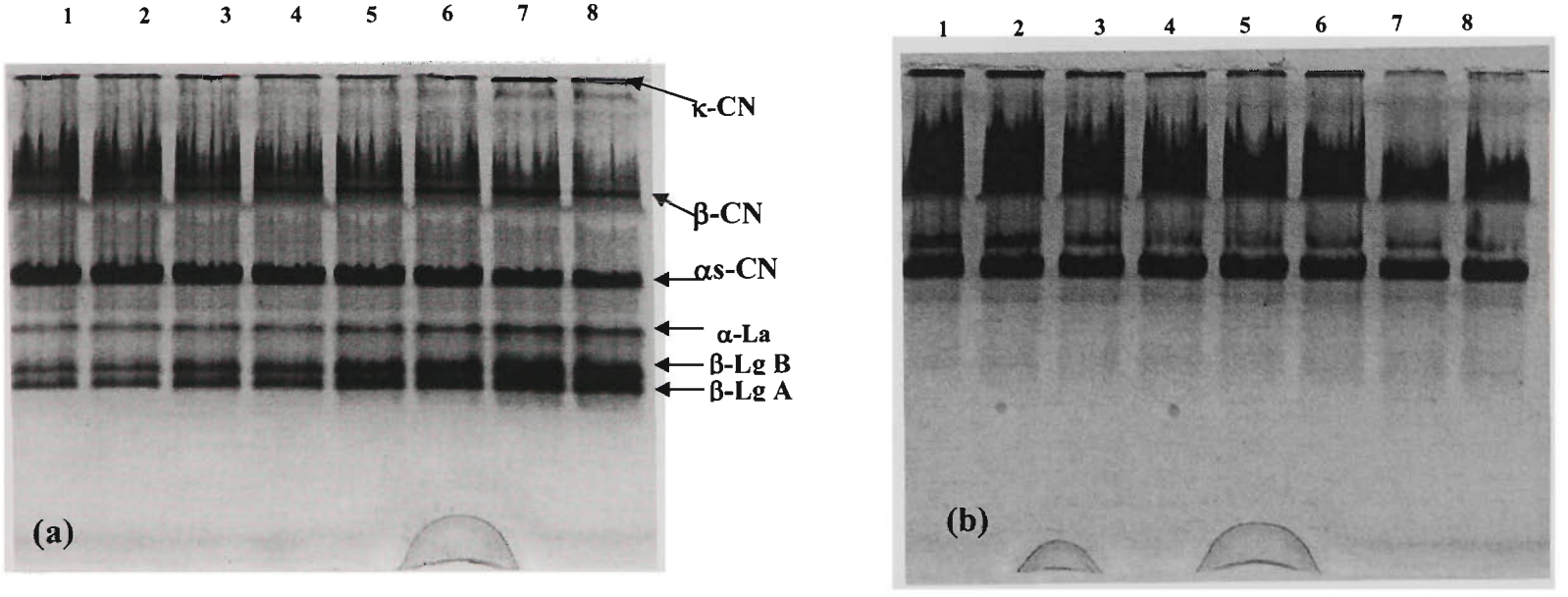

Figure 6.2: Protein profiles of unheated (a) and heated (b) milk blends made at 14% total solids with CN to WP ratios of 4:1 (lanes 1 and 2), 3:1 (lanes 3 and 4), 2:1 (lanes 5 and 6) and 1:1 (lanes 7 and 8) in native-PAGE. κ-CN = κ-casein, β-CN = β-casein, αs-CN = αs-casein, α-La = α-Lactalbumin, β-Lg A, B = β-Lactoglobulin A, B

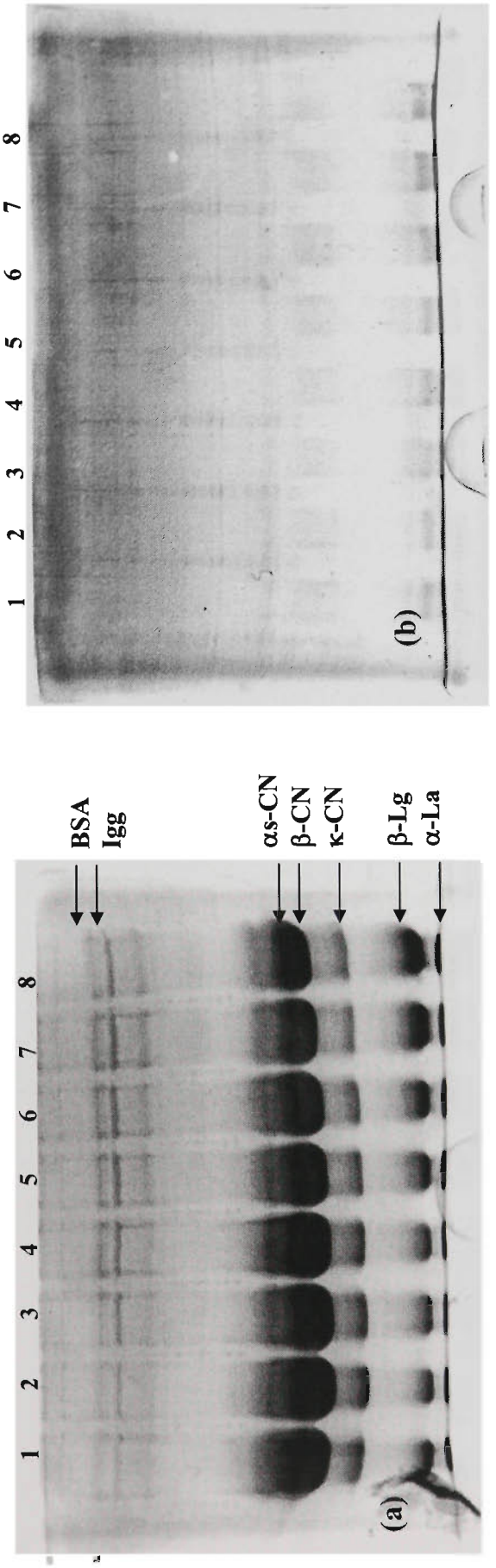

Figure 6.3: Protein profiles of heated milk blends (a) and the supernatant of milk adjusted to pH 4.60 (b) made at 9% total solids with CN to WP ratios of 4:1 (lanes 1 and 2), 3:1 (lanes 3 and 4), 2:1 (lanes 5 and 6) and 1:1 (lanes 7 and 8) in SDS-PAGE. BSA = Bovine serum albumin, Igg = Immunoglobulin, CN = casein, α-La = α-Lactalbumin, β-Lg = β-Lactoglobulin

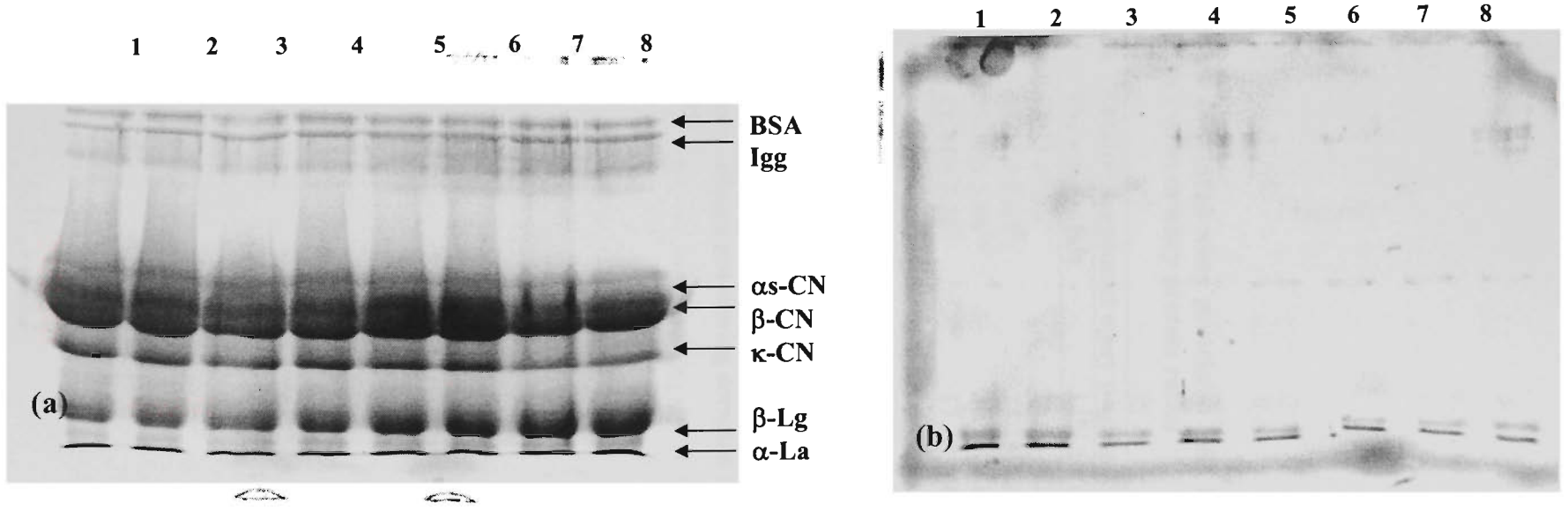

Figure 6.4: Protein profiles of heated milk blends (a) and the supernatant of heated milk adjusted to pH 4.60 (b) made at 14% total solids with CN to WP ratios of 4:1 (lanes 1 and 2), 3:1 (lanes 3 and 4), 2:1 (lanes 5 and 6) and 1:1 (lanes 7 and 8) in SDS-PAGE. BSA = Bovine serum albumin, Igg = Immunoglobulin, CN = casein, α-La = α-Lactalbumin, β-Lg = β-Lactoglobulin

Table 6.5: Soluble denatured whey protein in milk blends at 9 and 14% total solids with various casein to whey protein ratios

CN to WP ratio	Soluble denatured whey protein (%)[1, 2, 3]	
	9% total solids	14% total solids
4:1	42.93 ±1.10[a]	26.22 ± 2.91[a]
3:1	37.22 ± 2.65[b]	23.23 ± 2.10[a,b]
2:1	35.02 ± 3.06[b,c]	20.90 ± 3.55[a,b]
1:1	29.44 ± 1.18[c]	17.28 ± 4.27[b]

[1] n = 3

[2]Values represent mean ± standard deviation

[2] Values within the same column not sharing common superscript differ significantly ($P < 0.05$)

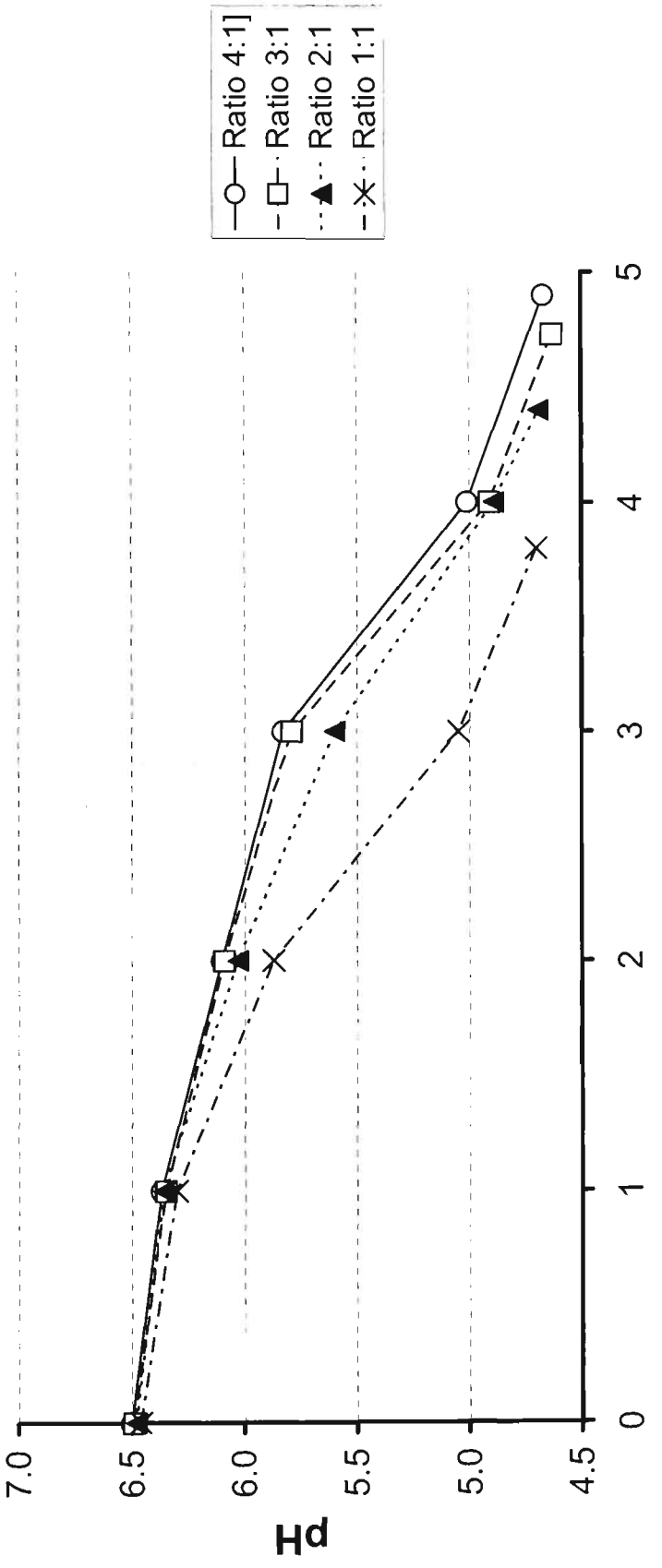

Figure 6.5: Time-pH curves during fermentation of yoghurt mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 9% total solids using non-EPS-producing starter cultures. The fermentation was terminated when the pH of 4.70 was achieved, n = 3.

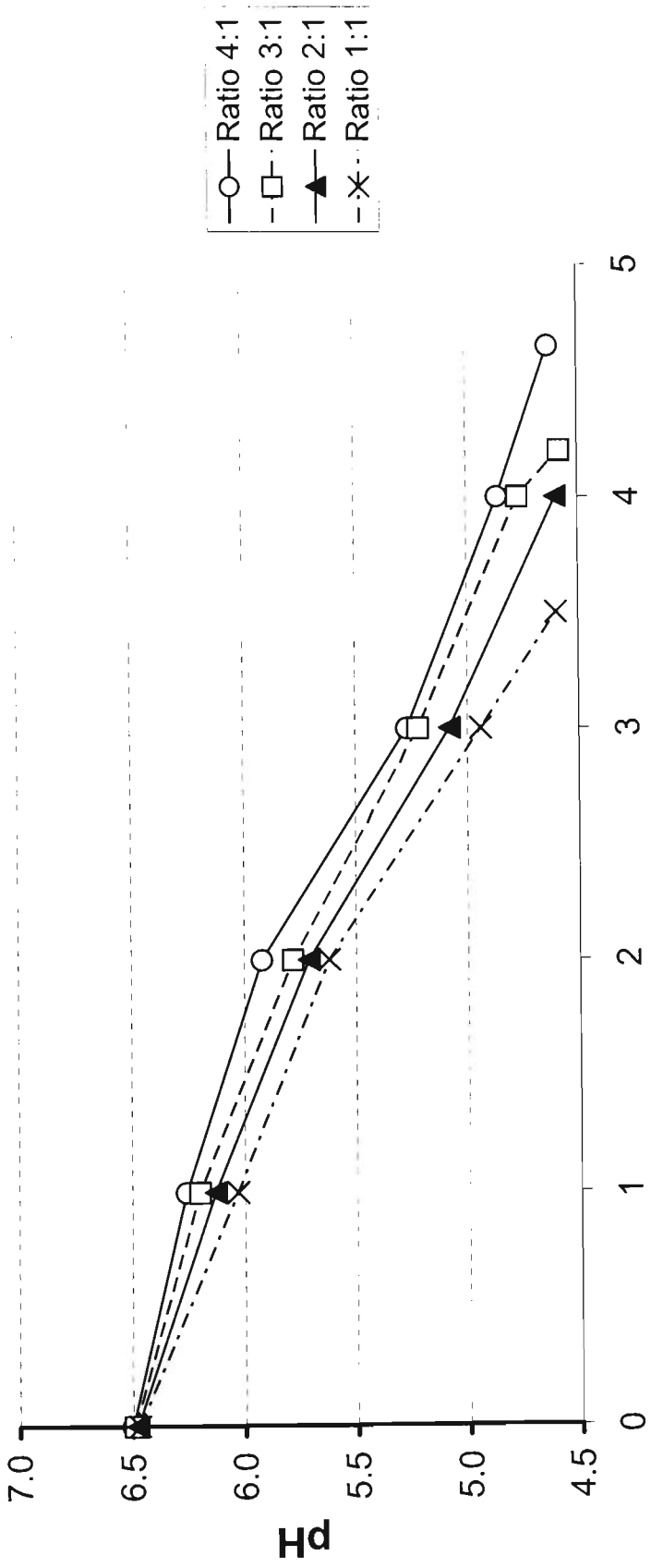

Figure 6.6: Time-pH curves during fermentation of set yoghurts mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 14% total solids using non-EPS-producing starter cultures. The fermentation was terminated when the pH of 4.70 was achieved, n = 3.

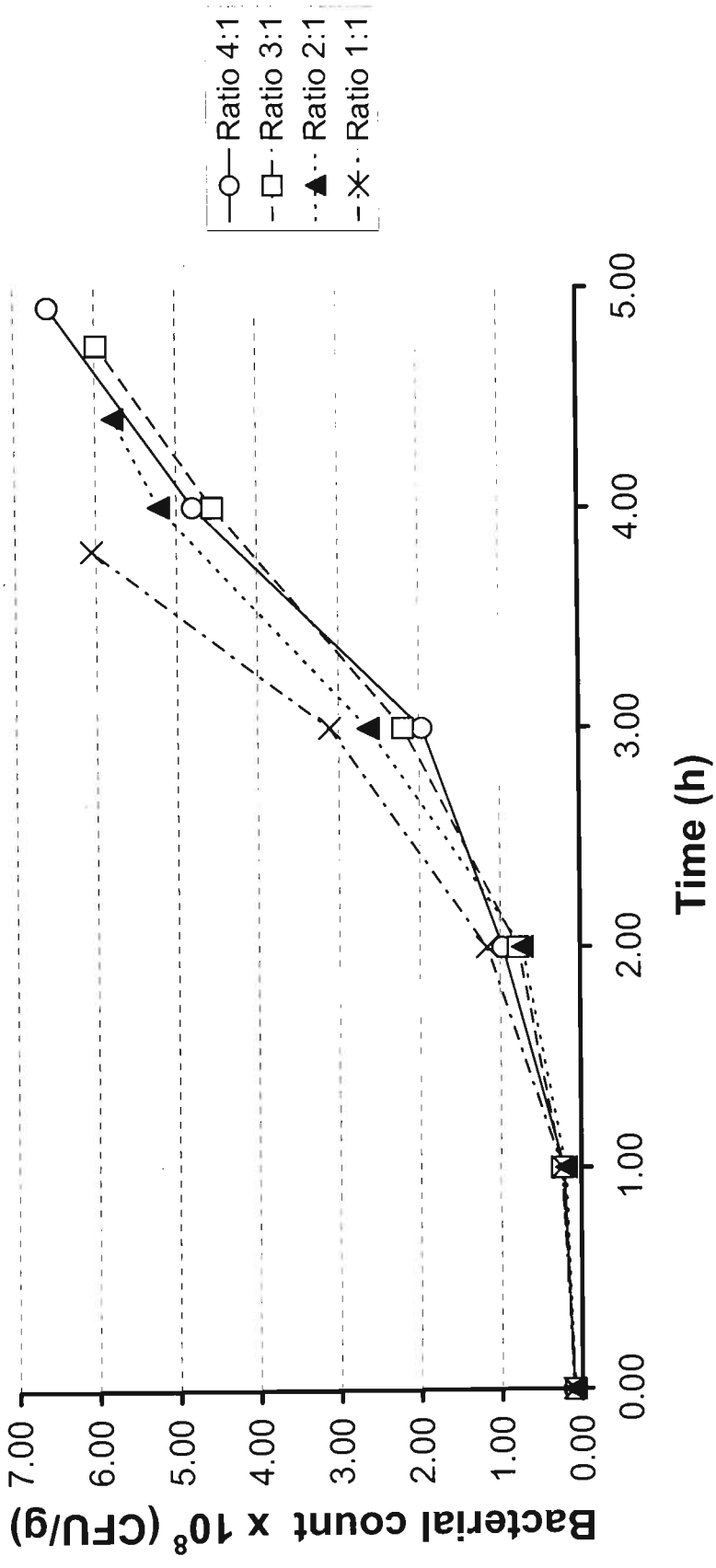

Figure 6.7: Growth curves of non-EPS-producing *S. thermophilus* during fermentation of yoghurt mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 9% total solids. The fermentation was terminated when the pH of 4.70 was achieved, n = 3.

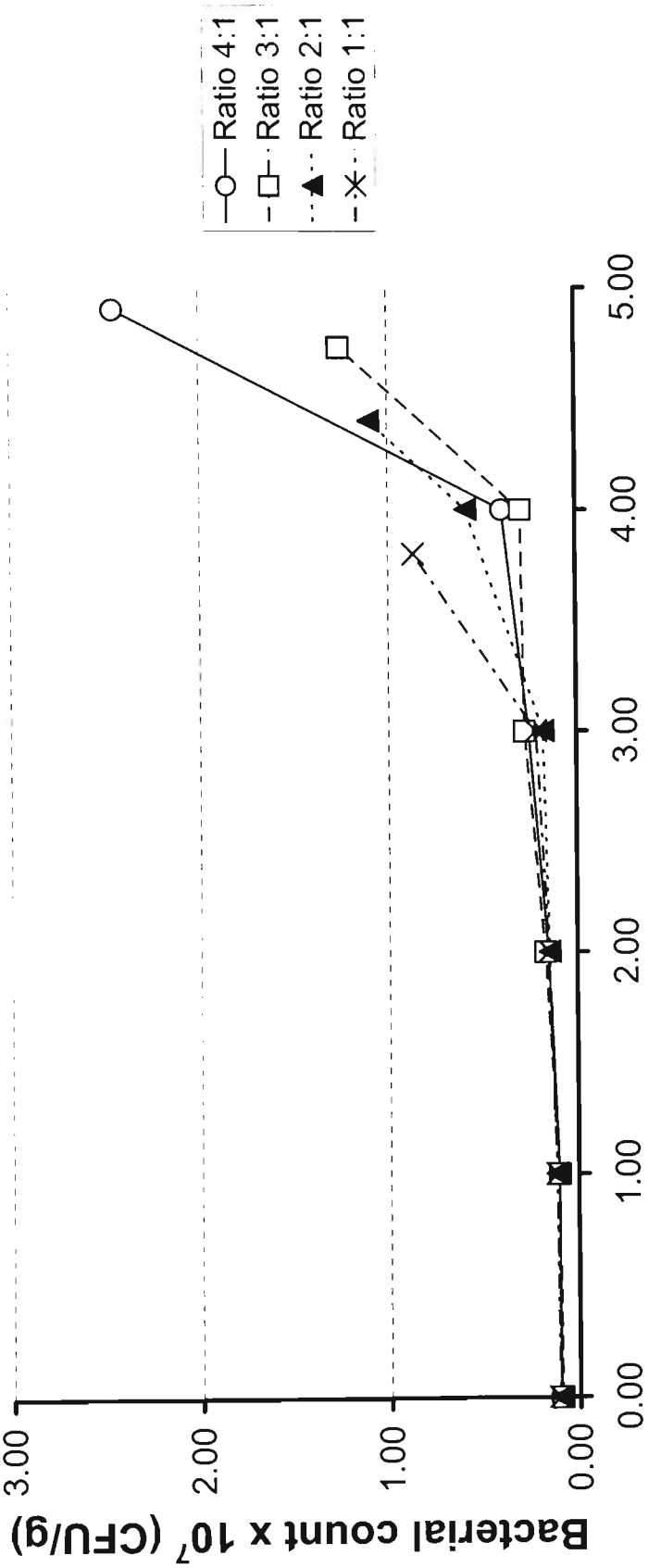

Figure 6.8: Growth curves of non-EPS-producing *L. delbrueckii* ssp. *bulgaricus* during fermentation of yoghurt mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 9% total solids. The fermentation was terminated when the pH of 4.70 was achieved, n = 3.

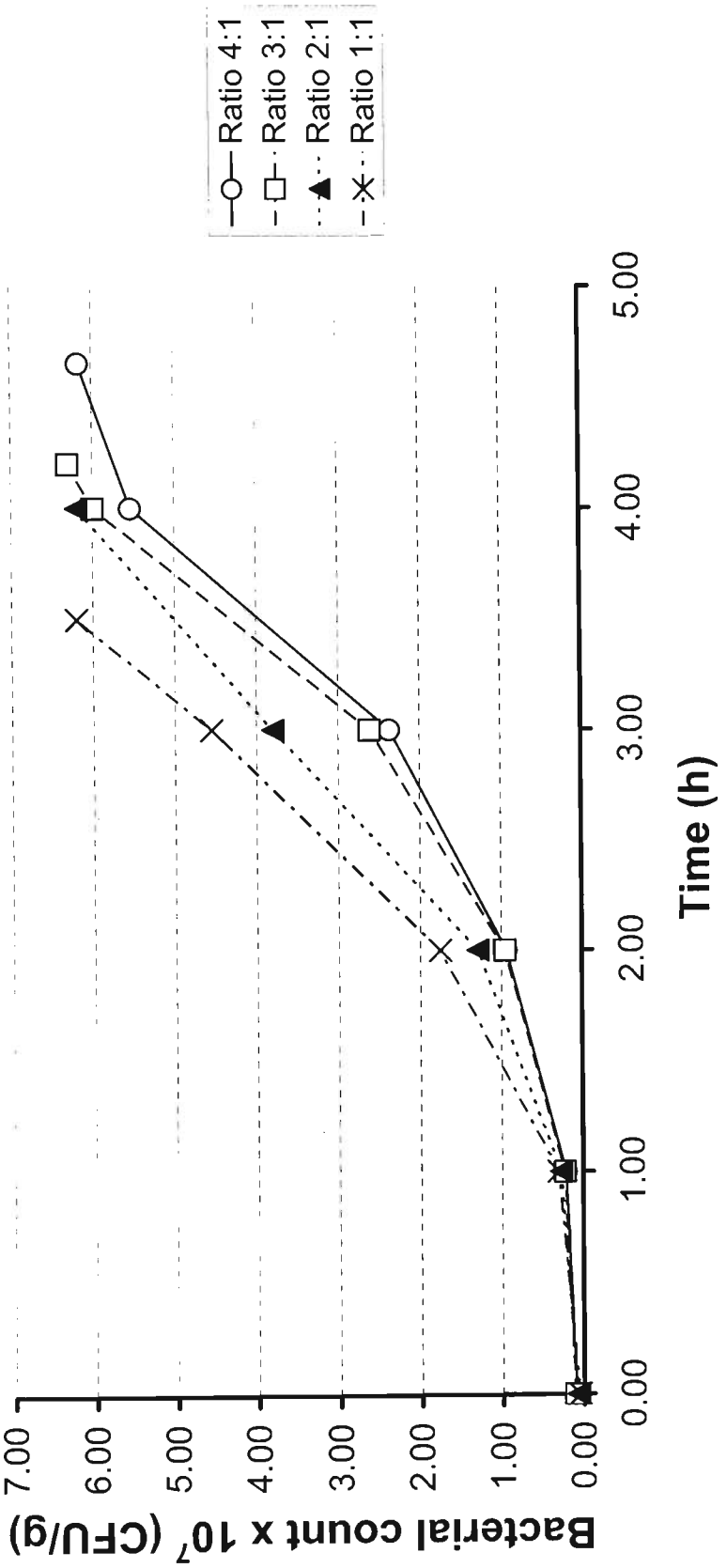

Figure 6.9: Growth curves of non-EPS-producing *S. thermophilus* during fermentation of yoghurt mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 14% total solids. The fermentation was terminated when the pH of 4.70 was achieved, n = 3.

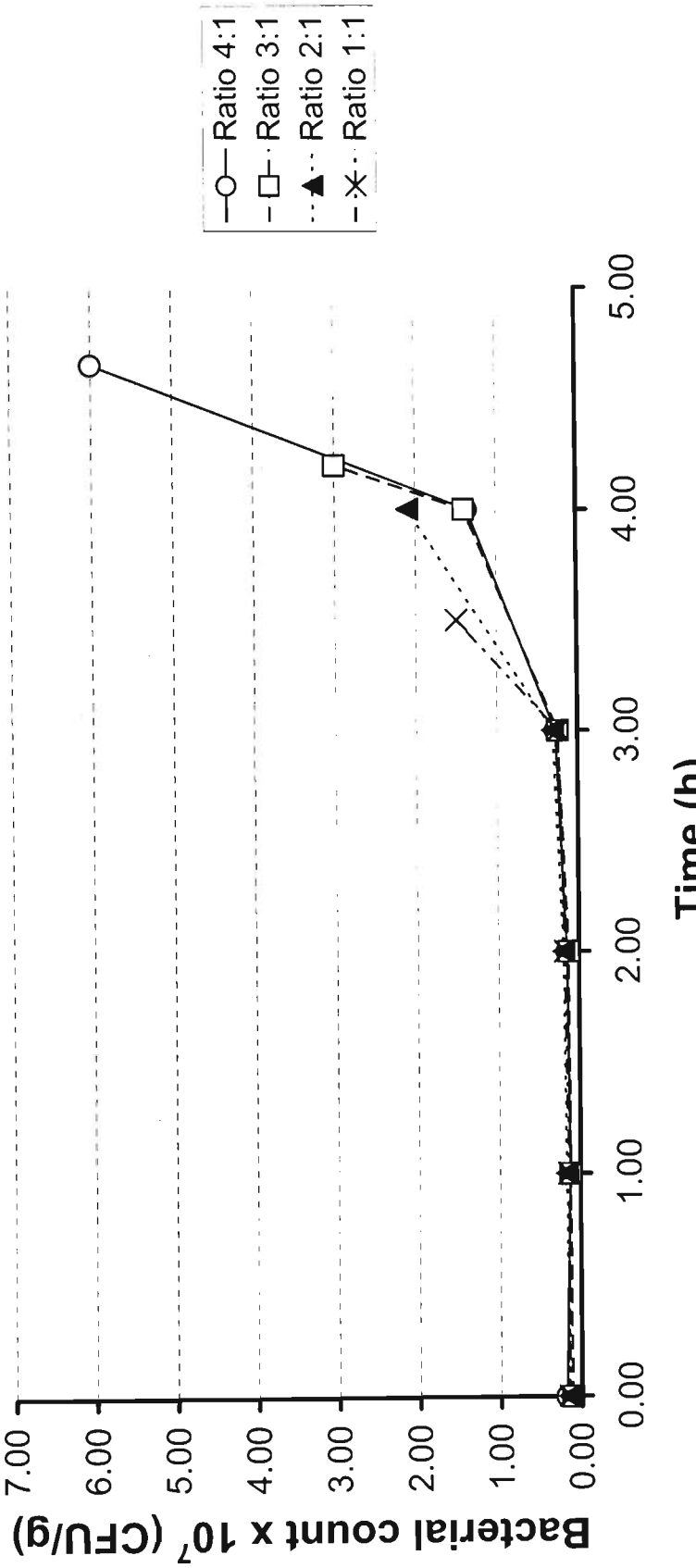

Figure 6.10: Growth curves of non-EPS-producing *L. delbrueckii* ssp. *bulgaricus* during fermentation of yoghurt mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 14% total solids. The fermentation was terminated when the pH of 4.70 was achieved, n = 3.

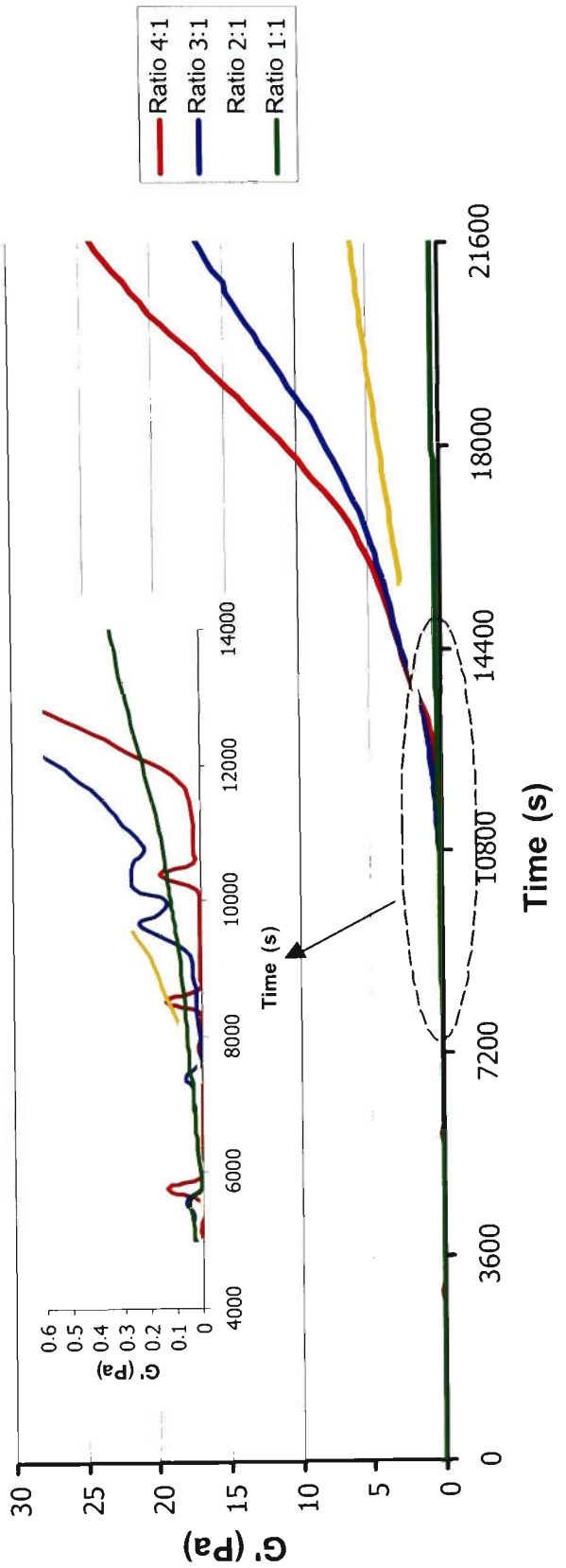

Figure 6.11: Development of storage modulus (G') during fermentation of yoghurt mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 9% solids using non-EPS-producing starter cultures, n = 3.

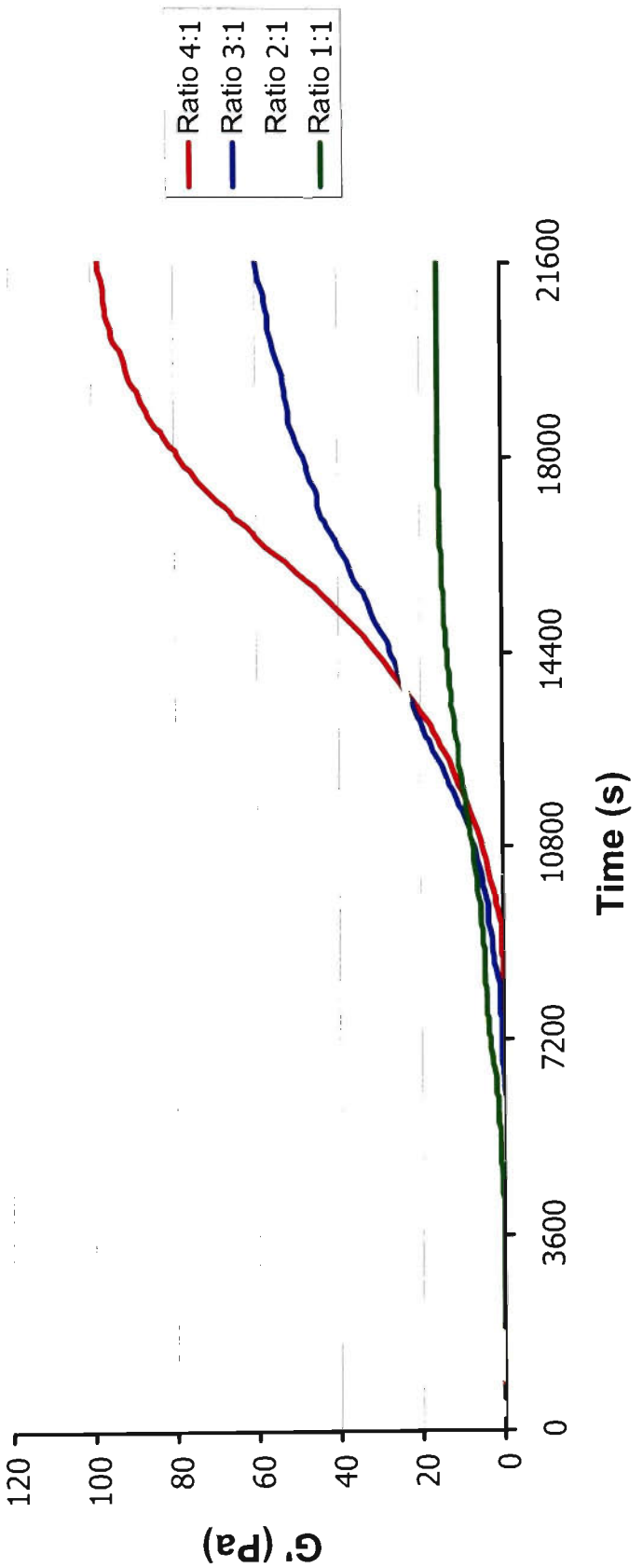

Figure 6.12: Development of storage modulus (G') during fermentation of yoghurt mix with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at 14% solids using non-EPS-producing starter cultures, n = 3.

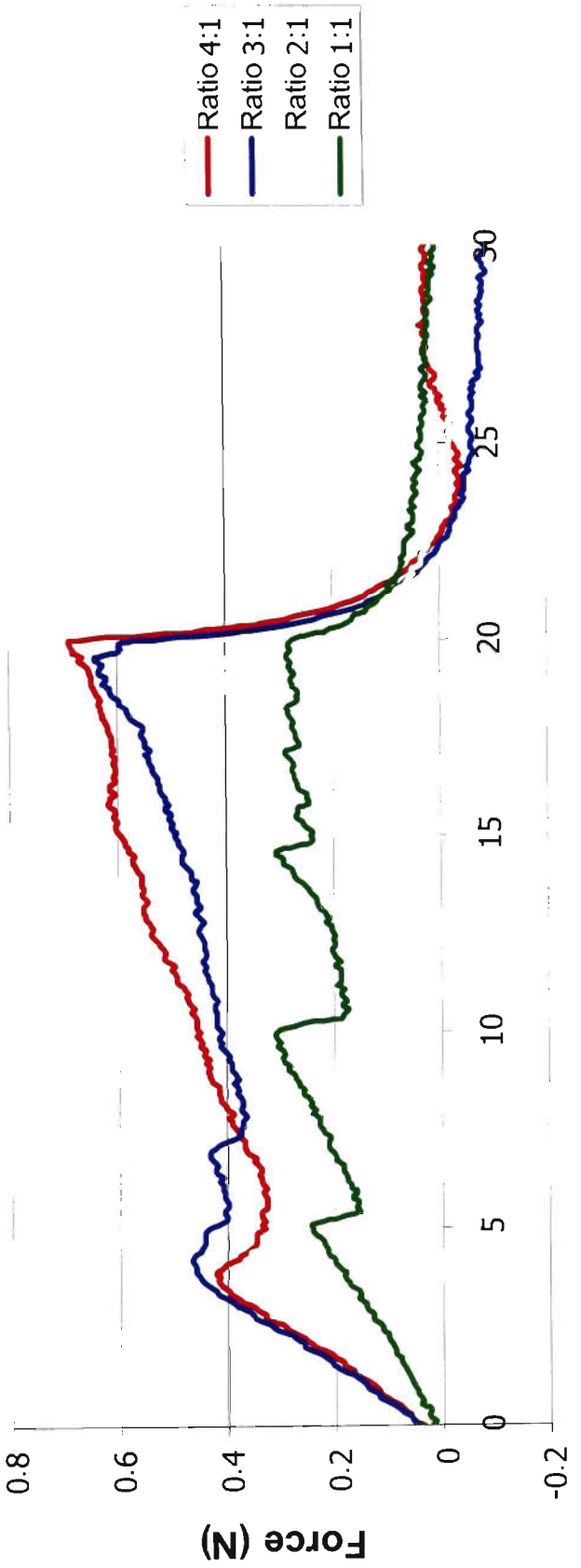

Figure 6.13: Force-Time curves during penetration test of set yoghurts made at 9% total solids with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-producing starter cultures after 1 day of storage, n = 3.

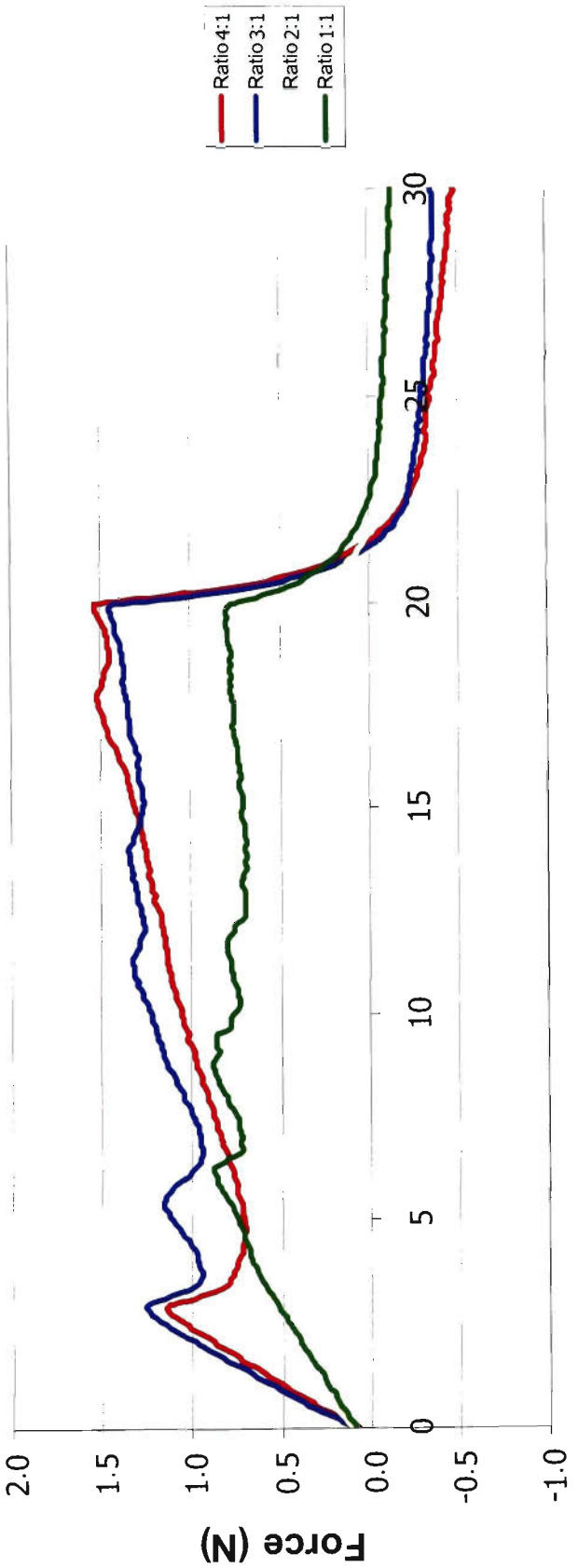

Figure 6.14: Force-Time curves during penetration test of set yoghurts made at 14% total solids with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-producing starter cultures after 1 day of storage, n = 3.

7.0 PHYSICAL PROPERTIES OF SET AND STIRRED YOGHURTS MADE AT 9% TOTAL SOLIDS OF VARYING CASEIN TO WHEY PROTEIN RATIOS USING EPS-PRODUCING STARTER CULTURES

7.1 Introduction

There has been an increasing trend in the use of starter cultures that are able to produce EPS (both capsular and ropy EPS) in yoghurt manufacture. This is due to their high water binding and texture promoting abilities. Both capsular and ropy EPS possess high water binding ability resulting in increased water retention in yoghurts (Wacher-Rodarte *et al.*, 1993; Hassan *et al.*, 1996b; Jaros *et al.*, 2002). Perry *et al.* (1997) reported that the moisture retention of mozzallela cheese increased by 2% as a result of using capsular EPS-producing starter cultures. However, fermented dairy products produced from EPS-producing starter cultures showed lower firmness (Hassan *et al.*, 1996a; Hess *et al.*, 1997). Jaros *et al.* (2002) observed an opposite result that set yoghurt made using EPS-producing starter cultures showed firmer texture compared to the control product. Marshall and Rawson (1999) observed that stirred yoghurts made with ropy EPS-producing starter cultures had higher apparent viscosity than those made using capsular and non-EPS-producing starter cultures. In addition, Hassan *et al.* (1995a, 2002b) observed an aggregation of casein micelles and changes in complex modulus during

fermentation were affected by capsular EPS-producing starter cultures but not ropy EPS- or non-EPS-producing starter cultures.

Two major concerns of using EPS-producing starter cultures in fermented dairy products are consistency in EPS production and degradation of EPS. An absence of EPS production in EPS producing strains of *S. thermophilus* and *L. casei* was reported by Cerning *et al.* (1988) and Mozzi *et al.* (1996), respectively. The loss of EPS producing ability in mesophilic lactic acid bacteria was hypothesized to be due to the loss of plasmid coding for EPS production (Broadbent *et al.*, 2003, Mozzi *et al.*, 1996). However, the loss of EPS-producing ability in *S. thermophilus* was reported to be reversed as the bacteria was grown in skim milk added with casein (Cernning *et al.*, 1988). Ariga *et al.* (1992) showed that this microorganism did not contain plasmid coding for EPS production. The EPS degradation during prolonged fermentation was observed by Degeest and De Vuyst (1999), Mozzi *et al.* (1996) and Pham *et al.* (2000). Mozzi *et al.* (1996) reported that the EPS concentration produced by *L. casei* decreased by almost 40% during prolonged fermentation from 24 to 72 h. Pham *et al.* (2000) observed the degradation of EPS produced by *Lactobacillus rhamnosus* R and a large spectrum of glycohydrolytic enzymes such as α-D-glucosidase, β-D-glucosidase, α-D-galactosidase, β-D-galactosidase, β-D-glucuronidase and α-L-rhamnosidase during prolonged fermentation. However, there is no report on degradation of EPS in yoghurt during cold storage. On the other hand, it is also possible that the EPS concentration in yoghurts may increase during storage that in turn may also affect physical properties of yoghurts.

The use of EPS-producing starter cultures in yoghurt manufacture has the potential to replace or reduce the use of stabilizers as well as dairy ingredients that are used for reducing syneresis and increasing firmness of set yoghurts, and for increasing viscosity of stirred yoghurts. Fortification with dairy ingredients not only increases the total solids level but also changes the ratios of CN to WP of milk depending on the types of ingredients used. Importantly, it affects production costs. Puvanenthiran *et al.* (2002) reported that the alteration of CN to WP ratio improved physical properties of set yoghurts.

By combining the use of EPS-producing starter cultures with the alteration of CN to WP ratio, it may be possible to maintain physical properties of yoghurts without the need for supplementation of milk or the use of stabilizers.

7.2 Aim

This chapter examines the physical properties of set- (firmness and syneresis) and stirred (apparent viscosity, flow curve, the area of hysteresis loop) yoghurts produced at 9% total solids level. The solids content is the minimum level required by the Australian Food Standard Code. The viable counts of starter cultures, concentrations of lactic acid and EPS were also monitored throughout the storage period of 28 days at 4°C.

7.3 Materials and Methods

7.3.1 Experimental design

This study evaluated chemical (concentrations of lactic acid and EPS), microbiological (the growth of starter cultures) and physical properties (firmness and syneresis of set yoghurts; flow curves, the area of hysteresis loop, apparent viscosity of stirred yoghurts) in twelve batches of yoghurts made at low solids content (9% w/w) as shown in Table 7.1.

Table 7.1: Summary of types of milk and starter cultures used for the production of yoghurts reported in Chapter 7

Variations	Types of milk (CN to WP ratio)	Starter cultures
1	4:1	**Non-EPS**: 1% (v/v) each of *S. thermophilus* ASCC 1342 (non-EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
2	3:1	
3	2:1	
4	1:1	
5	4:1	**Capsular EPS**: 1% each of *S. thermophilus* ASCC 285 (capsular EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
6	3:1	
7	2:1	
8	1:1	
9	4:1	**Ropy EPS** 1% each of *S. thermophilus* ASCC 1275 (ropy EPS) and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466 (non-EPS)
10	3:1	
11	2:1	
12	1:1	

All parameters were monitored at day 1, 7, 14, 21 and 28 during storage (4°C). the experiment was carried out in triplicate.

7.3.2 Starter culture

Non-EPS-producing *S. thermophilus* ASCC 1342 and non-EPS-producing *L. delbrueckii* ssp. *bulgaricus* ASCC 1466, capsular EPS-producing *S. thermophilus* ASCC 285, and ropy EPS-producing *S. thermophilus* ASCC 1275 were used in this study. The maintenance and activation of these bacteria were carried out as described in Chapter 3, Section 3.1.

7.3.3 Enumeration of starter cultures

The counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* at inoculation and 1 day after storage (4°C) were determined as described in Chapter 3, Section 3.2.

7.3.4 Yoghurts manufacture

Twelve batches each of set and stirred yoghurts were made at 9% solids content with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 as in Chapter 6, Section 6.3.2 using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures according to the procedure of yoghurt manufacture described in Chapter 3, Section 3.3.

7.3.5 Determination of lactic acid concentration in yoghurt

The concentration of lactic acid in yoghurt was determined using a high performance liquid chromatography (HPLC) according to the procedures described in Chapter 3, Section 3.6.

7.3.6 EPS isolation, purification and quantification in yoghurt

The EPS in yoghurt was isolated, purified and quantified according to the procedures given in Chapter 3, Section 3.7.

7.3.7 Determination of spontaneous syneresis in undisturbed set yoghurts

The level of spontaneous syneresis of undisturbed set yoghurts was determined using the siphon method according to the procedures described in Chapter 3, Section 3.8.

7.3.8 Firmness of set yoghurt

The firmness of set yoghurts was determined using TA-XT.2 Texture analyzer. The measurement was carried out according to procedure described in Chapter 3, Section 3.12 immediately after the sample was removed from the walk-in-cooler (4°C).

7.3.9 Flow curves, the area of hysteresis loop and apparent viscosity of stirred yoghurt

The flow curve and apparent viscosity of stirred yoghurt were constructed separately using a RS 50 RheoStress (Haake Rheometer, Karlsruhe, Germany) using a coaxial measuring cell, Z20 DIN sensor (gap = 0.85 mm). The measurements were carried out according to the procedures described in Chapter 3, Sections 3.14 and 3.15.

7.3.10 Statistical analysis

The data were analysed using one-way analysis of variance at 95% confidence level with SPSS version 10 for Windows (SPSS inc., NY, USA). The mean values were compared using Tukey's test.

7.4 Results and Discussion

7.4.1 Lactic acid concentration

The concentration of lactic acid in yoghurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during storage of 28 days is shown in Table 7.2. The data is also presented as three separate bar graphs: Figures 7.1a (non-EPS), 7.1b (capsular EPS) and 7.1c (ropy EPS). At day 1, there was a significant reduction ($P < 0.05$) in lactic acid concentration from around 0.90 to 0.70% (w/w) as the ratio of CN to WP was decreased from 4:1 to 1:1 (Figure 7.1a). This was observed in all types of products (Figures 7.1a to 7.1c). This could be due to the difference in buffering system of

milk blends (Section 6.4.3). Shorter fermentation time as well as the decrease in lactic acid concentration in products made with low CN to WP ratios confirmed lower buffering system of milk blends at low CN to WP ratios. The type of starter cultures did not affect the concentration of lactic acid. A slight increase in the concentration of lactic acid ($P < 0.05$) was observed in some samples (Non-EPS-2:1, 1:1/capsular EPS-4:1, 3:1, 2:1/ropy EPS-4:1, 1:1) after the first week of storage. This is believed to be due to an activity of starters during storage.

7.4.2 Viable counts

Table 7.3 shows the viable counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in yoghurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during storage of 28 days. At day 1, the viable counts of *S. thermophilus* in yoghurts were similar, whereas those of *L. delbrueckii* ssp. *bulgaricus* decreased as the ratio of CN to WP was reduced. This trend was observed in both types of products produced using non-EPS- or EPS-producing starter cultures. As discussed earlier (Chapter 6, Section 6.4.3), a decrease in the buffering system of milk blends made at low CN to WP ratios affected fermentation time and limited the growth of *L. delbrueckii* ssp. *bulgaricus*. An increase in the available nutrients as the proportion of WPC was increased may also contribute to the result. During storage, the counts of *S. thermophilus* in product made using non-EPS- and capsular EPS-producing starter cultures gradually decreased from day 1, whereas the counts in those made using ropy EPS-producing starter cultures increased slightly during the first week of storage and declined thereafter. The viable counts of *L. delbrueckii* ssp. *bulgaricus*

decreased gradually from day 1. At day 28, the counts of *L. delbrueckii* ssp. *bulgaricus* in products made using non-EPS-producing starter cultures with the CN to WP ratios of 4:1 or 1:1 showed the lowest bacterial counts (< 10^6 cfu g^{-1}). Conversely, higher numbers of *L. delbrueckii* ssp. *bulgaricus* were found in products produced using EPS-producing starter cultures.

7.4.3 EPS concentration

Table 7.4 shows EPS concentration in yoghurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during 28 days of storage. Figures 7.2a, 7.2b and 7.2c represent bar graphs which are reproduced from data in Table 7.4. In general, the concentration of EPS in yoghurts made using EPS-producing starter cultures ranged from 30 to 70 mg L^{-1} (Table 7.4; Figures 7.2b and 7.2c). Other researchers have reported the concentration of EPS produced by lactic acid bacteria in fermented milk products ranging from 40 to 400 mg L^{-1} (Marshall and Rawson, 1999; De Vuyst *et al.*, 2003; Toba *et al.*, 1991). These differences may be due to individual strains, level of inoculation, differences in fermentation conditions and methods of isolation, purification and quantification of EPS. In our preliminary study (Chapter 4, Section 4.4.2; Table 4.4), the EPS concentration in the yoghurt produced with RSM at 12% solids using ropy EPS-producing starter cultures was higher (~ 100 mg L^{-1}) than that at 9% solids (~ 40 mg L^{-1}) (Figure 7.2c). This may be explained by an increase in available nutrients in the 12% products as compared to that at 9%. Low

concentration of EPS of ~ 10 mg L^{-1} was observed in yoghurts produced using non-EPS-producing starter cultures (Table 7.3; Figure 7.2a). This is consistent with the result from previous Chapter (Section 4.4.2). The CSLM-micrographs in Chapter 9 confirmed that this non-EPS-producing starter culture produced EPS. However, the physical properties of set and stirred yoghurts made using this non-EPS-producing starter cultures were substantially different from those made using EPS-producing starter cultures as discussed below (Sections 7.4.3 to 7.4.6; Tables 7.5 to 7.8; Figures 7.3 to 7.7).

At day 1 of storage, the concentration of EPS in yoghurts produced from ropy EPS-producing starter cultures (Figure 7.2c) was slightly higher, but not significantly different ($P > 0.05$, Table 7.4) from those produced using capsular EPS-producing starter cultures (Figure 7.2c). The highest EPS concentration was observed in the product that was made with the CN to WP ratio of 2:1 using ropy EPS-producing starter cultures (~ 48 mg L^{-1}). Hassan *et al.* (2001) have reported that the addition of WPC into medium increased the size of capsule produced by capsular EPS-producing starter cultures. Similarly, Zisu and Shah (2003) reported that the addition of 0.5% of WPC to 10% RSM helped increase EPS production by ropy EPS strain. However, the EPS concentration in products made using non-EPS- and capsular EPS-producing starter cultures did not increase as a result of increasing proportion of WP ($P > 0.05$, Table 7.4). Interestingly, there was no decrease in the EPS concentration with a reduction in the fermentation time of yoghurts as a result of lowering the CN to WP ratio from 4:1 to 1:1 (Section 6.4.3; Figures 6.5 and 6.6).

Degeest and De Vuyst (1999) and Pham *et al.* (2000) observed degradation of EPS during prolonged fermentation (72 h) of EPS-producing starter cultures in a complex medium. These studies reported a correlation between a large spectrum of glycohydrolytic enzymes and the decrease in EPS concentration during prolonged fermentation. However, in our study, the EPS concentration did not decrease. The concentration of EPS in batches made using ropy EPS-producing starter cultures, particularly in the product made at the CN to WP ratio of 3:1 increased from 40 to 75 mg L^{-1}. This may suggest that there was no degradation of EPS in yoghurts during storage.

7.4.4 Syneresis in set yoghurts

The level of spontaneous syneresis in set yoghurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during storage of 28 days is shown in Table 7.5. The data is also presented as three separate bar graphs; Figures 7.3a, 7.3b and 7.3c show the level of syneresis in set yoghurts made using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures, respectively. The level of syneresis decreased significantly as the ratio of CN to WP was reduced from 4:1 to 1:1 ($P < 0.05$, Table 7.5). No syneresis was detected in the yoghurt at the CN to WP ratio of 1:1. This phenomenon was observed in all products (Figures 7.3a to 7.3c). The reduction in

syneresis as the ratio of CN to WP was decreased was similar to that reported by Puvanenthiran *et al.* (2002). Guzmán-González *et al.* (1999) reported that yoghurts supplemented with WPC had lower level of syneresis than the control yoghurt. Bhullar *et al.* (2002) and Remuef *et al.* (2003) also reported similar results. Additionally, they observed that yoghurts produced from milk supplemented with WPC had a denser protein matrix than those from unfortified milk. Harwalkar and Kalab (1986) reported a relationship between the reduction in syneresis and an increase in the density of protein matrix as a result of increased solids content in yoghurts. Puvanenthiran *et al.* (2002) suggested that an increase in the compactness of yoghurt microstructure with the reduction in the CN to WP ratios led to high level of the immobilization of free water in the yoghurt gel.

The level of syneresis in yoghurts made at a CN to WP ratio of 4:1 using EPS-producing starter cultures (4 to 5% w/w) was significantly lower than those with non-EPS-producing starter cultures (7% w/w) (Table 7.5; Figures 7.3a to 7.3c). Similar trends regarding the effect of EPS on a reduction in syneresis of yoghurt have been reported by other researchers (Marshall and Rawson, 1999; Wacher-Rodarte *et al.*, 1993). This may be due to the high water-binding capacity of EPS (Cerning, 1990; De Vuyst *et al.*, 1999) as well as due to the modification of yoghurt microstructure by the use of EPS-producing starter cultures as reported by Hassan *et al.* (1995a, 1995b, 2002b). The microstructure of set yoghurts made with varying CN to WP ratios using non-EPS- and EPS-producing starter cultures (both capsular and ropy) will be discussed in Chapter 9. Recently, Ruas-Madiedo and Zoon (2003)

studied the effects of rheological properties of serum containing EPS produced from *Lactobacillus lactis* strains on the permeability of skim milk gels. They concluded that the effect of EPS on permeability of serum through skim milk gel was mainly due to the flow behaviour (viscosity) of serum, not from the microstructure of milk gel. Although there was no information regarding type of EPS provided, it was assumed that *Lactobacillus lactis* strains produced ropy EPS. It is important to point out that the permeability test, the filtration of serum containing EPS through glass membrane, of Ruas-Madiedo and Zoon (2003) could have been influenced from gravitational force. This testing condition would be different from that occur during fermentation of yoghurt. In addition, the EPS produced from capsular EPS-producing starter cultures does not increase viscosity of serum as much as that of ropy EPS-producing starter cultures (Hassan *et al.*, 1996a; Petersen *et al.*, 2000). If the conclusion of Ruas-Madiedo and Zoon (2003) was correct, the level of spontaneous syneresis of samples made using capsular EPS-producing starter cultures would be similar to that of non-EPS products. However, yoghurts made using capsular EPS-producing starter cultures at the CN to WP ratio of 4:1 had the lowest level of syneresis as compared to those using non-EPS- and ropy EPS-producing starter cultures. It is interesting to note that there was only a slight difference in the syneresis of yoghurts made using capsular EPS- and ropy EPS-producing starter cultures at the CN to WP ratios of 2:1 and 1:1 (Table 7.5; Figures 7.3b and 7.3c). A greater difference in syneresis was observed in products at the CN to WP ratios of 4:1 and 3:1. It is possible that the influence of WP in products made at the CN to WP ratios of 2:1 and 1:1 surpassed the water-binding ability of

capsular EPS and ropy EPS. There was no change in the level of syneresis during storage, regardless of the type of starter cultures or CN to WP ratios.

7.4.5 Firmness of set yoghurts

The firmness of set yoghurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during storage of 28 days is shown in Table 7.6. The data is also presented as three separate bar graphs; Figures 7.4a, 7.4b and 7.4c show the firmness of set yoghurts made using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures, respectively. The firmness of yoghurts made at the CN to WP ratios of 4:1 and 3:1 using capsular EPS- and ropy EPS-producing starter cultures was significantly lower than those produced from non-EPS-producing starter cultures ($P < 0.05$, Table 7.6). At the CN to WP ratios of 2:1 and 1:1, there was no significant difference in firmness among products made with non-EPS- and EPS-producing starter cultures (both capsular and ropy) ($P > 0.05$, Table 7.6). Similar trends of a decrease in the firmness of set yoghurts made using EPS-producing starter cultures were reported by Hassan *et al.* (1996b), Hess *et al.* (1997) and Marshall and Rawson (1999). The incompatibility between EPS and proteins may explain this phenomenon. The EPS and proteins have like charges at pH above the isoelectric point of proteins (de Kruif and Tuinier, 2001) during yoghurt fermentation resulting in a repulsive force between EPS and proteins. The incompatibility between EPS and proteins may result in depletion induced attraction of casein micelles by EPS leading to the formation of acid milk gel filled with EPS particles (Tolstoguzov,

1997; de Kruif and Tuinier, 2001). This may cause a difference in protein aggregation and the structure of protein network between yoghurts made using non-EPS- and EPS-producing starter cultures.

The discussion on the effects of varying CN to WP ratios on gel firmness of yoghurt has already been given in Chapter 6, Sections 6.4.4 and 6.4.5. Briefly, the firmness decreased with the reduction in CN to WP ratios. This is likely to be due to the interaction between WP and CN resulting in the formation of whey protein coated layer on the surface of casein micelles. This may prevent casein to form clusters, aggregates and network and may result in the decrease in firmness as the ratios of CN to WP were reduced.

The yoghurts made with EPS-producing starter cultures (capsular and ropy) did not show any significant changes in firmness during storage ($P > 0.05$, Table 7.6, Figures 7.4b and 7.4c). On the contrary, the products produced with non-EPS-producing starter cultures (especially at CN to WP ratios of 4:1 and 3:1) showed an increase in firmness during the first week of storage and after that it remained constant (Figure 7.4a). It is possible that the presence of EPS could disrupt the continuity of protein network. Any change of protein networks due to an increased acidity will be minimixed.

7.4.6 Flow curves and the area of hysteresis loop of stirred yoghurt

The flow curves (one loop of upward curve and downward curve) of stirred yogurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures are shown in Figures 7.5a, 7.5b and 7.5c, respectively. The flow curves of stirred yoghurts made using the three different types of starter cultures were similar. It showed structural degradation after shearing (the presence of hysteresis loop). Stirred yoghurts made with ropy EPS-producing starter cultures (Figure 7.5c) had higher shear stress (on increasing shear rate corresponding to the upward curve) than those made using the other types of starter cultures. Several researchers have found that yoghurts made with ropy EPS-producing starter cultures had higher apparent viscosity than those made with non-EPS- or capsular EPS-producing starter cultures (Wacher-Rodarte *et al.*, 1993; Hassan *et al.*, 1996b; Hassan *et al.*, 2003a). Stirred yoghurts made using non-EPS-producing starter cultures (Figure 7.5a) had a similar shear stress to those made using capsular EPS-producing starter cultures (Figure 7.5b). For yoghurts made with EPS- or non-EPS-producing starter cultures, a CN to WP ratio of 1:1 gave a shear stress that was distinctively lower than that found at higher CN to WP ratios. It is possible that the difference in shear stress as a result of varying CN to WP ratios of stirred yoghurt was due to level of soluble denatured whey protein. As shown in Table 6.5, the soluble denatured whey protein decreased with decreasing CN to WP ratios. This means that there was an increase in denatured whey protein covered casein surface when decreasing CN to WP ratios. This prevents casein network formation causing a decrease in firmness, shear stress and viscosity.

Stirred yoghurts made with ropy EPS-producing starter cultures (Figure 7.5c) had a higher shear stress than those with capsular EPS-producing starter cultures (Figure 7.5b). The difference in shear stress between stirred yoghurts made with capsular EPS- and ropy EPS-producing starter cultures may be caused by the difference in physico-chemical properties (structure, charge, volume) of its EPS.

The area of hysteresis loop between the up and down shear rate vs. shear stress curves of stirred yoghurts made with various CN to WP ratios using non-EPS-, capsular EPS- and ropy EP-producing starter cultures is shown in Table 7.7. The data in Table 7.7 is also presented as three separate bar graphs in Figures 7.6a (non-EPS), 7.6b (capsular EPS) and 7.6c (ropy EPS). The hysteresis loop area of stirred yoghurts made with ropy EPS-producing starter cultures was significantly higher than that with non-EPS- and capsular EPS-producing starter cultures ($P < 0.05$, Table 7.7). The presence of ropy EPS could be solely responsible for the results observed as all stirred yoghurts had the same level of total solids and were made using the same procedure and given the incompatibility between EPS and milk proteins (de Kruif and Tuinier, 2001). According to Morris (1995), most polysaccharides exist in solution as random coils and can form entangled networks depending on their numbers (proportion to concentration) and molecular volume (size). The entangled network causes an increase in the viscosity of solution (Sworn, 2004).

Hassan *et al.* (2002b) observed the microstructure before and after stirring of milk fermented with a single strain of *L. delbrueckii* ssp. *bulgaricus* RR (ropy starter culture). They reported that the EPS segregated from the proteins into pores forming the pools of EPS in the stirred gel compared to the set gel. They suggested that stirring promoted the interactions between molecules of ropy EPS. However, they did not specify whether the interactions are chemical or physical (entanglement) type. However, it is likely that the interactions, as stated by Hassan *et al.* (2002b), are physical or the entanglement type of polysaccharide interactions. Although the stirred yoghurt in this study were made by passing set product through a sieve, the entangled networks of ropy EPS are expected to occur in product in our study as well.

Theoretically, the area of hysteresis loop between upward and downward flow curves represents the structural breakdown of stirred yoghurt during shearing (Halmos and Tiu, 1981; Ramaswamy and Basak, 1991; Hassan *et al.*, 1996a, 2003a). The formation of entangled networks of ropy EPS, as an additional structure in stirred yoghurt, may explain the increase in the area of hysteresis loop of products made using ropy EPS-producing starter cultures. It also implies that there was less or no entangled network of EPS formed in the products made using capsular EPS-producing starter cultures as the value of hysteresis loop was comparable to that made with non-EPS-starter cultures.

The area of hysteresis loop of stirred yoghurts made using non-EPS- (Figures 7.6a) or capsular EPS-producing starter cultures (Figure 7.6b) decreased as the CN to WP ratio was decreased. As the presence of capsular EPS did not contribute to the hysteresis loop area, the results can be interpreted as a decrease in the structural breakdown of proteins during shearing. This could also mean that there was a significant damage of protein structure in initial stirred yoghurts made at low CN to WP ratios compared to those made at high ratios prior to measurement. In our study, stirred yoghurt was produced by pressing the gel of set yoghurt with a spoon through a sieve. Applying the same procedure with the soft gel (set yoghurt made at a CN to WP ratio of 1:1) could result in higher structural damage of proteins than that with a firm gel (set yoghurt made at a CN to WP ratio of 4:1). Stirred yoghurt made with a CN to WP ratio of 3:1 and using ropy EPS-producing starter cultures had the highest hysteresis loop area.

During storage, a slight increase in the area of hysteresis loop was observed in stirred yoghurts made using ropy EPS-producing starter cultures (Table 7.7; Figures 7.6a to 7.6c). The change in the loop area correlated with the increase in its EPS concentration (Table 7.4). This relationship was not observed in the products made with non-EPS- or capsular EPS-producing starter cultures. This could be due to the ability of ropy EPS in the formation of entangled network. Furthermore, the loop area decreased slightly in stirred yoghurts made using either non-EPS- or EPS-producing starter cultures (both capsular and ropy) after day 14 and 21. However, as

this phenomenon was also observed in stirred yoghurt made using non-EPS-producing starter cultures, the decrease in loop area may be due to changes in milk proteins.

7.4.7 The apparent viscosity of stirred yoghurts

The apparent viscosity of stirred yoghurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures is shown in Table 7.8. The data is also presented in the form of bar graphs in Figures 7.7a (non-EPS), 7.7b (capsular EPS) and 7.7c (ropy EPS). The apparent viscosity of yoghurts made with ropy EPS-producing starter cultures with the CN to WP ratios of 3:1 and 2:1 (~1.10 Pa s) was significantly higher ($P < 0.05$, Table 7.8) than those with non-EPS- or capsular EPS-producing starter cultures (~0.60 to ~0.90). These results were similar to those of the area of hysteresis loops in previous section (Section 7.4.6). The entanglement of ropy EPS was also believed to be responsible for an increase in apparent viscosity of stirred yoghurts apart from its effects on the area of hysteresis loop as discussed above (Section 7.4.6). An additional structure of entangled ropy EPS would increase resistance to flow or apparent viscosity of stirred yoghurt. The apparent viscosity of stirred yoghurts made using non-EPS- and capsular EPS-producing starter cultures decreased when the CN to WP ratios were reduced from 4:1 to 1:1. (Table 7.8; Figures 7.7a to 7.7b). The increase in structural damage of protein network in samples made at low CN to WP ratios was considered as the cause of this phenomenon. During storage, the apparent viscosity of samples made using ropy EPS-producing starter cultures with

the CN to WP ratios of 3:1 and 2:1 increased slightly which could be related to the increase in EPS concentration as mentioned above.

7.5 Conclusions

A decrease in the ratio of CN to WP caused a decrease in the level of syneresis and firmness of set yoghurts made at 9% total solids content. The use of EPS-producing starter cultures further reduced the level of syneresis and gel firmness. In stirred yoghurts, there was an increase in the shear stress, apparent viscosity and the area of hysteresis loop in products made using ropy EPS-producing starter cultures. The rheological characteristics of stirred yoghurts appear to depend on the concentration of ropy EPS. Furthermore, there was no degradation of EPS during storage. Based on these results, set yoghurt made at 9% solids should be produced using non-EPS-producing starter cultures with a CN to WP ratio of 3:1. Stirred yoghurt should be made using ropy EPS-producing starter cultures with a CN to WP ratio of 3:1. However, this study dealt only with yoghurts made at 9% solids content. Increasing solids level of yoghurts to 14% may result in different patterns of results.

Table 7.2: Lactic acid concentration in yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Lactic acid concentration (%, w/w)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$0.93 \pm 0.03^{g,A}$	$0.99 \pm 0.06^{e,A}$	$0.95 \pm 0.07^{d,e,A}$	$0.96 \pm 0.09^{d,e,A}$	$0.95 \pm 0.03^{d,A}$
	3:1	$0.85 \pm 0.04^{d,e,f,g,A}$	$0.88 \pm 0.04^{d,e,A}$	$0.87 \pm 0.08^{b,c,d,e,A}$	$0.90 \pm 0.06^{c,d,e,A}$	$0.84 \pm 0.01^{b,c,d,A}$
	2:1	$0.75 \pm 0.06^{b,c,d,A}$	$0.85 \pm 0.01^{c,d,A,B}$	$0.79 \pm 0.05^{a,b,c,d,A,B}$	$0.89 \pm 0.03^{b,c,d,e,B}$	$0.80 \pm 0.06^{a,b,c,A,B}$
	1:1	$0.69 \pm 0.04^{a,b,c,A}$	$0.75 \pm 0.04^{a,b,c,A,B}$	$0.69 \pm 0.03^{a,A}$	$0.80 \pm 0.03^{a,b,c,d,B}$	$0.78 \pm 0.05^{a,b,c,A,B}$
Capsular EPS-producing starter cultures	4:1	$0.85 \pm 0.03^{f,g,A}$	$1.01 \pm 0.04^{e,B,C}$	$1.02 \pm 0.02^{e,C}$	$0.97 \pm 0.09^{e,A,B,C}$	$0.89 \pm 0.04^{c,d,A,B}$
	3:1	$0.80 \pm 0.05^{c,d,e,f,A}$	$0.93 \pm 0.06^{d,e,B}$	$0.94 \pm 0.03^{d,e,B}$	$0.86 \pm 0.03^{a,b,c,d,e,A,B}$	$0.80 \pm 0.06^{a,b,c,A}$
	2:1	$0.72 \pm 0.01^{b,c,A}$	$0.82 \pm 0.04^{a,b,c,d,A,B}$	$0.86 \pm 0.02^{b,c,d,e,B}$	$0.77 \pm 0.07^{a,b,c,A,B}$	$0.77 \pm 0.08^{a,b,c,A,B}$
	1:1	$0.60 \pm 0.01^{a,A}$	$0.72 \pm 0.04^{a,b,A}$	$0.71 \pm 0.02^{a,b,A}$	$0.69 \pm 0.07^{a,A}$	$0.69 \pm 0.08^{a,A}$
Ropy EPS-producing starter cultures	4:1	$0.89 \pm 0.02^{e,f,g,A,B}$	$0.90 \pm 0.01^{d,e,A,B}$	$0.99 \pm 0.05^{e,B}$	$0.86 \pm 0.08^{b,c,d,e,A}$	$0.89 \pm 0.02^{c,d,A,B}$
	3:1	$0.84 \pm 0.02^{d,e,f,g,A}$	$0.91 \pm 0.05^{d,e,A}$	$0.92 \pm 0.06^{c,d,e,A}$	$0.89 \pm 0.03^{c,d,e,A}$	$0.88 \pm 0.04^{c,d,A}$
	2:1	$0.75 \pm 0.04^{b,c,d,e,A}$	$0.84 \pm 0.03^{b,c,d,A}$	$0.78 \pm 0.09^{a,b,c,A}$	$0.79 \pm 0.08^{a,b,c,d,A}$	$0.85 \pm 0.04^{b,c,d,A}$
	1:1	$0.69 \pm 0.01^{a,b,A}$	$0.70 \pm 0.04^{a,A}$	$0.79 \pm 0.05^{a,b,c,d,B}$	$0.72 \pm 0.02^{a,b,A,B}$	$0.72 \pm 0.03^{a,b,A,B}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e,f,g}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

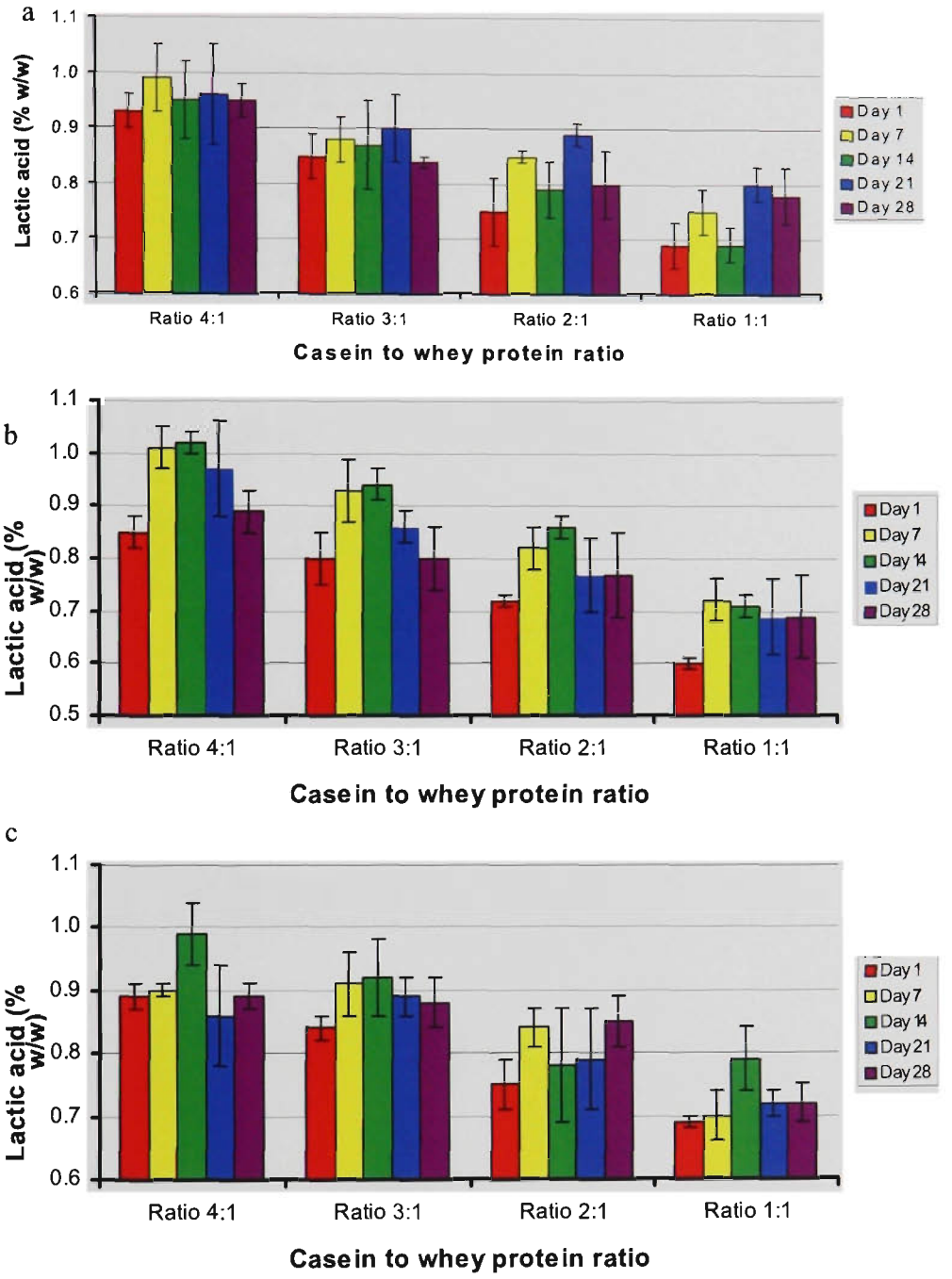

Figure 7.1: Lactic acid concentration in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 7.1). Error bars represent standard deviation (n = 3).

Table 7.3: Viable counts[1] ($\log_{10}$(cfu g^{-1})) of *S. thermophilus* (ST) and *L. delbrueckii* ssp. *bulgaricus* (LB) in yoghurts prepared with various CN to WP ratios using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during storage (4°C)

Starter culture	Casein to whey protein ratios	Storage period									
		Day 1		Day 7		Day 14		Day 21		Day 28	
		ST	LB	ST	LB	ST	LB	ST	LB	ST	LB
Non-EPS-producing starter cultures	4:1	8.89	7.73	8.86	7.79	8.41	7.86	8.00	6.88	7.88	5.45
	3:1	8.94	7.53	8.88	7.86	8.56	7.85	7.73	7.63	7.90	7.51
	2:1	8.94	7.65	8.86	7.68	8.75	7.54	8.58	6.74	8.38	6.68
	1:1	8.78	7.01	8.89	7.62	8.70	6.91	8.64	6.61	8.58	5.29
Capsular EPS-producing starter cultures	4:1	8.85	7.64	8.84	7.69	8.61	7.74	7.71	6.67	7.44	6.37
	3:1	8.79	7.71	8.56	7.69	8.53	7.66	8.35	7.11	8.18	6.68
	2:1	8.84	7.51	8.70	7.46	8.56	7.59	8.61	6.95	8.52	6.47
	1:1	8.63	7.59	8.44	7.32	8.41	7.51	8.36	6.51	7.72	6.46
Ropy EPS-producing starter cultures	4:1	8.82	7.73	9.05	7.82	8.70	8.09	8.37	7.69	8.53	7.50
	3:1	8.80	7.85	8.84	7.67	9.33	8.03	8.53	7.46	8.49	7.67
	2:1	8.85	7.76	9.03	7.64	8.69	7.59	8.58	7.74	7.73	7.22
	1:1	8.81	7.11	8.89	7.45	8.90	7.09	8.67	7.28	7.72	7.47

[1] n = 3

Table 7.4: EPS concentration in yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	EPS concentration (mg L^{-1})[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$9.10 \pm 2.11^{a,A}$	$10.30 \pm 1.19^{a,A}$	$9.48 \pm 0.09^{a,A}$	$10.44 \pm 1.34^{a,A}$	$8.54 \pm 3.71^{a,A}$
	3:1	$8.03 \pm 0.71^{a,A}$	$9.74 \pm 0.14^{a,A}$	$9.68 \pm 0.97^{a,A}$	$8.27 \pm 0.86^{a,A}$	$9.68 \pm 1.40^{a,A}$
	2:1	$9.20 \pm 1.04^{a,A,B}$	$9.04 \pm 0.51^{a,A,B}$	$8.86 \pm 0.81^{a,A,B}$	$9.70 \pm 0.83^{a,B}$	$7.96 \pm 0.71^{a,A}$
	1:1	$7.95 \pm 1.20^{a,A}$	$10.01 \pm 0.26^{a,A}$	$8.85 \pm 1.90^{a,A}$	$8.28 \pm 2.47^{a,A}$	$8.76 \pm 1.69^{a,A}$
Capsular EPS-producing starter cultures	4:1	$32.41 \pm 0.73^{b,c,B}$	$28.89 \pm 0.94^{b,A}$	$30.86 \pm 2.96^{b,c,A,B}$	$36.90 \pm 1.00^{b,c,C}$	$33.51 \pm 0.71^{b,B}$
	3:1	$32.54 \pm 0.31^{b,c,A,B}$	$29.37 \pm 0.93^{b,A}$	$33.43 \pm 3.92^{b,c,B}$	$30.34 \pm 1.55^{b,A,B}$	$32.40 \pm 1.06^{b,A,B}$
	2:1	$29.16 \pm 2.84^{b,A}$	$28.83 \pm 1.27^{b,A}$	$28.53 \pm 3.43^{b,A}$	$31.48 \pm 0.87^{b,A}$	$31.86 \pm 5.66^{b,A}$
	1:1	$38.35 \pm 3.56^{b,c,d,A}$	$31.35 \pm 3.80^{b,A}$	$32.85 \pm 4.22^{b,c,A}$	$36.15 \pm 2.17^{b,c,A}$	$36.06 \pm 1.80^{b,A}$
Ropy EPS-producing starter cultures	4:1	$41.57 \pm 8.19^{c,d,e,A}$	$47.78 \pm 3.42^{d,A}$	$49.69 \pm 13.75^{d,e,A}$	$53.97 \pm 5.63^{d,e,A,B}$	$66.45 \pm 5.40^{d,B}$
	3:1	$43.20 \pm 3.04^{d,e,A}$	$47.72 \pm 2.02^{d,A,B}$	$60.06 \pm 11.79^{e,B,C}$	$63.21 \pm 12.47^{e,B,C}$	$75.41 \pm 6.70^{e,C}$
	2:1	$48.62 \pm 9.99^{e,A,B}$	$41.48 \pm 4.78^{c,A}$	$58.41 \pm 8.79^{e,B}$	$50.13 \pm 11.59^{d,A,B}$	$62.31 \pm 2.69^{c,d,B}$
	1:1	$36.64 \pm 4.15^{b,c,d,A}$	$42.47 \pm 3.81^{c,A,B}$	$42.39 \pm 4.02^{c,d,A,B}$	$44.70 \pm 2.72^{c,d,B}$	$57.33 \pm 2.38^{c,C}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B,C}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

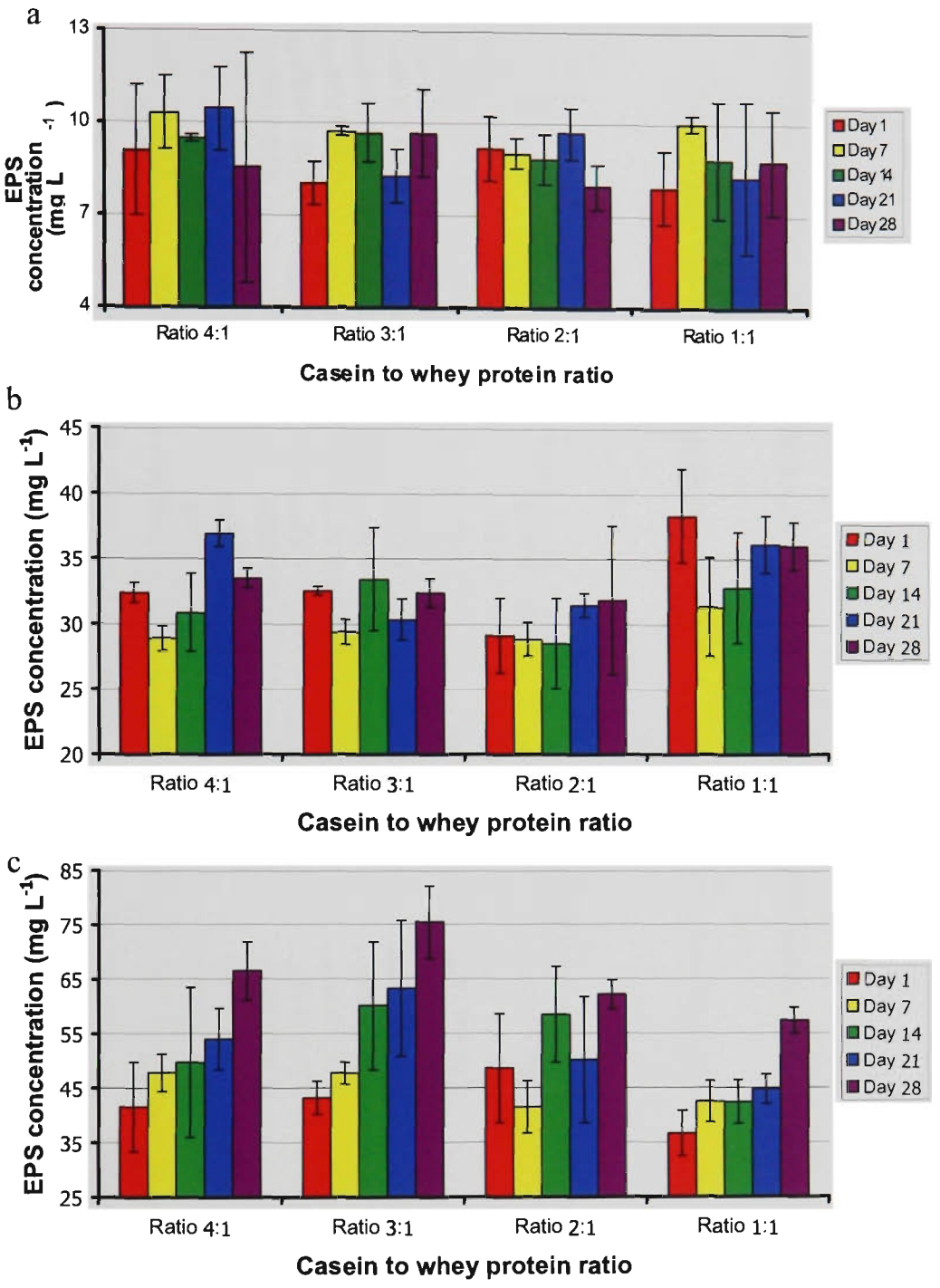

Figure 7.2: EPS concentration in yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 7.3). Error bars represent standard deviation (n = 3).

Table 7.5: Syneresis in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Syneresis (%, w/w)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$7.31 \pm 0.31^{f,A}$	$6.71 \pm 0.56^{f,A}$	$6.80 \pm 0.45^{e,A}$	$6.95 \pm 0.83^{f,A}$	$6.86 \pm 0.82^{d,A}$
	3:1	$3.04 \pm 0.29^{c,d,A}$	$4.11 \pm 1.18^{d,e,A}$	$3.33 \pm 0.15^{d,A}$	$4.95 \pm 0.67^{e,A}$	$4.91 \pm 1.70^{c,d,A}$
	2:1	$1.56 \pm 0.69^{b,A}$	$1.52 \pm 0.39^{a,b,c,A}$	$1.83 \pm 0.39^{b,c,A}$	$2.07 \pm 0.19^{b,c,d,A}$	$1.99 \pm 0.32^{a,b,A}$
	1:1	$0.04 \pm 0.05^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$
Capsular EPS-producing starter cultures	4:1	$3.39 \pm 0.52^{d,A}$	$3.21 \pm 0.33^{c,d,e,A}$	$2.75 \pm 0.52^{c,d,A}$	$3.04 \pm 0.22^{c,d,A}$	$3.05 \pm 0.42^{b,c,A}$
	3:1	$3.12 \pm 0.59^{c,d,A}$	$2.46 \pm 0.22^{b,c,d,A}$	$2.15 \pm 0.16^{c,d,A}$	$2.53 \pm 0.47^{c,d,A}$	$2.94 \pm 0.64^{a,b,c,A}$
	2:1	$1.26 \pm 0.85^{a,b,A}$	$0.87 \pm 0.49^{a,b,A}$	$0.59 \pm 0.10^{a,b,A}$	$0.79 \pm 0.74^{a,b,c,A}$	$1.39 \pm 0.73^{a,b,A}$
	1:1	$0.19 \pm 0.23^{a,b,A}$	$0.26 \pm 0.33^{a,b,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$
Ropy EPS-producing starter cultures	4:1	$4.68 \pm 1.00^{e,A,B}$	$5.43 \pm 0.61^{e,f,A,B}$	$6.12 \pm 1.49^{e,B}$	$4.94 \pm 0.70^{e,f,B}$	$4.20 \pm 0.73^{b,c,A}$
	3:1	$2.75 \pm 0.76^{d,A}$	$1.83 \pm 0.48^{a,b,c,A}$	$1.62 \pm 0.59^{b,c,A}$	$2.31 \pm 0.79^{d,A}$	$1.96 \pm 0.92^{a,b,c,A}$
	2:1	$0.67 \pm 0.22^{a,b,A}$	$0.93 \pm 0.58^{a,b,c,A}$	$0.80 \pm 0.73^{a,A}$	$0.53 \pm 0.61^{a,b,A}$	$1.06 \pm 0.88^{a,b,A}$
	1:1	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.07 \pm 0.08^{a,A}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e,f}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

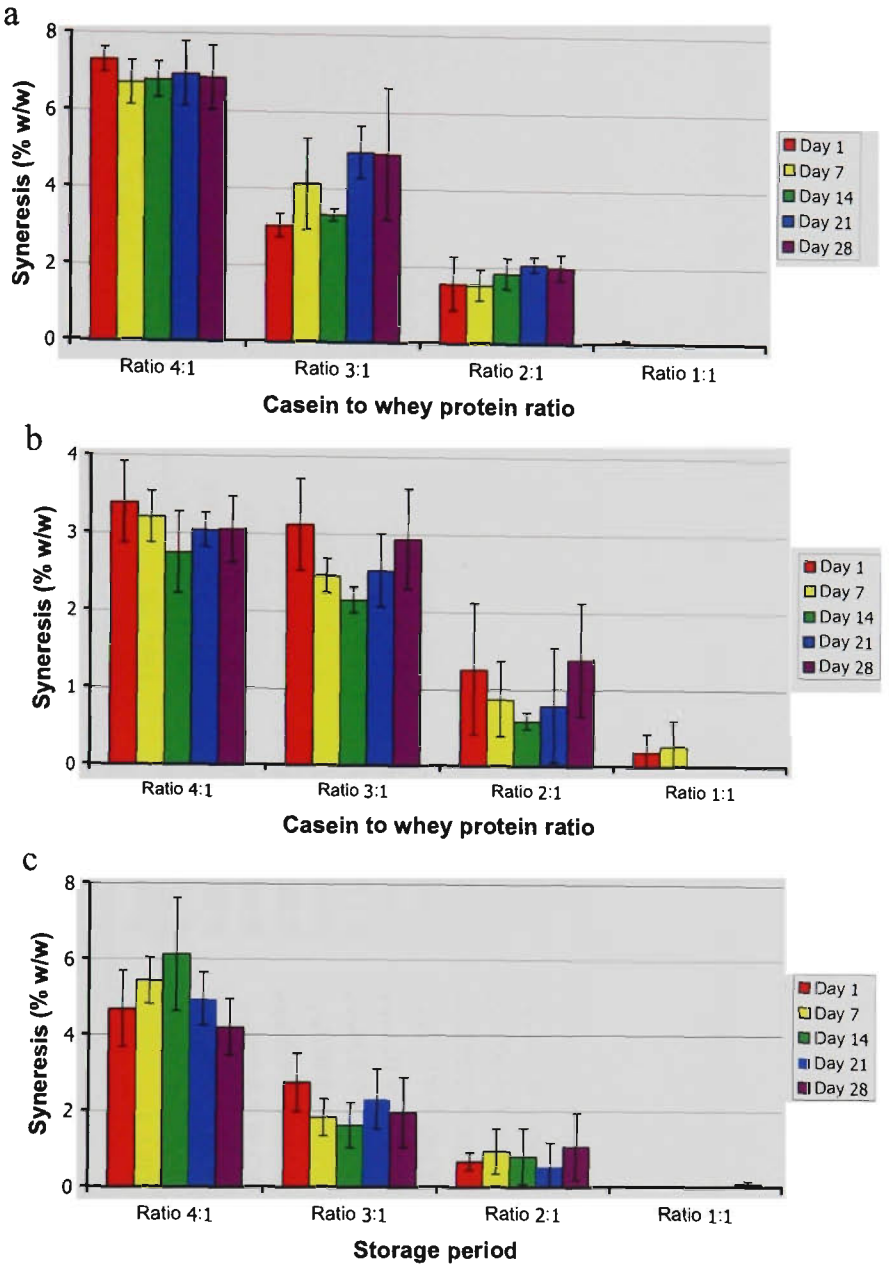

Figure 7.3: Syneresis in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 7.4). Error bars represent standard deviation (n = 3).

Table 7.6: Firmness in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Firmness (N)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$0.517 \pm 0.053^{c,A}$	$0.752 \pm 0.062^{f,B}$	$0.704 \pm 0.058^{d,B}$	$0.735 \pm 0.002^{f,B}$	$0.747 \pm 0.020^{g,B}$
	3:1	$0.499 \pm 0.087^{c,A}$	$0.624 \pm 0.018^{e,A,B}$	$0,633 \pm 0.005^{d,A,B}$	$0.680 \pm 0.049^{e,A,B}$	$0.610 \pm 0.023^{f,B}$
	2:1	$0.376 \pm 0.031^{b,A}$	$0.420 \pm 0.041^{d,A,B}$	$0.469 \pm 0.019^{c,B}$	$0.416 \pm 0.006^{d,A,B}$	$0.453 \pm 0.026^{e,B}$
	1:1	$0.294 \pm 0.036^{a,b,A}$	$0.293 \pm 0.032^{a,b,c,A}$	$0.275 \pm 0.024^{a,A}$	$0.298 \pm 0.005^{a,b,A}$	$0.294 \pm 0.002^{a,b,A}$
Capsular EPS-producing starter cultures	4:1	$0.391 \pm 0.019^{b,A}$	$0.378 \pm 0.027^{c,d,A}$	$0.378 \pm 0.018^{b,A}$	$0.377 \pm 0.012^{c,d,A}$	$0.387 \pm 0.002^{d,A}$
	3:1	$0.365 \pm 0.034^{a,b,A}$	$0.358 \pm 0.014^{c,d,A}$	$0.371 \pm 0.012^{b,A}$	$0.350 \pm 0.010^{c,A}$	$0.367 \pm 0.013^{c,d,A}$
	2:1	$0.315 \pm 0.027^{a,b,A}$	$0.303 \pm 0.006^{a,b,c,A}$	$0.311 \pm 0.024^{a,b,A}$	$0.333 \pm 0.013^{b,c,A}$	$0.332 \pm 0.003^{b,c,A}$
	1:1	$0.262 \pm 0.032^{a,A}$	$0.233 \pm 0.014^{a,A}$	$0.252 \pm 0.014^{a,A}$	$0.271 \pm 0.013^{a,A}$	$0.259 \pm 0.020^{a,A}$
Ropy EPS-producing starter cultures	4:1	$0.306 \pm 0.061^{a,b,A}$	$0.350 \pm 0.038^{c,d,A}$	$0.339 \pm 0.056^{a,b,A}$	$0.352 \pm 0.018^{c,A}$	$0.358 \pm 0.015^{c,d,A}$
	3:1	$0.338 \pm 0.007^{a,b,A}$	$0.342 \pm 0.024^{b,c,d,A}$	$0.330 \pm 0.042^{a,b,A}$	$0.336 \pm 0.006^{b,c,A}$	$0.361 \pm 0.001^{c,d,A}$
	2:1	$0.290 \pm 0.025^{a,b,A}$	$0.311 \pm 0.021^{a,b,c,A}$	$0.326 \pm 0.012^{a,b,A}$	$0.347 \pm 0.010^{b,c,A}$	$0.303 \pm 0.031^{a,b,A}$
	1:1	$0.260 \pm 0.039^{a,A}$	$0.262 \pm 0.010^{a,b,A}$	$0.288 \pm 0.003^{a,A}$	$0.273 \pm 0.016^{a,A}$	$0.304 \pm 0.011^{a,b,A}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e,f,g}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

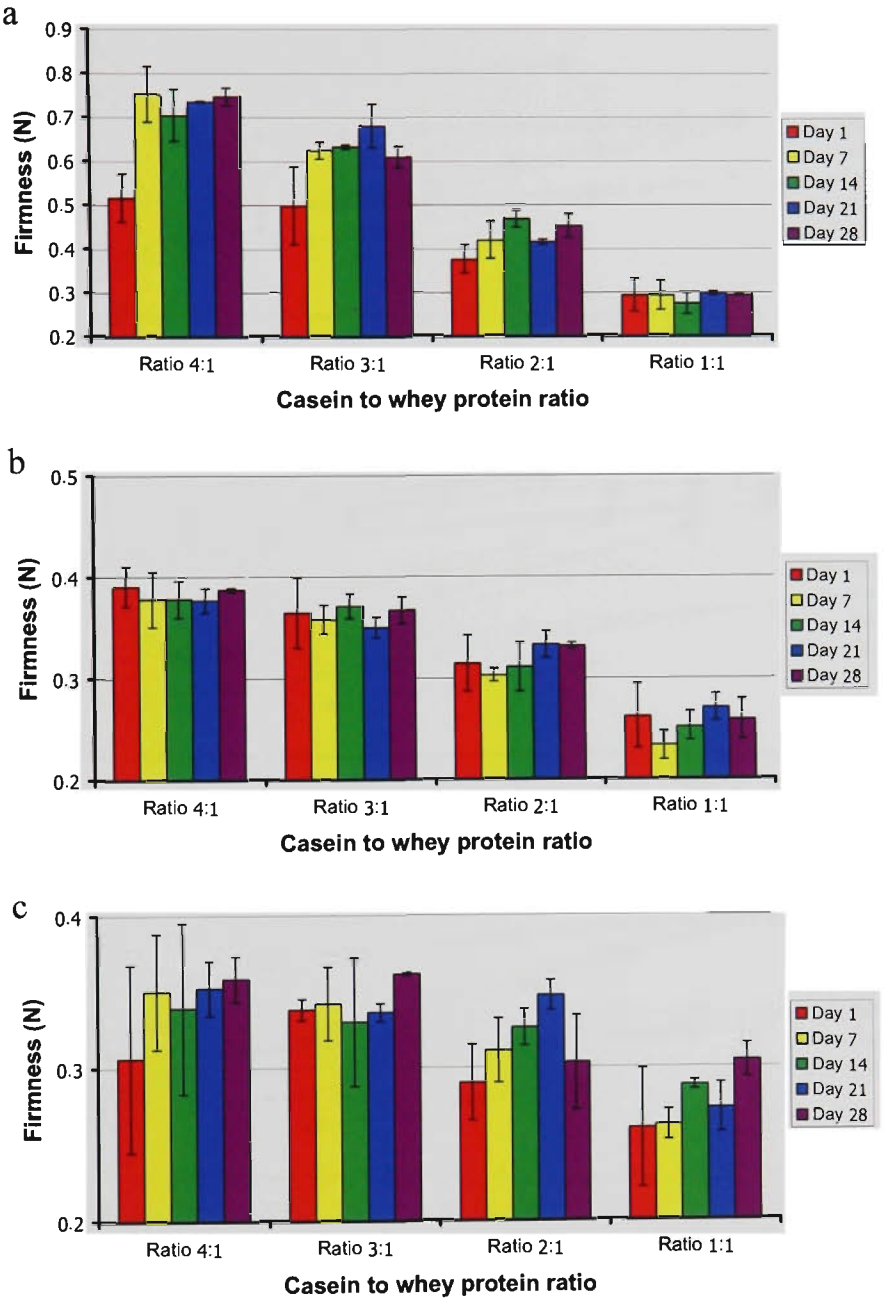

Figure 7.4: Firmness of set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c) -producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 7.5). Error bars represent standard deviation (n = 3).

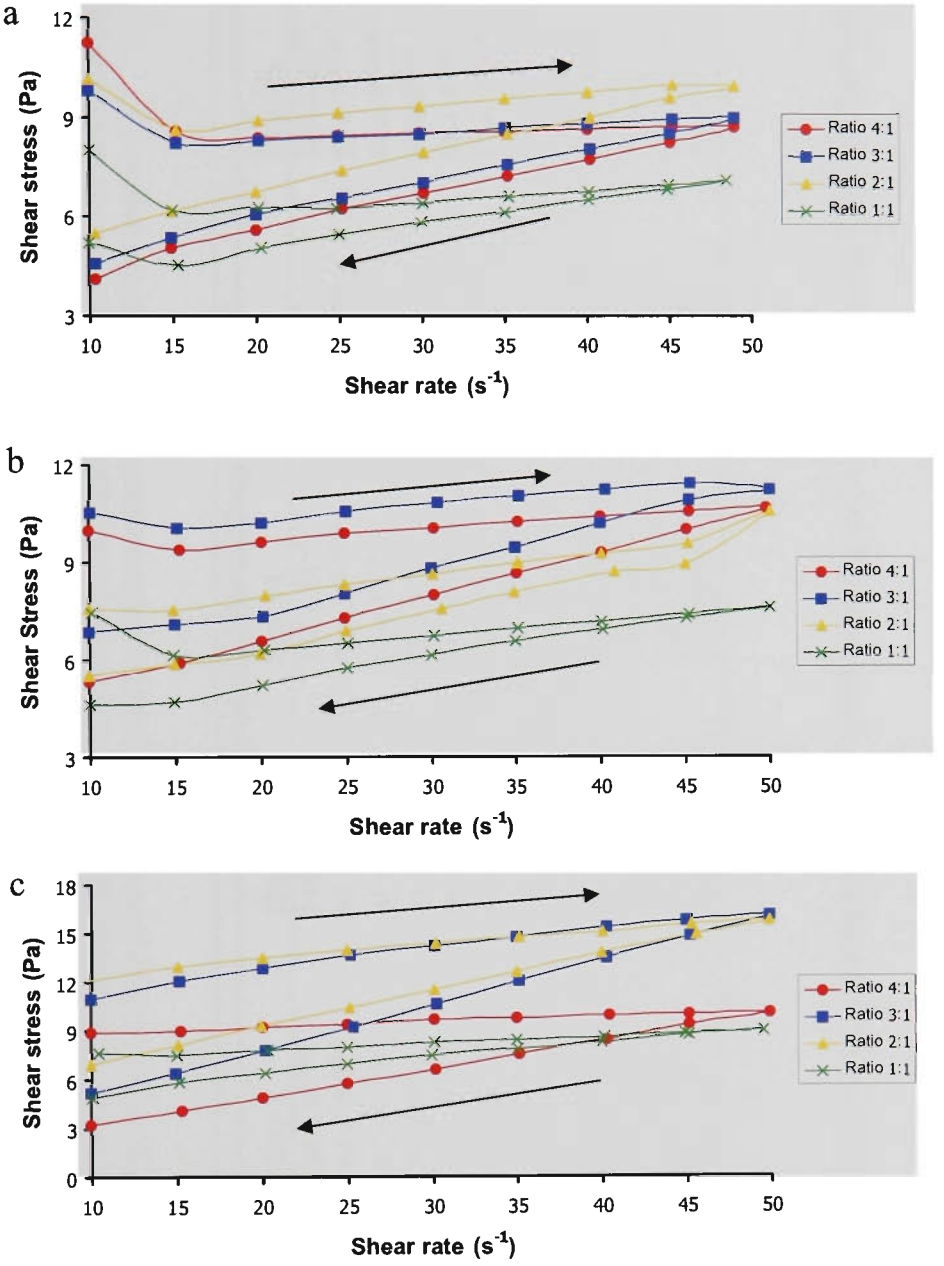

Figure 7.5: Flow curves of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c) - producing starter cultures after 1 day of storage at 4°C (n = 3).

Table 7.7: The area of hysteresis loop between upward and downward curves of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Area of hysteresis loop (Pa s^{-1})[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$81 \pm 8^{b,c,d,A}$	$110 \pm 9^{b,c,A}$	$112 \pm 14^{c,d,e,A}$	$95 \pm 5^{a,b,A}$	$105 \pm 10^{c,A}$
	3:1	$64 \pm 2^{a,b,c,A}$	$82 \pm 3^{a,b,c,A}$	$79 \pm 3^{a,b,c,d,A}$	$82 \pm 10^{a,b,A}$	$85 \pm 7^{a,b,c,A}$
	2:1	$57 \pm 3^{a,b,A}$	$61 \pm 5^{a,b,A}$	$62 \pm 3^{a,b,A}$	$68 \pm 4^{a,A}$	$64 \pm 1^{a,b,c,A}$
	1:1	$34 \pm 3^{a,A}$	$41 \pm 1^{a,A}$	$39 \pm 5^{a,A}$	$44 \pm 6^{a,A}$	$57 \pm 8^{a,b,A}$
Capsular EPS-producing starter cultures	4:1	$82 \pm 6^{b,c,d,A}$	$90 \pm 9^{a,b,c,A}$	$95 \pm 4^{b,c,d,e,A}$	$100 \pm 2^{a,b,c,A}$	$91 \pm 3^{b,c,A}$
	3:1	$76 \pm 9^{b,c,d,A}$	$85 \pm 7^{a,b,c,A}$	$100 \pm 3^{b,c,d,e,A}$	$92 \pm 8^{a,b,A}$	$87 \pm 5^{a,b,c,A}$
	2:1	$48 \pm 1^{a,b,A}$	$60 \pm 3^{a,b,A,B}$	$60 \pm 7^{a,b,A,B}$	$70 \pm 3^{a,B}$	$65 \pm 5^{a,b,c,A,B}$
	1:1	$30 \pm 7^{a,A}$	$40 \pm 8^{a,A}$	$42 \pm 6^{a,A}$	$40 \pm 8^{a,A}$	$44 \pm 4^{a,A}$
Ropy EPS-producing starter cultures	4:1	$106 \pm 8^{d,e,A}$	$128 \pm 34^{c,A}$	$121 \pm 21^{d,e,A}$	$165 \pm 25^{c,d,A}$	$108 \pm 9^{c,d,A}$
	3:1	$126 \pm 8^{e,A}$	$124 \pm 20^{c,A}$	$131 \pm 18^{e,A}$	$176 \pm 19^{d,B}$	$163 \pm 9^{e,B}$
	2:1	$96 \pm 16^{c,d,e,A}$	$117 \pm 4^{b,c,A,B}$	$128 \pm 7^{d,e,A,B}$	$146 \pm 28^{b,c,d,B}$	$154 \pm 20^{d,e,B}$
	1:1	$31 \pm 2^{a,A}$	$40 \pm 6^{a,A}$	$67 \pm 13^{a,b,c,B}$	$48 \pm 8^{a,A,B}$	$55 \pm 9^{a,b,A,B}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B,C}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

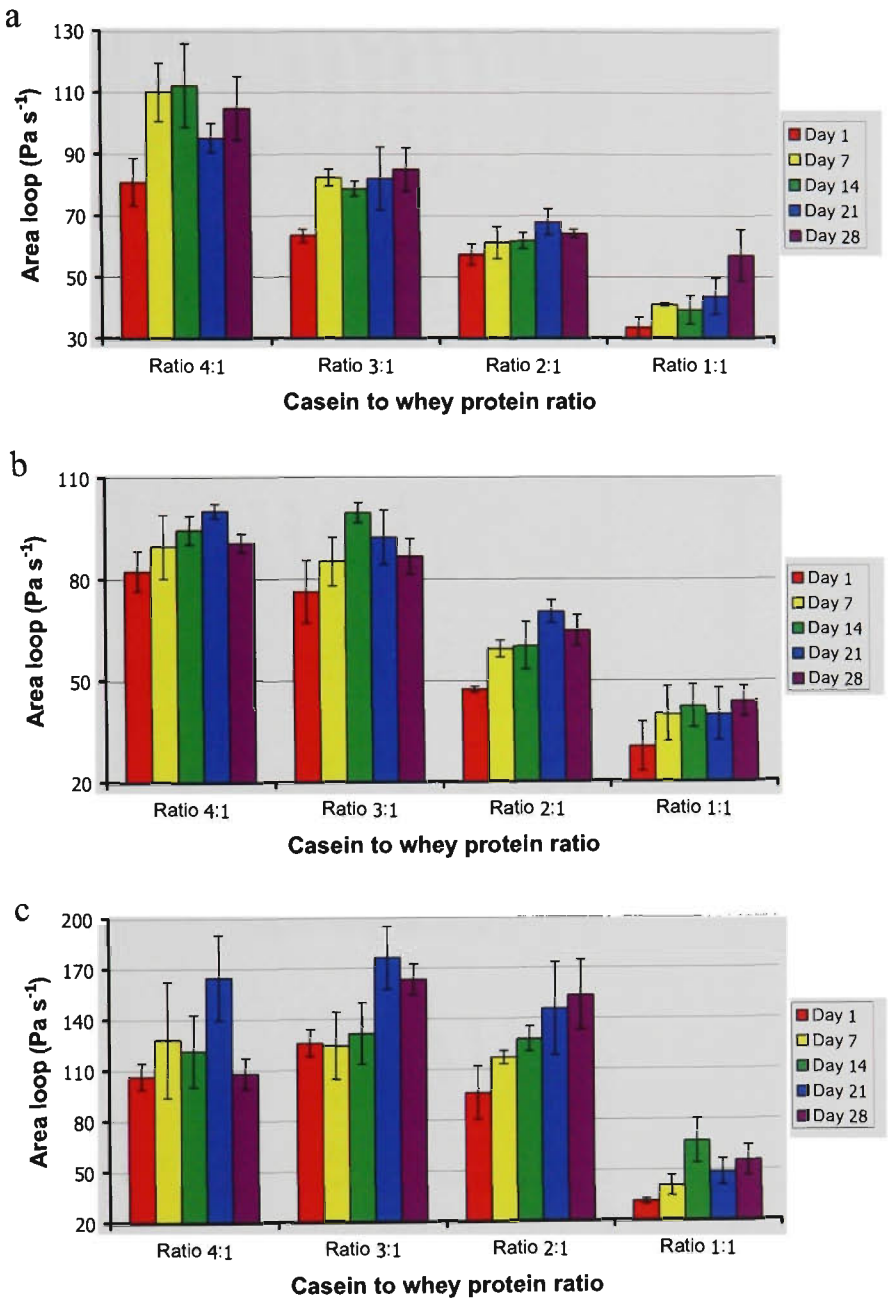

Figure 7.6: The area of hysteresis loop between upward and downward flow curves of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c) -producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 7.6). Error bars represent standard deviation (n = 3).

Table 7.8: Apparent viscosity of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Apparent viscosity (Pa s)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$0.89 \pm 0.02^{d,e,A}$	$0.90 \pm 0.06^{d,e,A}$	$0.81 \pm 0.09^{a,b,c,d,A}$	$0.84 \pm 0.05^{a,b,c,A}$	$0.90 \pm 0.03^{c,d,e,A}$
	3:1	$0.75 \pm 0.08^{b,c,d,A}$	$0.83 \pm 0.05^{b,c,d,e,A}$	$0.76 \pm 0.03^{a,b,c,A}$	$0.83 \pm 0.07^{a,b,c,A}$	$0.84 \pm 0.04^{b,c,d,e,A}$
	2:1	$0.66 \pm 0.01^{b,c,A}$	$0.72 \pm 0.04^{a,b,c,d,A}$	$0.70 \pm 0.10^{a,b,c,A}$	$0.81 \pm 0.12^{a,b,c,A}$	$0.80 \pm 0.13^{a,b,c,d,A}$
	1:1	$0.59 \pm 0.06^{a,b,A}$	$0.65 \pm 0.05^{a,b,A}$	$0.67 \pm 0.01^{a,b,c,A}$	$0.71 \pm 0.10^{a,b,A}$	$0.59 \pm 0.05^{a,A}$
Capsular EPS-producing starter cultures	4:1	$0.80 \pm 0.07^{c,d,e,A}$	$0.86 \pm 0.10^{c,d,e,A}$	$0.86 \pm 0.11^{c,d,A}$	$0.90 \pm 0.05^{a,b,c,A}$	$0.89 \pm 0.04^{b,c,d,e,A}$
	3:1	$0.81 \pm 0.04^{c,d,e,A}$	$0.86 \pm 0.04^{c,d,e,A}$	$0.85 \pm 0.07^{b,c,d,A}$	$0.90 \pm 0.06^{a,b,c,A}$	$0.85 \pm 0.08^{b,c,d,e,A}$
	2:1	$0.62 \pm 0.02^{a,b,A}$	$0.69 \pm 0.05^{a,b,c,A,B}$	$0.77 \pm 0.05^{a,b,c,B,C}$	$0.81 \pm 0.02^{a,b,c,C}$	$0.84 \pm 0.04^{b,c,d,e,C}$
	1:1	$0.46 \pm 0.07^{a,A}$	$0.59 \pm 0.03^{a,A}$	$0.58 \pm 0.08^{a,A}$	$0.64 \pm 0.11^{a,A}$	$0.65 \pm 0.11^{a,b,A}$
Ropy EPS-producing starter cultures	4:1	$0.87 \pm 0.05^{d,e,A}$	$0.75 \pm 0.08^{a,b,c,d,A}$	$0.83 \pm 0.06^{b,c,d,A}$	$0.95 \pm 0.20^{b,c,A}$	$0.75 \pm 0.06^{a,b,c,A}$
	3:1	$0.91 \pm 0.01^{d,e,A}$	$1.15 \pm 0.07^{f,B}$	$1.03 \pm 0.05^{d,e,A,B}$	$1.09 \pm 0.09^{c,A,B}$	$1.05 \pm 0.05^{e,B}$
	2:1	$0.93 \pm 0.04^{e,A}$	$1.00 \pm 0.08^{e,f,A,B}$	$1.12 \pm 0.03^{e,B}$	$1.00 \pm 0.04^{c,A,B}$	$0.99 \pm 0.07^{d,e,A,B}$
	1:1	$0.61 \pm 0.09^{a,b,A}$	$0.71 \pm 0.07^{a,b,c,d,A}$	$0.62 \pm 0.13^{a,b,A}$	$0.64 \pm 0.04^{a,A}$	$0.69 \pm 0.07^{a,b,A}$

[1]n= 3

[2]Mean values within the same column not sharing common superscript (^{a,b,c,d,e,f}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3]Mean values within the same row not sharing common superscript ($^{\wedge,B}$) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

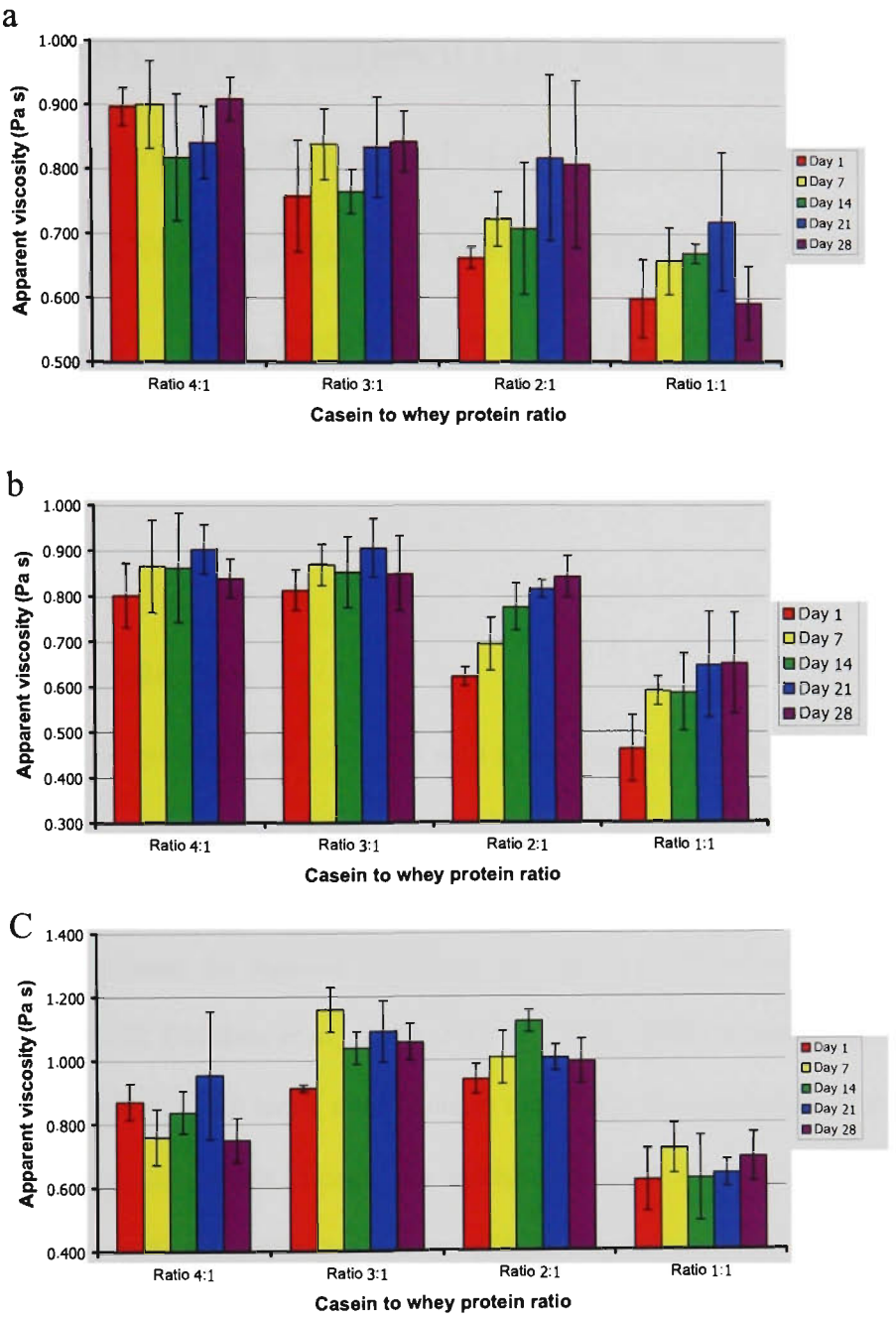

Figure 7.7: Apparent viscosity of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c) - producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 7.7). Error bars represent standard deviation (n = 3).

8.0 PHYSICAL PROPERTIES OF SET AND STIRRED YOGHURTS MADE AT 14% TOTAL SOLIDS WITH VARYING CASEIN TO WHEY PROTEIN RATIOS AND USING EPS-PRODUCING STARTER CULTURES DURING STORAGE

8.1 Introduction

In general, yoghurts are produced with a minimum solids level of 14 to 16% (w/w) (Tamime and Deeth, 1986; Shah, 2003). This improves physical properties (firmness, viscosity and syneresis) of yoghurts and also enhanced the growth of starter cultures. As reported by several researchers (Marshall *et al.*, 1995; De Vuyst *et al.*, 1998; Grobben *et al.*, 2000; Zisu and Shah, 2003), a number of factors affect EPS production by lactic acid bacteria including pH, incubation time, and carbon and nitrogen sources. Hence, an increase in total solids content, which would increase carbon and nitrogen content of milk blends, may promote the production of EPS by *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in yoghurt.

Chapter 3 discussed physical properties of set yoghurts made at 12% solids level. Chapter 7 focused on physical properties of yoghurts made at 9% (w/w) total solids content. This is the minimum solids level required by the Australian Food Standard

Code. The firmness and concentration of EPS in products made at 12% solids with RSM (the CN to WP ratio of 4:1) were higher than those made at 9%. The increase in total solids from 9 to 14% also decreased fermentation time. However, it increased the storage modulus at the end of fermentation (6 h) and firmness after overnight storage (4°C) of yoghurt gels made with non-EPS-producing starter cultures (Chapter 6). This may suggest the effect of increased solids content on these parameters. Due to the difference in experimental design, the comparison in physical properties of yoghurts supplemented with WPC (Chapter 4) and those made with varying the ratios of CN to WP (Chapter 7) was not possible.

In this chapter, physical properties of yoghurts were studied as carried out in Chapter 7, except that the yoghurts were made at 14% solids content. The yoghurts made using EPS-producing starter cultures with high level of solids content (14% or higher) may show different patterns of physical properties as compared to those with low solids (e.g. 9% solids content).

8.2 Aim

The objective of this chapter was to examine the physical properties of yoghurts made at 14% solids content as affected by the combined effects of various CN to WP ratios and EPS-producing starter cultures during the storage period of 28 days at 4°C.

8.3 Materials and Methods

8.3.1 Experimental design

The study was carried out as in Chapter 7, except the yoghurts were produced at higher solids content (14%).

8.3.2 Starter culture

Non-EPS-producing *S. thermophilus* ASCC 1342 and non-EPS-producing *L. delbrueckii* ssp. *bulgaricus* ASCC 1466, capsular EPS-producing *S. thermophilus* ASCC 285, and ropy EPS-producing *S. thermophilus* ASCC 1275 were used in this study. The maintenance and activation of these bacteria were carried out as described in Chapter 3, Section 3.1.

8.3.3 Enumeration of starter cultures

The viable counts of starter cultures in yoghurt during storage at day 1, 7, 14, 21 and 28 were determined according to the procedures described in Chapter 3, Section 3.2.

8.3.4 Yoghurts manufacture

Twelve batches each of set and stirred yoghurts made at 14% solids content with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 as in Chapter 6, Section 6.3.2 using non-

EPS-, capsular EPS- or ropy EPS-producing starter cultures according to the procedure of yoghurt manufacture as described in Chapter 3, Section 3.3.

8.3.5 Determination of lactic acid concentration of yoghurt

The concentration of lactic acid in yoghurt was determined according to the procedure described in Chapter 3, Section 3.6.

8.3.6 EPS isolation, purification and quantification in yoghurt

EPS in yoghurt was isolated, purified and quantified according to the procedure given in Chapter 3, Section 3.7.

8.3.7 Determination of spontaneous syneresis of undisturbed set yoghurt

The level of spontaneous syneresis of undisturbed set yoghurt was determined using the siphon method according to the procedure described in Chapter 3, Section 3.8.

8.3.8 Firmness of set yoghurt

The firmness of a set yoghurts was defined as the maximum force in a TPA curve determined by a penetration test as described in Chapter 3, Section 3.12 immediately after the sample was removed from the walk-in-cooler (4°C).

8.3.9 Flow curves and the area of hysteresis loop and apparent viscosity of stirred yoghurt

The flow curve and apparent viscosity of stirred yoghurt were constructed separately using a RS 50 RheoStress (Haake Rheometer, Karlsruhe, Germany) with a coaxial measuring cell, Z20 DIN sensor (gap = 0.85 mm). The measurements were carried out according to the procedure described in Chapter 3, Section 3.14 and 3.15.

8.4 Results and Discussion

8.4.1 Lactic acid concentration

The lactic acid concentration in yoghurts made with various CN to WP ratios and types of starter cultures during storage of 28 days is shown in Table 8.1. The data is also presented as three separate bar graphs. Figures 8.1a, 8.1b and 8.1c show lactic acid concentrations in yoghurts made using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures, respectively. The concentration of lactic acid decreased significantly ($P < 0.05$, Table 8.1) from ~1.3% in yoghurts made at the CN to WP ratio of 4:1 to ~0.8 % of those made at the ratio of 1:1. This pattern was previously

observed in products made at 9% solids as the lactic acid concentration decreased from ~1.0% in yoghurts made at the CN to WP ratio of 4:1 to 0.6% in those made at the CN to WP ratio of 1:1 (Section 7.4.1; Figure 7.1). A reduction in buffering system in yoghurts as a result of decreasing CN to WP ratios could be the explanation as already discussed in Section 7.4.1.

Increasing the total solids content of yoghurts from 9 to 14% elevated the lactic acid concentration. An increase in lactose content as a result of increasing total solids level of milk blends could promote the growth of bacteria and acid production. The level of lactic acid in products made using non-EPS- and ropy EPS-producing starter cultures was comparable. During storage, the level of lactic acid increased slightly, particularly in products made with capsular EPS-producing starter culture.

8.4.2 Viable counts

The viable counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in yoghurts are shown in Table 8.2. The decrease in the CN to WP ratio did not affect the growth of the microorganisms. The counts of non-EPS- and ropy EPS-producing *S. thermophilus* were higher than those of capsular EPS-producing strain (~ 9.10 vs. ~8.80 log (cfu g^{-1})). Yoghurts made using non-EPS- and capsular EPS-producing starter cultures had similar viable counts of *L. delbrueckii* ssp. *bulgaricus*. However, the counts were higher in products made using ropy EPS-producing starter cultures than those with non-EPS- and capsular EPS-producing starter cultures. The counts

of both *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* reduced gradually during storage. However, the total viable counts were still higher than 10^7 cfu g^{-1} after 28 days of storage.

8.4.3 EPS concentration

The concentration of EPS in yoghurts made at the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during storage for 28 days is shown in Table 8.3. The data is also presented in the form of bar graph. Figure 8.2 shows EPS concentration in yoghurts made using non-EPS (a)-, capsular EPS (a)- and ropy EPS (c)-producing starter cultures, respectively. The concentration of EPS in products made using non-EPS-producing starter cultures was ~ 20 mg L^{-1} (Table 8.3; Figure 8.2a). The concentration was higher than in the same products made at 9% solids, ~ 10 mg L^{-1} (Figure 7.2a). This may suggest that an increase in lactose content as well as other nitrogeneous nutrients as a result of increasing solids level promoted EPS production. The EPS concentration of yoghurts made using non-EPS-producing starter cultures was not affected by either the reduction in the CN to WP ratios or the storage period.

Decreasing the CN to WP ratios from 4:1 to 1:1 reduced the concentration of EPS significantly ($P < 0.05$) from ~ 120 to ~ 45 mg L^{-1} and from ~110 to ~80 mg L^{-1} in the yoghurts made using capsular EPS- and ropy EPS-producing starter cultures (Table 8.3), respectively. The concentration of both capsular and ropy EPS was

much higher in products made at 14% solids with the CN to WP ratios of 4:1, 3:1 and 2:1 as compared to those at 9% (Section 7.4.3; Figures 7.2b and 7.2c). This could be due to the increased availability of nutrient and decreased fermentation time. In 14% products, the level of nutrients in the form of peptides ad amino acids through additional milk solids was higher than that in 9% yoghurts. Increased nutrients could enhance acid production resulting in a reduction in fermentation time in products made at 14% as compared to those at 9% (Section 6.4.3, Figures 6.5 and 6.6). The decrease in fermentation time could also be a major limiting factor controlling the production of EPS in products made at 14%. Longer fermentation time in yoghurts made at 9% solids would allow time for EPS-producing *S. thermophilus* to produce similar level of EPS among products made with different CN to WP ratios.

Furthermore, the EPS concentration in yoghurts made using ropy EPS-producing starter cultures at the CN to WP ratios of 4:1 and 3:1 increased significantly ($P < 0.05$, Table 8.3) from ~120 (day 1) to ~160 (day 28) mg L^{-1} and from ~ 80 (day 1) to 140 (day 14) mg L^{-1}, respectively. An increase in EPS concentration during storage of yoghurt made at CN to WP ratio of 4:1 and 3:1 could be due to the ability of microbial enzyme on CN degradation. Similarly, an increase in EPS concentration was observed in yoghurts made at 9% solids with ropy EPS-producing starter cultures (Section 7.4.3, Figure 7.2c). This confirmed that the storage condition did not promote the degradation of EPS.

8.4.4 Syneresis in set yoghurts

The syneresis in set yoghurts during 28 days of storage is shown in Table 8.4. Figure 8.3 shows bar graphs which are reproduced from data in Table 8.4 of the syneresis in set yoghurts made using non-EPS (a)-, capsular EPS (b)- and ropy EPS (c)-producing starter cultures, respectively. The levels of syneresis reduced significantly as the CN to WP ratio was decreased and there was no syneresis detected in products made at the CN to WP ratio of 1:1 ($P < 0.05$; Table 8.4). This pattern was previously observed in the products made at 9% solids. However, the use of EPS-producing starter cultures (both capsular- and ropy EPS) did not further reduce the level of syneresis in the products made at 14% solids compared with those with non-EPS-producing starter cultures as observed in the 9% products (Section 7.4.4; Figures 7.3a to 7.3c). This may be due to the increase in density of protein matrix as the total solids content was increased that immobilized free water in the protein structure. A comparison of the microstructure of products made at 9% and 14% solids will be discussed in Chapter 9.

The level of syneresis decreased by around 2% in set yoghurt made at 14% using non-EPS-producing starter cultures at a CN to WP ratio of 4:1 compared to that made at 9% solids. This shows the effect of increased solids content on syneresis. This is in agreement with Harwalkar and Kalab (1986) and it has been already discussed in Section 7.4.4. During storage, the level of syneresis in all products was not significantly different. However, the level of syneresis in products made with

capsular and ropy EPS-producing starter cultures at 14% solids were comparable to that of products made at 9%. This showed that EPS effectively reduced syneresis in set yoghurts made at lower total solids more. Based on these findings, the total solids of set yoghurt could be reduced with the optimized use of EPS-producing starter cultures, while the syneresis of the product can be kept at an acceptable level.

8.4.5 Firmness of set yoghurts

The firmness of set yoghurts during 28 days of storage is shown in Table 8.5. The data is also presented as three separate bar graphs. Figure 8.4 shows the firmness of set yoghurts made using non-EPS (a)-, capsular EPS (b)- and ropy EPS (c)-producing starter cultures, respectively. The firmness decreased significantly ($P < 0.05$, Table 8.5) from ~1.40 to ~0.80 N as the CN to WP ratio was reduced from 4:1 to 1:1 regardless of the type of starter cultures (non-EPS, capsular EPS and ropy EPS products). This is due to the effect of heat treatment on casein-whey protein interaction that has already been discussed in Section 7.4.5. It is interesting to note that the firmness of all products made at 14% solids was much higher than those at 9%. However, the use of EPS-producing starter cultures (both capsular and ropy) did not reduce the firmness in products made at 14% as observed earlier in products made at 9% (Section 7.4.5; Figure 7.4) and 12% (Section 4.4.3, Figure 4.1). This is likely to be due to the effect of increased milk solids on the changes in microstructure of yoghurts. According to Hassan *et al.* (2003), their CLSM-micrographs of yoghurts showed separation of EPS (concentrating in voids space of protein network) from protein network. Protein network appeared to be denser in

yoghurt produced with EPS-producing starters than that of non-EPS producing starter cultures. They explained the phenomenon as incompatibility between protein and EPS. The changes in microstructure as observed in our study (Chapter 9) correlated with a reduction in incompatibility as the concentration increased from 9% to 14% total solids. There was less difference in microstructure between yoghurt made using EPS producing starter cultures and that of non-EPS producing starter cultures. This hypothesis was supported by the work of Harwalkar and Kalab (1986). They reported that the increase in total solids and protein contents affected gel structure and physical properties of products. The microstructure of products made at 9% and 14% solids will be discussed in Chapter 9. During storage, there was no change in firmness, except in batches made using ropy EPS-producing starter cultures at the CN to WP ratio of 4:1 and 3:1, where the firmness increased significantly ($P < 0.05$, Table 8.5). The increase in firmness may be due to the increase in EPS concentration (Figure 8.4c) as well as that of lactic acid (Figure 8.3).

8.4.6 Flow curves and hysteresis loop area of stirred yoghurts

The flow curves of stirred yoghurts made with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures after 1 day of storage at 4°C are shown in Figures 8.5a, 8.5b and 8.5c, respectively. The shear stress (up curve) of all products showed similar patterns of decreasing from the starting point (shear rate 10 s^{-1}) to a certain value and remained relatively constant until the end of the upward curve (shear rate 50 s^{-1}). However, the stirred yoghurts made with the CN to WP ratios of 4:1 and 3:1 using ropy EPS-producing

starter cultures (Figure 8.5c) showed the highest level of shear stress (~50 Pa) when compared in the steady region (i.e. between shear rate 25 to 50 s^{-1}), as compared to those made with non-EPS- (Figure 8.5a) and capsular EPS-producing starter cultures (Figure 8.5b). The latter samples had similar levels of shear stress (~35 Pa). The flow curve of the stirred yoghurt made with the CN to WP ratio of 1:1 showed the lowest shear stress regardless of the types of starter culture used. This was also observed in stirred products at 9% solids. The decrease in the CN to WP ratios between 4:1 to 2:1 did not affect shear stress of products made using capsular EPS- or non-EPS-producing starter cultures. This could be due to the condition used in constructing flow curves. Observation of flow curves between the shear rate of 10 and 50 s^{-1} may not cover all possible flow behaviour of stirred yoghurt (up to 10^3 s^{-1}). Nevertheless, according to Steffe (1992), foods should be tested between shear rate of 10^1 and 10^2 s^{-1}.

There was a reduction in the level of shear stress in samples made using ropy EPS-producing starter cultures. It appears that the level of shear stress in stirred yoghurts made with ropy EPS-producing starter cultures (Figure 8.5c) depended on the concentration of ropy EPS (Table 8.3; Figure 8.2c). The ability of ropy EPS on its entanglement formation would explain the result. Hassan *et al.* (1996b) observed similar behaviour. Other researchers observed a correlation between the concentration of EPS and the apparent viscosity of fermented milk (Cerning *et al.*, 1988; Cerning 1990; Marshall and Rawson, 1999).

The area of hysteresis loop between the up and down curves of stirred yoghurts show a decreasing pattern as the CN to WP ratio was decreased (Table 8.6). An increase in structural damage of products made at lower CN to WP ratios would be the explanation as this has already been discussed in Chapter 7, Section 7.4.6. The hysteresis loop areas of stirred yoghurts made using non-EPS-producing starter cultures were comparable to those with capsular EPS-producing starter cultures (Figures 8.6a and 8.6b) when compared at the same CN to WP ratio. During storage, there was no change in the area of hysteresis loop area in products made with non-EPS- or capsular EPS-producing starter cultures.

A substantial reduction in the value of hysteresis loop was observed only in products made using ropy EPS-producing starter cultures as the ratios of CN to WP were reduced from 4:1 to 1:1; the values reduced from ~990 Pa s^{-1} at the ratio of 4:1 to ~500 Pa s^{-1} at the ratio of 1:1. This pattern correlated with the concentration of ropy EPS (Figure 8.2). Similar results were observed in stirred product made at 9% solids with ropy EPS-producing starter cultures. Nonetheless, the value did not alter during storage whether the concentration of EPS increased (the CN to WP ratios of 4:1 and 3:1) or decreased (the CN to WP ratios of 2:1 and 1:1) as previously observed in products at 9% solids (Figure 7.6). These results suggest that the concentration of ropy EPS had a significant impact on the gel structure of stirred yoghurts. It also suggested that any changes during storage in physical properties of

stirred yoghurts due to the alteration of EPS concentration may be suppressed in products made at high solids content.

8.4.7 Apparent viscosity of stirred yoghurts

The apparent viscosity of stirred yoghurts during 28 days storage is shown in Table 8.7. Figure 8.7 represents bar graphs of the apparent viscosity of stirred yoghurts made using non-EPS (a)-, capsular EPS (b)- or ropy EPS (c)-producing starter cultures, respectively, which are reproduced from data in Table 8.7. The apparent viscosity in products made with ropy EPS-producing starter cultures (ranging from 4.5 to 2.5 Pa s) was in general higher than those made using non-EPS- and capsular EPS-producing starter cultures (ranging from 2.8 to 1.5 Pa s^{-1}). The apparent viscosity of the products made with non-EPS- and capsular EPS-producing starter cultures was comparable (Table 8.7). This pattern was previously observed in products made at 9% solids (Section 7.4.6; Figures 7.7a and 7.7b) and could be explained by the influence of entanglement of ropy EPS in the structure of stirred yoghurt. Some products showed an increase in the apparent viscosity during storage. A decrease in the apparent viscosity as the ratio of CN to WP was reduced was only observed in products made using ropy EPS-producing starter cultures.

There appears to have a correlation between EPS concentration and apparent viscosity of stirred products made using ropy EPS-producing starter cultures. Importantly, although the product made at 9% solids using ropy EPS-producing

starter cultures at the CN to WP ratio of 3:1 showed a substantial increase in apparent viscosity (Figure 7.7c) compared to those with non-EPS-producing starter cultures, the values were much lower than those in stirred yoghurt made at 14% using the non-EPS-producing starter cultures (~ 1.1 Pa s vs. ~3.0 Pa s).

8.5 Conclusion

The increase in solids level to 14% increased the growth of starter cultures, EPS and lactic acid concentration, firmness of set yoghurts, shear stress, apparent viscosity and the area of hysteresis loop of stirred yoghurts, but decreased the level of syneresis in set yoghurts. EPS did not have any effects on the reduction in firmness as observed in set yoghurts made at 9% solids. The use of ropy EPS-producing starter cultures significantly increased the value of shear stress, the area of hysteresis loop and apparent viscosity of stirred yoghurts. Based on these results set yoghurts should be made at 14% solids with or without the use of EPS-producing starter cultures. Stirred yoghurt can be produced at lower solids content than 14% by using EPS-producing starter cultures with RSM.

Table 8.1: Lactic acid concentration in yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Lactic acid concentration (%, w/w)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$1.25 \pm 0.06^{f,g,A}$	$1.29 \pm 0.02^{b,A}$	$1.38 \pm 0.04^{d,A}$	$1.38 \pm 0.11^{d,e,A}$	$1.38 \pm 0.07^{d,e,f,A}$
	3:1	$1.15 \pm 0.02^{e,f,A}$	$1.22 \pm 0.06^{b,A}$	$1.23 \pm 0.05^{c,d,A}$	$1.26 \pm 0.07^{b,c,d,A}$	$1.24 \pm 0.03^{c,d,e,A}$
	2:1	$1.00 \pm 0.02^{b,c,d,A}$	$1.09 \pm 0.03^{a,b,B}$	$1.19 \pm 0.08^{c,d,C}$	$1.16 \pm 0.07^{c,d,e,C}$	$1.22 \pm 0.03^{b,c,d,C}$
	1:1	$0.85 \pm 0.08^{a,A}$	$0.95 \pm 0.04^{a,A}$	$0.98 \pm 0.05^{a,A}$	$0.93 \pm 0.04^{a,A}$	$0.98 \pm 0.04^{a,A}$
Capsular EPS-producing starter cultures	4:1	$1.20 \pm 0.07^{f,g,A}$	$1.31 \pm 0.04^{b,A}$	$1.34 \pm 0.06^{d,A}$	$1.42 \pm 0.05^{e,A}$	$1.43 \pm 0.01^{f,A}$
	3:1	$1.02 \pm 0.03^{c,d,A}$	$1.17 \pm 0.03^{a,b,A,B}$	$1.19 \pm 0.07^{b,c,A,B}$	$1.31 \pm 0.02^{d,e,B,C}$	$1.38 \pm 0.12^{e,f,C}$
	2:1	$0.87 \pm 0.07^{a,b,A}$	$1.13 \pm 0.10^{a,b,B}$	$1.22 \pm 0.02^{b,c,B,C}$	$1.29 \pm 0.01^{c,d,e,B,C}$	$1.33 \pm 0.02^{c,d,e,f,C}$
	1:1	$0.86 \pm 0.04^{a,A}$	$0.99 \pm 0.09^{a,b,A}$	$0.95 \pm 0.04^{a,A}$	$1.05 \pm 0.07^{a,b,c,A}$	$1.06 \pm 0.08^{a,b,A}$
Ropy EPS-producing starter cultures	4:1	$1.32 \pm 0.06^{g,A}$	$1.31 \pm 0.04^{b,A}$	$1.34 \pm 0.07^{d,A}$	$1.33 \pm 0.09^{d,e,A}$	$1.35 \pm 0.05^{d,e,f,A}$
	3:1	$1.15 \pm 0.10^{d,e,A}$	$1.26 \pm 0.10^{a,b,B}$	$1.24 \pm 0.12^{b,c,A,B}$	$1.37 \pm 0.03^{d,e,C}$	$1.33 \pm 0.13^{c,d,B}$
	2:1	$1.09 \pm 0.01^{d,e,A}$	$1.11 \pm 0.10^{a,b,A}$	$1.12 \pm 0.05^{b,A}$	$1.16 \pm 0.02^{a,b,c,d,A}$	$1.16 \pm 0.01^{b,c,A}$
	1:1	$0.94 \pm 0.06^{a,b,c,A}$	$1.05 \pm 0.11^{a,b,B}$	$0.97 \pm 0.01^{a,A,B}$	$0.99 \pm 0.01^{a,b,A,B}$	$0.99 \pm 0.01^{a,A,B}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e,f,g}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B,C}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

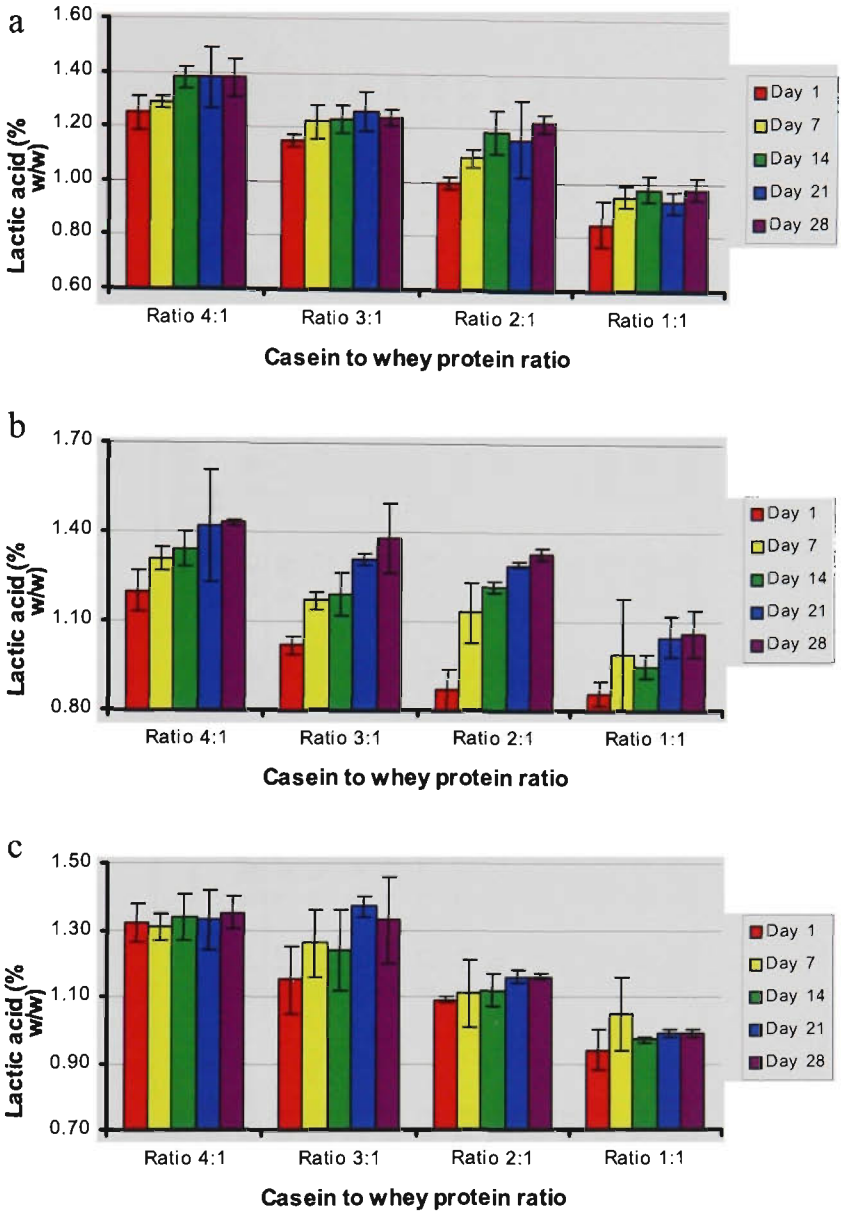

Figure 8.1: Lactic acid concentration in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 8.1). Error bars represent standard deviation (n = 3).

Table 8.2: Viable counts[1] (log 10 (cfu g^{-1})) of *S. thermophilus* (ST) and *L. delbrueckii* ssp. *bulgaricus* (LB) in yoghurts prepared with various CN to WP ratios using non-EPS-, capsular EPS- and ropy EPS-producing starter cultures during storage (4°C)

Starter culture	Casein to whey protein ratios	Storage period									
		Day 1		Day 7		Day 14		Day 21		Day 28	
		ST[a]	LB[b]	ST	LB	ST	LB	ST	LB	ST	LB
Non-EPS-producing starter cultures	4:1	9.10	7.65	9.23	7.95	9.08	7.67	8.98	7.74	8.96	7.26
	3:1	9.11	7.83	9.12	7.83	9.22	7.69	9.12	7.89	9.00	7.26
	2:1	9.19	7.69	9.18	7.68	9.02	7.81	9.15	7.88	8.94	7.20
	1:1	9.32	7.86	9.02	7.39	8.98	7.65	8.92	7.37	8.70	7.36
Capsular EPS-producing starter cultures	4:1	8.73	7.77	8.85	7.86	8.18	7.52	7.60	7.66	7.70	7.20
	3:1	8.84	7.93	8.23	8.12	8.06	7.86	7.62	7.72	7.47	7.45
	2:1	8.80	7.84	8.48	8.09	8.23	7.88	8.01	7.70	7.95	7.64
	1:1	8.70	8.01	9.15	8.04	8.89	7.73	8.87	7.93	8.89	7.44
Ropy EPS-producing starter cultures	4:1	9.27	8.13	9.21	7.98	9.20	7.82	8.96	7.64	8.68	7.39
	3:1	9.17	8.20	9.14	8.19	9.22	8.04	8.91	7.82	8.75	7.77
	2:1	9.10	8.16	8.91	8.03	8.61	7.86	8.51	8.23	8.87	7.65
	1:1	9.09	8.13	8.92	8.16	8.55	8.03	8.70	8.11	8.85	7.93

[1] n = 3

Table 8.3: EPS concentration in yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	EPS concentration (mg L^{-1})[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$20.78 \pm 3.05^{a,A}$	$19.32 \pm 4.09^{a,A}$	$23.53 \pm 3.83^{a,A}$	$25.38 \pm 2.79^{a,A}$	$22.99 \pm 7.52^{a,b,A}$
	3:1	$20.68 \pm 1.45^{a,A}$	$20.71 \pm 3.93^{a,A}$	$23.53 \pm 1.13^{a,A}$	$21.27 \pm 2.97^{a,A}$	$24.06 \pm 1.95^{a,b,A}$
	2:1	$20.40 \pm 1.46^{a,A}$	$19.42 \pm 2.08^{a,A}$	$24.19 \pm 3.12^{a,A}$	$23.55 \pm 3.60^{a,A}$	$23.55 \pm 1.66^{a,b,A}$
	1:1	$19.56 \pm 2.11^{a,A,B}$	$21.63 \pm 3.17^{a,A,B}$	$22.42 \pm 3.61^{a,B}$	$19.02 \pm 2.79^{a,A,B}$	$19.84 \pm 4.76^{a,A}$
Capsular EPS-producing starter cultures	4:1	$114.63 \pm 10.34^{d,A}$	$110.31 \pm 7.66^{d,A}$	$107.95 \pm 7.64^{d,e,A}$	$103.63 \pm 2.11^{e,A}$	$111.27 \pm 9.73^{e,f,A}$
	3:1	$85.53 \pm 5.08^{c,A}$	$78.74 \pm 9.23^{c,A}$	$83.87 \pm 7.97^{c,d,A}$	$89.70 \pm 12.43^{d,e,A}$	$91.08 \pm 4.58^{d,e,A}$
	2:1	$57.87 \pm 9.60^{b,A}$	$58.16 \pm 8.88^{b,c,A,B}$	$80.59 \pm 9.46^{c,C}$	$76.20 \pm 11.61^{c,d,B,C}$	$80.82 \pm 8.12^{c,d,B,C}$
	1:1	$47.56 \pm 4.51^{b,A}$	$45.59 \pm 6.73^{b,A}$	$54.59 \pm 7.82^{b,A}$	$45.90 \pm 2.60^{b,A}$	$47.28 \pm 5.99^{b,c,A}$
Ropy EPS-producing starter cultures	4:1	$109.80 \pm 9.25^{d,A}$	$123.42 \pm 6.06^{d,A,B}$	$142.27 \pm 14.09^{f,A,B}$	$148.34 \pm 11.01^{f,B}$	$147.42 \pm 12.65^{g,B}$
	3:1	$82.17 \pm 2.77^{c,A}$	$116.68 \pm 6.39^{d,B}$	$137.46 \pm 13.25^{e,f,B}$	$106.35 \pm 9.51^{e,A,B}$	$121.33 \pm 17.77^{f,g,B}$
	2:1	$92.01 \pm 5.37^{c,B}$	$80.84 \pm 10.58^{c,A}$	$72.71 \pm 8.11^{b,c,A}$	$73.29 \pm 2.26^{c,A}$	$70.37 \pm 8.28^{c,d,A}$
	1:1	$83.09 \pm 9.48^{c,A}$	$82.52 \pm 8.49^{c,A}$	$72.51 \pm 8.97^{b,c,A}$	$57.79 \pm 8.32^{c,A}$	$66.74 \pm 11.58^{c,d,A}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e,f}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B,C}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

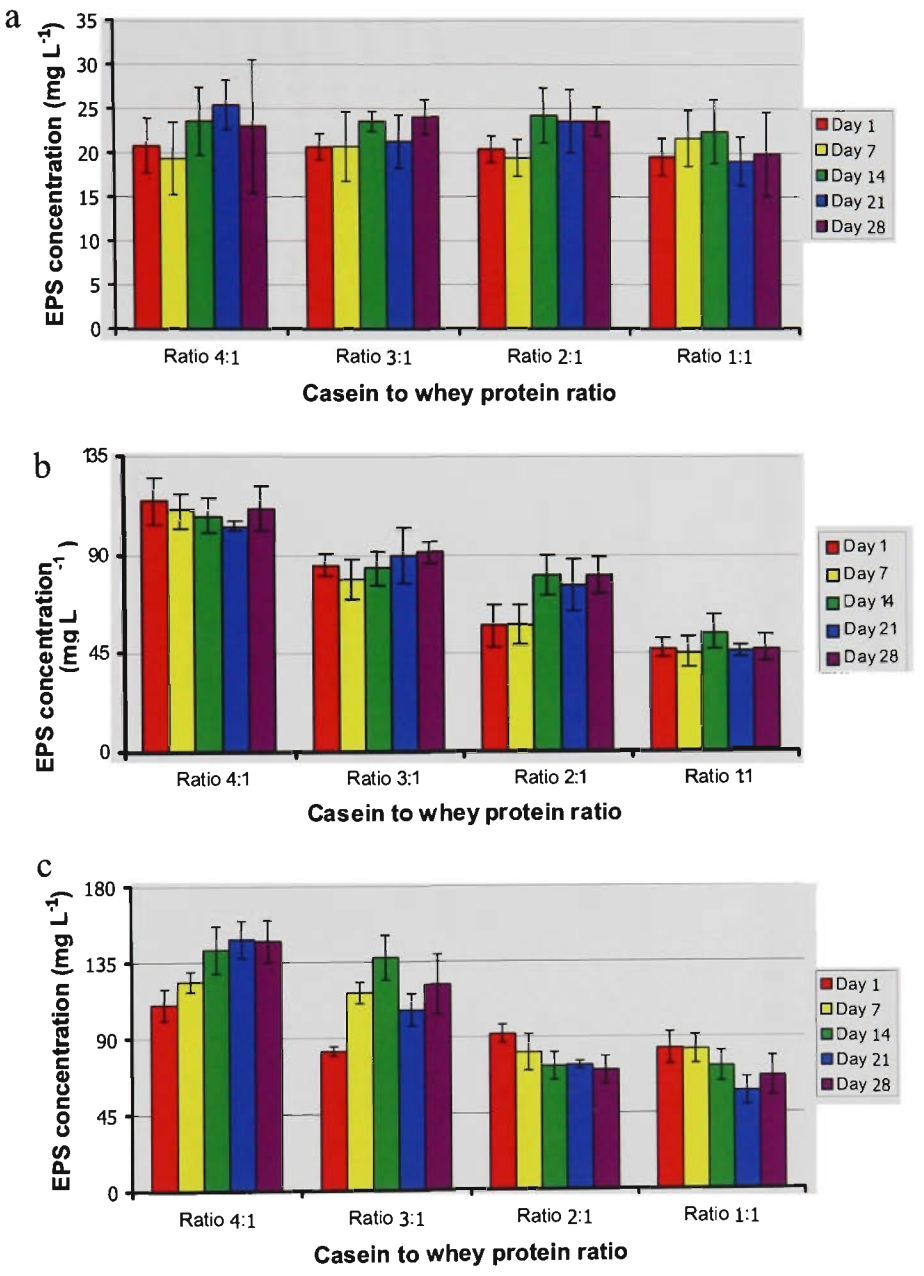

Figure 8.2: EPS concentration in yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c) - producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 8.3). Error bars represent standard deviation (n = 3).

Table 8.4: Syneresis in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Syneresis (% w/w)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$5.11 \pm 1.37^{g,A}$	$5.01 \pm 1.45^{c,d,A}$	$4.40 \pm 0.90^{d,e,A}$	$4.19 \pm 0.84^{e,A}$	$4.24 \pm 1.62^{d,A}$
	3:1	$2.95 \pm 0.63^{e,f,A}$	$3.13 \pm 0.35^{b,c,d,A}$	$2.95 \pm 0.59^{b,c,d,A}$	$3.08 \pm 0.68^{c,d,e,A}$	$3.17 \pm 0.62^{c,A}$
	2:1	$1.35 \pm 0.31^{b,c,A}$	$1.31 \pm 0.56^{a,b,A}$	$1.47 \pm 0.54^{a,b,A}$	$1.46 \pm 0.47^{a,b,c,A}$	$1.73 \pm 0.82^{a,b,c,A}$
	1:1	$0.13 \pm 0.14^{a,A}$	$0.09 \pm 0.14^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.27 \pm 0.13^{a,A}$
Capsular EPS-producing starter cultures	4:1	$3.11 \pm 0.84^{c,d,A}$	$3.72 \pm 0.81^{b,c,d,A}$	$3.75 \pm 0.88^{d,A}$	$3.60 \pm 0.94^{e,A}$	$2.92 \pm 0.79^{c,A}$
	3:1	$2.94 \pm 0.71^{b,c,d,A}$	$2.78 \pm 0.40^{b,c,A}$	$2.54 \pm 0.63^{b,c,d,A}$	$1.85 \pm 0.63^{b,c,d,A}$	$2.02 \pm 0.51^{a,b,c,A}$
	2:1	$0.47 \pm 0.16^{a,b,A}$	$0.49 \pm 0.10^{a,A}$	$0.54 \pm 0.23^{a,A}$	$0.44 \pm 0.10^{a,b,A}$	$0.40 \pm 0.09^{a,b,A}$
	1:1	$0.00 \pm 0.00^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.18 \pm 0.31^{a,A}$	$0.00 \pm 0.00^{a,A}$	$0.06 \pm 0.12^{a,A}$
Ropy EPS-producing starter cultures	4:1	$4.20 \pm 0.72^{f,A}$	$4.81 \pm 1.34^{d,A}$	$5.83 \pm 0.59^{e,A}$	$4.49 \pm 0.56^{e,A}$	$5.59 \pm 1.03^{d,A}$
	3:1	$1.88 \pm 0.87^{c,d,A}$	$2.67 \pm 0.88^{b,c,d,A}$	$3.25 \pm 0.67^{c,d,A,B}$	$3.07 \pm 0.54^{d,e,B}$	$3.02 \pm 0.96^{b,c,A,B}$
	2:1	$0.64 \pm 0.57^{a,b,A}$	$1.34 \pm 0.53^{a,b,A}$	$1.65 \pm 0.69^{a,b,c,A}$	$1.74 \pm 0.57^{b,c,d,A}$	$1.57 \pm 0.49^{a,b,c,A}$
	1:1	$0.00 \pm 0.00^{a,A}$	$0.03 \pm 0.07^{a,A}$	$0.07 \pm 0.17^{a,A}$	$0.03 \pm 0.07^{a,A}$	$0.00 \pm 0.00^{a,A}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e,f,g}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B,C}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

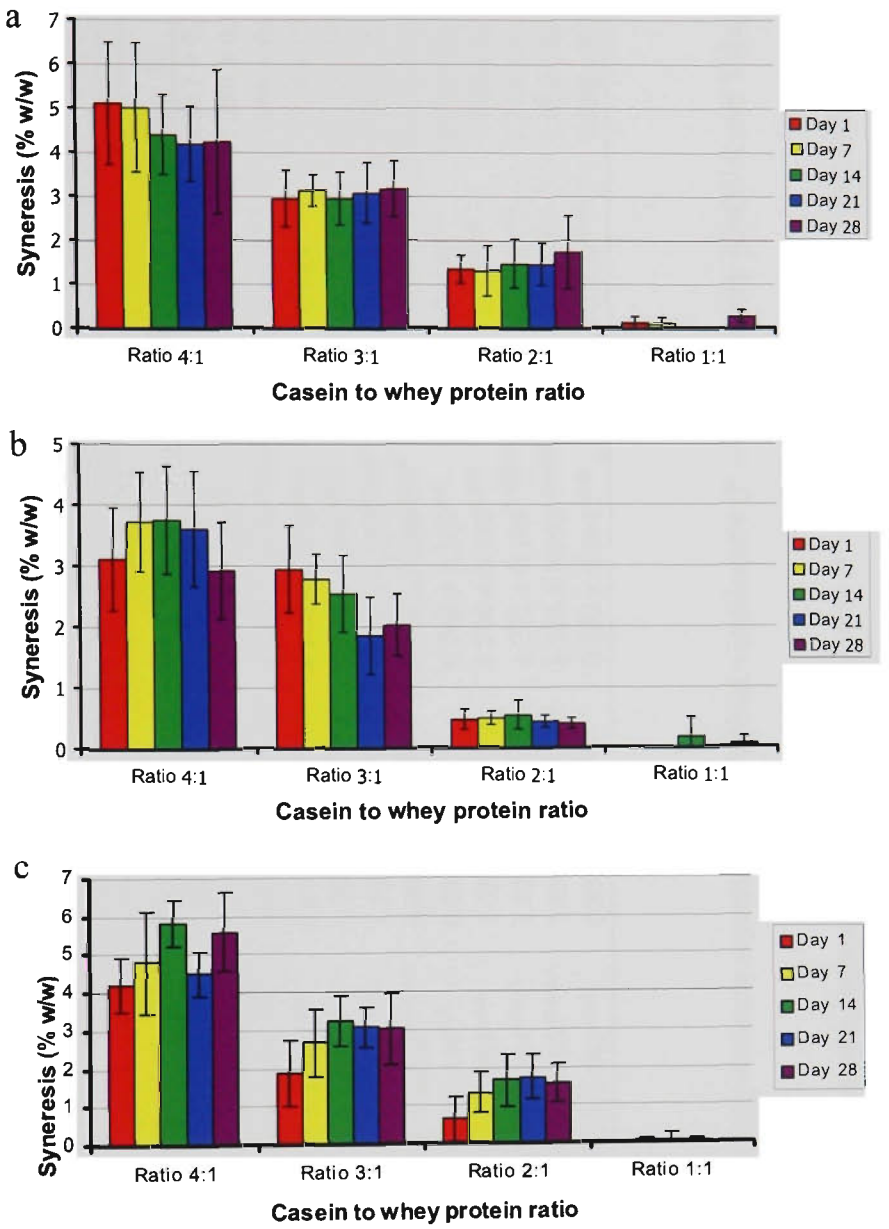

Figure 8.3: Syneresis in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 8.4). Error bars represent standard deviation (n = 3).

Table 8.5: Firmness in set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Firmness (N)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$1.346 \pm 0.173^{d,A}$	$1.450 \pm 0.356^{b,c,d,A}$	$1.396 \pm 0.298^{c,d,e,A}$	$1.494 \pm 0.155^{d,e,A}$	$1.517 \pm 0.238^{d,e,A}$
	3:1	$1.073 \pm 0.238^{b,c,d,A}$	$1.072 \pm 0.345^{b,c,d,A}$	$1.090 \pm 0.150^{a,b,c,d,A}$	$1.226 \pm 0.275^{b,c,d,A}$	$1.280 \pm 0.310^{c,d,e,A}$
	2:1	$0.855 \pm 0.083^{a,b,A}$	$0.994 \pm 0.159^{a,b,c,A,B}$	$1.033 \pm 0.118^{a,b,c,A,B}$	$1.053 \pm 0.086^{b,c,B}$	$1.068 \pm 0.044^{a,b,c,d,B}$
	1:1	$0.782 \pm 0.084^{a,b,A}$	$0.874 \pm 0.100^{a,b,A}$	$0.881 \pm 0.072^{a,b,A}$	$0.846 \pm 0.064^{a,b,A}$	$0.859 \pm 0.084^{a,b,A}$
Capsular EPS-producing starter cultures	4:1	$1.165 \pm 0.091^{c,d,A,B}$	$1.188 \pm 0.176^{a,b,c,d,A}$	$1.257 \pm 0.163^{d,e,B}$	$1.274 \pm 0.120^{c,d,A,B}$	$1.185 \pm 0.156^{b,c,d,e,A}$
	3:1	$1.174 \pm 0.106^{b,c,d,A}$	$1.299 \pm 0.047^{c,d,A}$	$1.228 \pm 0.117^{b,c,d,A}$	$1.201 \pm 0.078^{b,c,d,A}$	$1.309 \pm 0.126^{b,c,d,e,A}$
	2:1	$0.910 \pm 0.083^{a,b,c,d,A}$	$0.980 \pm 0.024^{a,b,c,A}$	$0.961 \pm 0.069^{a,b,c,A}$	$0.988 \pm 0.147^{a,b,c,A}$	$0.993 \pm 0.115^{a,b,c,d,A}$
	1:1	$0.691 \pm 0.160^{a,A}$	$0.695 \pm 0.056^{a,A}$	$0.788 \pm 0.107^{a,A}$	$0.730 \pm 0.070^{a,A}$	$0.763 \pm 0.134^{a,A}$
Ropy EPS-producing starter cultures	4:1	$1.236 \pm 0.089^{c,d,A}$	$1.555 \pm 0.159^{d,A,B}$	$1.746 \pm 0.176^{e,A,B}$	$1.779 \pm 0.159^{e,B}$	$1.739 \pm 0.243^{e,A,B}$
	3:1	$1.059 \pm 0.234^{a,b,c,A}$	$1.125 \pm 0.297^{a,b,c,A}$	$1.272 \pm 0.199^{b,c,d,B}$	$1.299 \pm 0.163^{c,d,B}$	$1.189 \pm 0.103^{a,b,c,d,e,}$
	2:1	$0.927 \pm 0.065^{a,b,c,d,A}$	$1.037 \pm 0.077^{a,b,c,d,A}$	$1.104 \pm 0.093^{a,b,c,d,A}$	$1.054 \pm 0.090^{b,c,A}$	$1.054 \pm 0.083^{a,b,c,d,A}$
	1:1	$0.813 \pm 0.065^{a,b,A}$	$0.875 \pm 0.067^{a,b,A}$	$0.932 \pm 0.061^{a,b,A}$	$0.900 \pm 0.038^{a,b,A}$	$0.888 \pm 0.033^{a,b,c,A}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

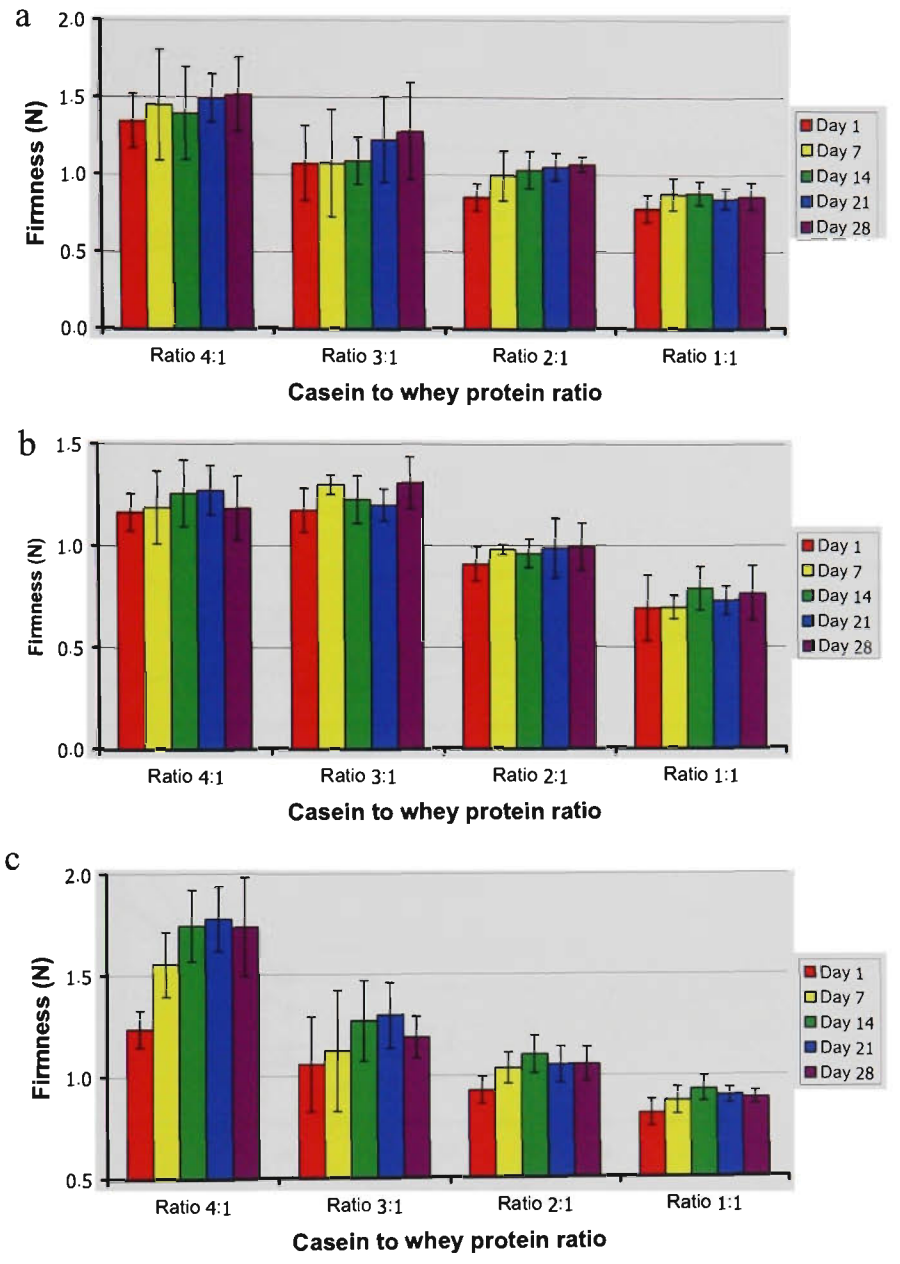

Figure 8.4: Firmness of set yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 8.5). Error bars represent standard deviation (n = 3).

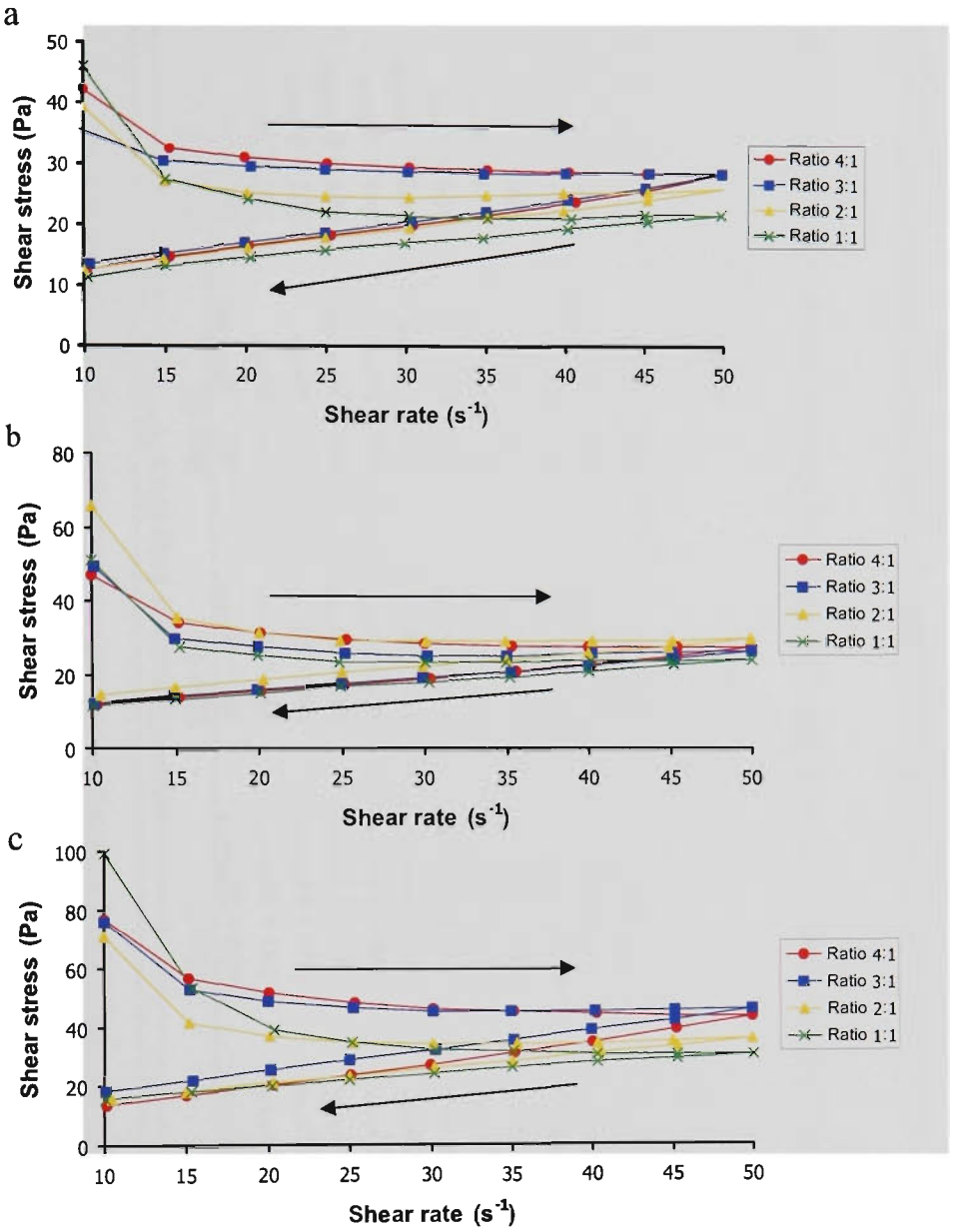

Figure 8.5: Flow curves of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures after 1 day of storage at 4°C (n = 3).

Table 8.6: The area of hysteresis loop between upward and downward curves of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Area of hysteresis loop (Pa s^{-1})[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$418 \pm 13^{b,c,A}$	$430 \pm 20^{a,b,c,d,A}$	$534 \pm 66^{c,d,B}$	$480 \pm 26^{b,c,d,A,B}$	$440 \pm 7^{b,A}$
	3:1	$371 \pm 61^{a,b,A}$	$393 \pm 28^{a,b,c,d,A}$	$395 \pm 46^{b,c,A}$	$379 \pm 62^{a,b,A}$	$367 \pm 55^{a,b,A}$
	2:1	$294 \pm 83^{a,b,A}$	$343 \pm 61^{a,b,c,A}$	$354 \pm 50^{b,A}$	$351 \pm 46^{a,b,A}$	$391 \pm 49^{a,b,A}$
	1:1	$286 \pm 41^{a,b,A}$	$362 \pm 113^{a,b,A}$	$383 \pm 50^{b,A,B}$	$406 \pm 78^{a,b,c,A,B}$	$469 \pm 83^{b,c,d,B}$
Capsular EPS-producing starter cultures	4:1	$433 \pm 22^{b,c,A}$	$460 \pm 53^{b,c,d,A}$	$445 \pm 44^{b,c,A}$	$400 \pm 58^{a,b,A}$	$445 \pm 39^{b,c,A}$
	3:1	$341 \pm 20^{a,b,A}$	$405 \pm 24^{a,b,c,d,A,B}$	$412 \pm 34^{b,c,B}$	$387 \pm 28^{a,b,A,B}$	$392 \pm 11^{a,b,A,B}$
	2:1	$311 \pm 67^{a,b,A}$	$373 \pm 55^{a,b,c,A,B}$	$378 \pm 78^{b,B,C}$	$394 \pm 85^{a,b,C}$	$409 \pm 72^{a,b,C}$
	1:1	$157 \pm 30^{a,A}$	$207 \pm 19^{a,A,B}$	$209 \pm 43^{a,A,B}$	$237 \pm 3^{a,B}$	$259 \pm 20^{a,B}$
Ropy EPS-producing starter cultures	4:1	$985 \pm 70^{d,A}$	$985 \pm 55^{f,A}$	$932 \pm 28^{f,A}$	$909 \pm 66^{f,A}$	$908 \pm 34^{f,A}$
	3:1	$684 \pm 98^{c,A}$	$684 \pm 43^{e,A}$	$693 \pm 22^{e,A}$	$683 \pm 35^{e,f,A}$	$734 \pm 60^{e,A}$
	2:1	$487 \pm 80^{b,c,A}$	$537 \pm 60^{c,d,e,A}$	$645 \pm 20^{d,e,A}$	$573 \pm 30^{c,d,e,A}$	$605 \pm 45^{c,d,e,A}$
	1:1	$484 \pm 77^{b,c,A}$	$511 \pm 36^{d,e,A}$	$588 \pm 45^{d,e,A}$	$549 \pm 53^{d,e,A}$	$559 \pm 67^{d,e,A}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B,C}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

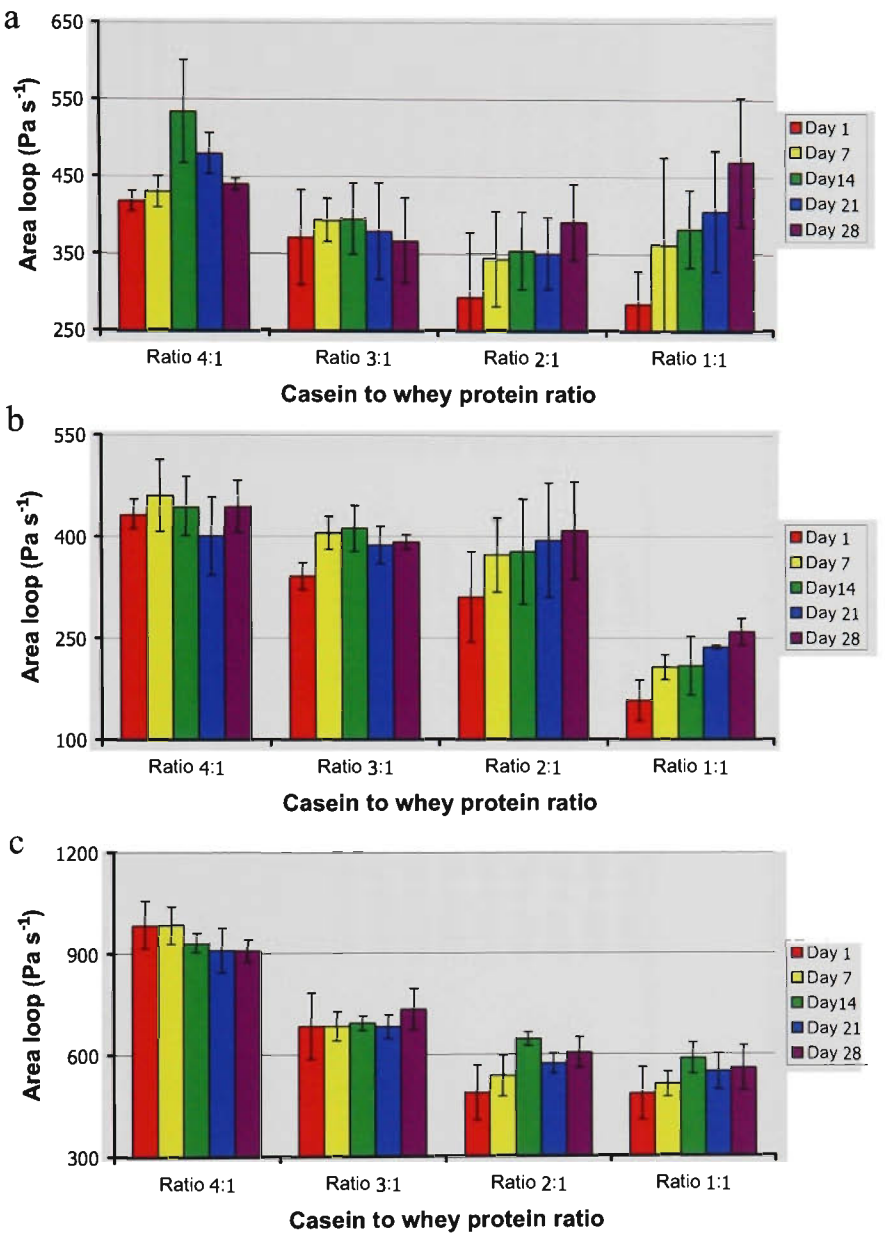

Figure 8.6: The area of hysteresis loop between upward and downward flow curves of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 8.6). Error bars represent standard deviation (n = 3).

Table 8.7: Apparent viscosity of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS-, or ropy EPS-producing starter cultures during 28 days of storage at 4°C

Starter cultures	Casein to whey protein ratio	Apparent viscosity (Pa s)[1, 2, 3]				
		Day 1	Day 7	Day 14	Day 21	Day 28
Non-EPS-producing starter cultures	4:1	$2.13 \pm 0.18^{a,b,A}$	$2.63 \pm 0.29^{b,c,d,A,B}$	$2.74 \pm 0.11^{b,c,A,B}$	$2.85 \pm 0.01^{a,b,c,d,B}$	$2.59 \pm 0.31^{b,c,A,B}$
	3:1	$2.05 \pm 0.29^{a,b,A}$	$2.40 \pm 0.37^{b,c,d,A}$	$2.50 \pm 0.16^{a,b,A}$	$2.44 \pm 0.01^{a,b,c,A}$	$2.42 \pm 0.26^{a,b,A}$
	2:1	$1.83 \pm 0.44^{a,A}$	$2.16 \pm 0.45^{a,b,A}$	$2.30 \pm 0.38^{a,b,A}$	$2.32 \pm 0.41^{a,b,A}$	$2.25 \pm 0.24^{a,b,A}$
	1:1	$1.85 \pm 0.12^{a,b,A}$	$2.30 \pm 0.24^{b,c,A,B}$	$2.49 \pm 0.11^{a,b,A,B}$	$2.53 \pm 0.24^{a,b,c,A,B}$	$2.64 \pm 0.15^{b,c,B}$
Capsular EPS-producing starter cultures	4:1	$2.27 \pm 0.14^{a,b,A}$	$2.64 \pm 0.14^{b,c,d,A}$	$2.60 \pm 0.09^{a,b,A}$	$2.50 \pm 0.56^{a,b,c,A}$	$2.22 \pm 0.16^{a,b,A}$
	3:1	$2.24 \pm 0.09^{a,b,A}$	$2.56 \pm 0.19^{b,c,A,B}$	$2.57 \pm 0.23^{a,b,A,B}$	$2.77 \pm 0.10^{a,b,c,d,B}$	$2.70 \pm 0.37^{b,c,A,B}$
	2:1	$1.88 \pm 0.06^{a,b,A}$	$2.29 \pm 0.31^{a,A}$	$2.45 \pm 0.17^{a,b,A}$	$2.29 \pm 0.38^{a,b,A}$	$2.49 \pm 0.34^{b,c,A}$
	1:1	$1.55 \pm 0.12^{a,A}$	$1.62 \pm 0.14^{a,A}$	$1.95 \pm 0.30^{a,A,B}$	$2.10 \pm 0.03^{a,B}$	$1.86 \pm 0.17^{a,A,B}$
Ropy EPS-producing starter cultures	4:1	$3.74 \pm 0.22^{c,A}$	$4.15 \pm 0.21^{f,A,B}$	$4.54 \pm 0.01^{e,B}$	$4.33 \pm 0.26^{e,B}$	$4.18 \pm 0.15^{e,A,B}$
	3:1	$3.48 \pm 0.02^{c,A}$	$3.57 \pm 0.34^{e,f,A}$	$3.55 \pm 0.05^{d,A}$	$3.59 \pm 0.01^{d,e,A}$	$3.67 \pm 0.01^{d,e,A}$
	2:1	$2.57 \pm 0.15^{b,A}$	$3.09 \pm 0.01^{d,e,A,B}$	$3.45 \pm 0.26^{c,d,B}$	$2.97 \pm 0.37^{b,c,d,A,B}$	$3.42 \pm 0.05^{d,e,B}$
	1:1	$2.50 \pm 0.31^{b,A}$	$2.66 \pm 0.29^{c,d,A}$	$2.91 \pm 0.06^{d,A}$	$2.93 \pm 0.01^{c,d,A}$	$3.00 \pm 0.18^{c,d,A}$

[1] n = 3

[2] Mean values within the same column not sharing common superscript (^{a,b,c,d,e}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

[3] Mean values within the same row not sharing common superscript (^{A,B}) differ significantly ($P < 0.05$, one-way ANOVA and Tukey's test).

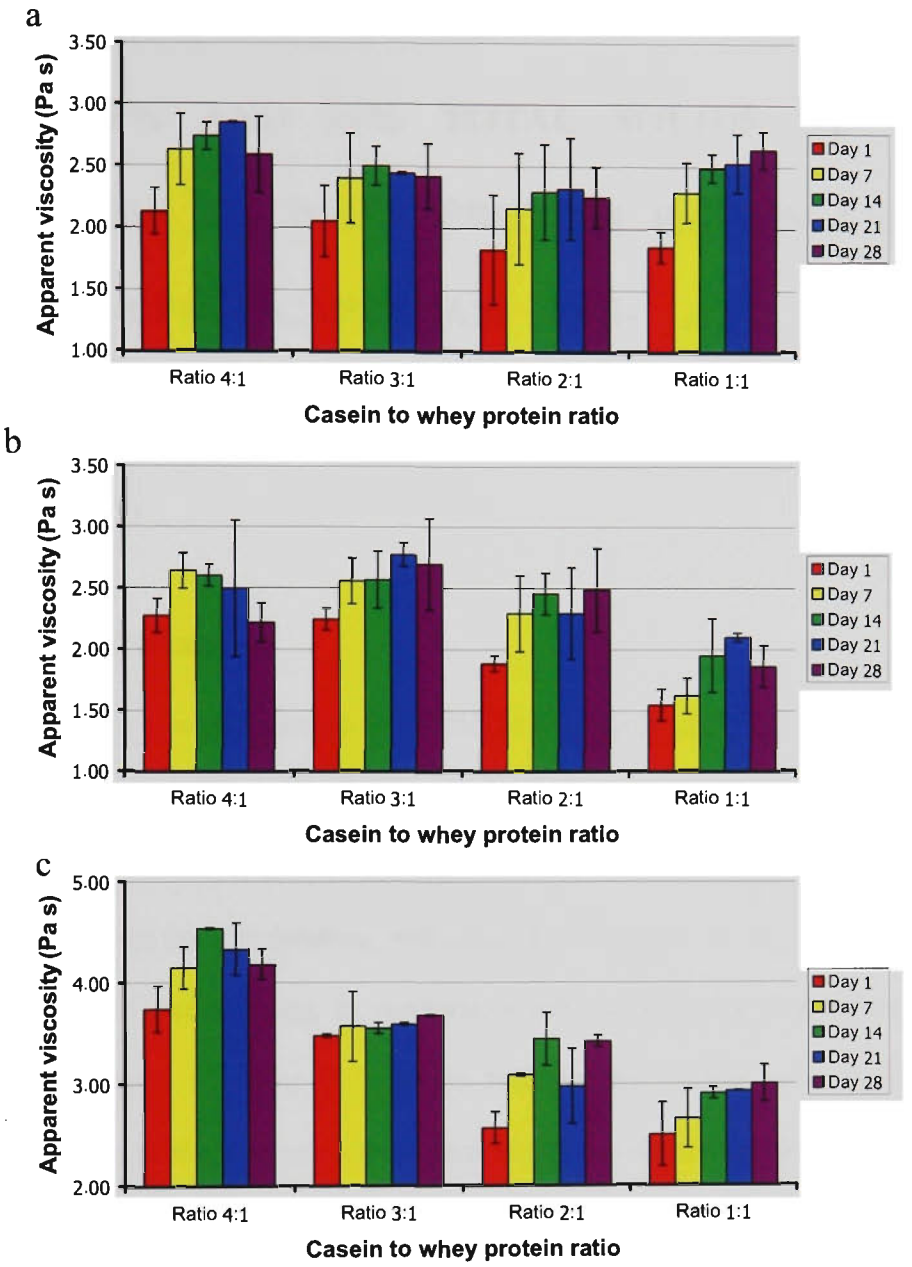

Figure 8.7: Apparent viscosity of stirred yoghurts made with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS (a), capsular EPS (b) and ropy EPS (c)-producing starter cultures during 28 days of storage at 4°C (reproduced from data in Table 8.7). Error bars represent standard deviation (n = 3).

9.0 MICROSTRUCTURE OF SET YOGHURTS MADE AT 9% AND 14% TOTAL SOLIDS BY VARYING CASEIN TO WHEY PROTEIN RATIOS AND USING NON-EPS-, CAPSULAR EPS- AND ROPY EPS-PRODUCING STARTER CULTURES

9.1 Introduction

Scanning electron microscopy (SEM) is commonly used to study the microstructure of food materials. Although the microstructure is revealed in great details due to high resolution of SEM, artefacts from chemical fixation are inevitable. Fixation procedures include defatting with chloroform or dehydration with solvents followed by critical point drying. In yoghurts, which contain considerable amount of water, replacement of water by solvents followed by critical point drying can cause artefacts to the microstructure, particularly for observing EPS (Kalab, 1993). The use of confocal scanning laser microscopy (CSLM) and cryo-SEM are suitable for examining the microstructure of yoghurts made using EPS-producing starter cultures. The microstructure of yoghurts taken by CSLM can be observed in hydrated state, in real time and in a 3 dimensional form as shown by Hassan *et al.* (1995a, 2002b, 2003a). However, CSLM microscopy has several limitations such as low resolution. Similarly, there are issues of specificity, stability, accessibility and affinity of dyes (Takeuchi and Frank, 2001; van de Velde *et al.*, 2003). van de Velde *et al.* (2002) showed that rhodamine B, which was used to stain starch

granules, accumulated in protein phases when the concentration of whey protein in the mixed starch granules-whey protein solution was increased. On the other hand, cryo-SEM or SEM in which the specimens are prepared with a cryo-preparation method offers high resolution with a minimum of chemical fixation procedures. However, the water on the surface of a fractured sample is removed by etching at sub-zero temperatures under vacuum. The images at the surface of specimen are still regarded as those of dehydrated specimen.

The results from Chapters 7 and 8 showed that total solids contents, ratios of CN to WP (4:1, 3:1, 2:1 and 1:1) as well as the type of starter cultures (non-EPS-, capsular EPS- or ropy EPS-producing starter cultures) had a significant impact on the physical properties of set yoghurts. The firmness and level of spontaneous syneresis decreased as the proportion of CN was reduced. The use of EPS-producing starter cultures further reduced the firmness and syneresis of products made at 9% solids, but not in those made at 14% solids. These results may be due to the changes in microstructure of yoghurts as a result of varying solids content, ratios of CN to WP as well as the use of EPS starter culture.

9.2 Aim

This chapter examined the microstructure of set yoghurts made at 9% and 14% total solids with the CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures during 28 days of storage, using CSLM. In addition, the microstructure was also examined using both conventional and cryo-SEM after day 1 of storage.

9.3 Materials and Methods

9.3.1 Experimental design

Twelve batches of set yoghurts made at 9 or at 14% solids were produced as the previous two experiments (Chapters 7 and 8). The microstructure was observed at day 1, 14 and 28 of storage (4°C) using confocal scanning laser microscope. A scanning electron microscope was also used for observing microstructure of yoghurts at 9 and 14% solids made using EPS-producing starter cultures at the CN to WP ratio of 4:1 (only at day 1 of storage). All yoghurts were made in triplicate.

9.3.2 Microorganisms

Non-EPS-producing *S. thermophilus* ASCC 1342 and *L. delbrueckii* ssp. *bulgaricus* ASCC 1466, capsular EPS-producing *S. thermophilus* ASCC 285, and ropy EPS-producing *S. thermophilus* ASCC 1275 were used in this study. The maintenance and activation of these bacteria were carried out as described in Chapter 3, Section 3.1.

9.3.3 Yoghurt manufacture

Set yoghurts made at 9 and 14% total solids contents using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 were produced according to the procedure described in Chapter 3, Section 3.3 and stored in a walk-in-cooler (4°C) for further analysis at day 1, 14 and 28.

9.3.4 Microstructure of set yoghurts

The microstructure of set yoghurts made at 9 and 14% total solids using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures with various CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 were examined at day 1, 14 and 28 using CSLM according to procedure described in Chapter 3, Section 3.16.1. The microstructure of products made using capsular EPS- or ropy EPS-producing starter cultures with the CN to WP ratio of 4:1 was also examined using both conventional SEM (Section 3.16.2) and cryo-SEM (Section 3.16.3).

9.4 Results and Discussions

9.4.1 CSLM micrograph and visibility of EPS

Figure 9.1 shows a series of CSLM micrographs taken through to the depth of 15 μm of 9% yoghurt with the CN to WP ratio of 4:1 made with ropy EPS-producing starter cultures. The green colour and tiny red dots represent protein matrix and EPS, respectively. EPS appeared to be present in lower amount (less red dots) and the pore sizes in protein matrix were seen to be smaller in the micrographs taken further

from the coverslip. The visibility of EPS may be explained by the limitation of accessibility of dye as suggested by Takeuchi and Frank (2001). The change in protein matrix may be due to the mechanical sample preparation (cutting the surface). Based on this result, CSLM-micrographs were taken at a distance of ~ 10 μm from the coverslip for the rest of this study.

Hassan *et al.* (2002b) used CSLM to show that larger pores in products made using EPS-producing starter cultures were filled with EPS. This could not be ascertained from our CSLM micrographs in Figure 9.1. In order to ascertain if the pores are filled up with EPS, the microstructure of yoghurts at the CN to WP ratio of 4:1 made with capsular EPS- and ropy EPS-producing starter cultures was studied by SEM with both conventional and cryo-fixation procedures after 1 day of storage.

9.4.2 Conventional SEM-micrograph of EPS yoghurts

Figure 9.2 shows the conventional SEM-micrographs of yoghurt with capsular EPS- (a) and ropy EPS- (b) producing starter cultures. There was an absence of EPS in the micrograph of the product made using capsular starter cultures (a), whereas EPS appeared as thick sheets attached to strands of protein in the yoghurt made using ropy starter cultures (b). The protein matrix appeared to be similar to those shown by other researchers (Heertje *et al.*, 1985; Harwalkar and Kalab, 1986;

Puvanenthiran *et al.*, 2002). In conventional sample preparation procedures, the water in yoghurt samples is replaced with ethanol or other solvents, followed by critical point drying. These solvents are commonly used for precipitation of carbohydrates including isolation of EPS. The shape of ropy EPS in the micrograph may be artefacts caused by chemical fixations, especially during dehydration with a series of ethanol solutions as suggested by Kalab (1993). The use of freeze-drying as used in this study instead of critical point drying might prevent artefacts. Therefore, the cryo-preparation method was chosen for preparing the yoghurt specimens for SEM.

9.4.3 Cryo-SEM-micrograph of EPS yoghurts

Figure 9.3 shows cryo-SEM-micrographs of set yoghurts at 9% solids made using capsular EPS- (a) and ropy EPS- (b) producing starter cultures. Although the micrographs were taken at different magnifications, both cryo-SEM-micrographs (Figures 9.3a and 9.3b) differed noticeably from those taken by the conventional SEM (Figures 9.2a and 9.2b). The artefacts caused by the chemical fixation may explain the difference. In cryo-SEM-micrographs, higher amount of EPS was observed inside the pores as thin sheets connected to strands of proteins (Figure 9.3a and 9.3b). The existence of EPS inside the pores was consistent with that reported by Hassan *et al.* (2002b), who observed that ropy EPS filled up the pores as observed by CSLM. By comparing between the cryo-SEM- and CSLM-micrographs of our results, it is obvious that only a small portion of EPS was shown

in CSLM-micrographs. The sample preparation technique, and dyes, as well as the nature of sample for CSLM microscopy may all contribute to this result.

9.4.4 CSLM-micrographs of yoghurts made at 9% during storage

Figure 9.4 shows the CSLM-micrographs of set yoghurts at 9% solids with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 using non-EPS-, capsular EPS- or ropy EPS-producing starter cultures at day 1 of storage. EPS appeared in the products made using non-EPS-producing starter cultures (Figure 9.4). This confirmed the results of EPS quantification as given in Chapters 3, 7 and 8 that non-EPS-producing starter cultures used produced a small amount of EPS (10 – 20 mg L^{-1}). Furthermore, the density of protein matrix increased when the ratios of CN to WP decreased from 4:1 to 1:1. Similar results were observed by Puvanenthiran *et al.* (2002) that the protein matrix observed with conventional SEM of set yoghurts became denser as the ratios of CN to WP were reduced. Bhullar *et al.* (2002) and Remuef *et al.* (2003) studied the effects of fortification on microstructure of yoghurts and observed that products fortified with WPC had a very dense protein matrix with numerous small pores. This phenomenon was observed in products made with the three types of starter cultures. The explanation for this might be that the increase in proportion of WP would result in greater interaction between β-lactoglobulin and κ-casein on the surface of casein micelles during heat treatment (Lucey and Singh, 1997). This interaction would prevent casein particles from forming clusters and protein networks (Lucey *et al.*, 1998a). In addition, the increase in the density of protein

matrix correlated with the reduction in the level of syneresis as shown in Chapters 7 and 8. This result was similar to that of Puvanenthiran *et al.* (2002) that the level of syneresis decreased when the protein matrix became denser as a result of reducing cn to WP ratios.

When the microstructure was compared among products made from different types of starter cultures (at the same CN to WP ratio), larger pore sizes were observed in products made from EPS-producing starter cultures (Figure 9.5) than those made using non-EPS-producing starter cultures. This was consistent with the results reported by Hassan *et al.* (1995a, 2002b). Further, the largest pore sizes were observed in set yoghurts made using ropy EPS-producing starter culture made at the CN to WP ratio of 4:1. In Chapters 4, 5, 7, 8, yoghurts made using EPS-producing starter cultures showed further reduction in syneresis as compared to those made using non-EPS-producing starter cultures. Harwalkar and Kalab (1986) observed a relationship between the reduction in syneresis of yoghurts and the increase in density of protein matrix. Our results did not agree with those of Harwalkar and Kalab (1986). Therefore, a reduction in syneresis in products made using EPS-producing starter cultures is likely to be due to the high water-binding ability of EPS as suggested by De Vuyst and Degeest (1999). During storage from day 1 (Figure 9.4) to 14 (Figure 9.5) and 28 (Figure 9.6), the pore sizes of products made using non-EPS- and EPS-producing starter cultures increased slightly. The pore sizes of set yoghurts made using ropy EPS-producing starter cultures increased substantially during storage, particularly at the CN to WP ratio of 4:1. This phenomenon is believed to correlate with EPS concentration during storage as

shown in Chapter 7. Nevertheless, the product made at a CN to WP ratio of 3:1 using ropy EPS-producing starter cultures, which had the highest EPS concentration at the end of storage, did not show the largest pore size. It may suggest the influence of CN to WP ratios and EPS on the changes of yoghurt microstructure during storage.

9.4.5 CSLM-micrographs of yoghurts made at 14% during storage

At 14% solids level (Figure 9.7), the protein matrix of yoghurts was denser than those made at 9%. The increase in the density of protein matrix when the ratios of CN to WP were reduced was similar to that observed in products made at 9% solids. This was observed regardless of the type of starter cultures used (non-EPS, capsular EPS or ropy EPS). Further, the microstructure of set yoghurts made using EPS-producing starter cultures did not appear to be much different from that made using non-EPS-producing starter cultures (at the same ratios of CN to WP) as observed in products made at 9% solids. No increase in the pore sizes was observed during storage from day 1 (Figure 7) to day 14 (Figure 8) and 28 (Figure 9). The similarity in microstructure of set yoghurts made at 14% solids as compared to those at 9% solids may explain their physical properties as reported in Chapter 8 that the use of EPS-producing starter cultures did not have an impact on firmness and syneresis of set yoghurts made at 14% total solids as observed in those made at 9% (Chapter 7).

9.5 Conclusions

Microstructure of set yoghurts was influenced by total solids content, the ratios of CN to WP as well as the type of starter cultures. Increasing solids content from 9 to 14% and decreasing the CN to WP ratios from 4:1 to 1:1 resulted in denser protein matrix. Largest and more irregular pore sizes were found in products made at 9% solids at the CN to WP ratio of 4:1 using ropy EPS-producing starter cultures. At 14% solids, the microstructure of products made using non-EPS- and EPS-producing starter cultures was similar (at the same ratio of CN to WP). During storage period, a remarkable increase in the pore sizes was only observed in the yoghurts made at 9% solids with the CN to WP ratio of 4:1 using ropy EPS-producing starter cultures.

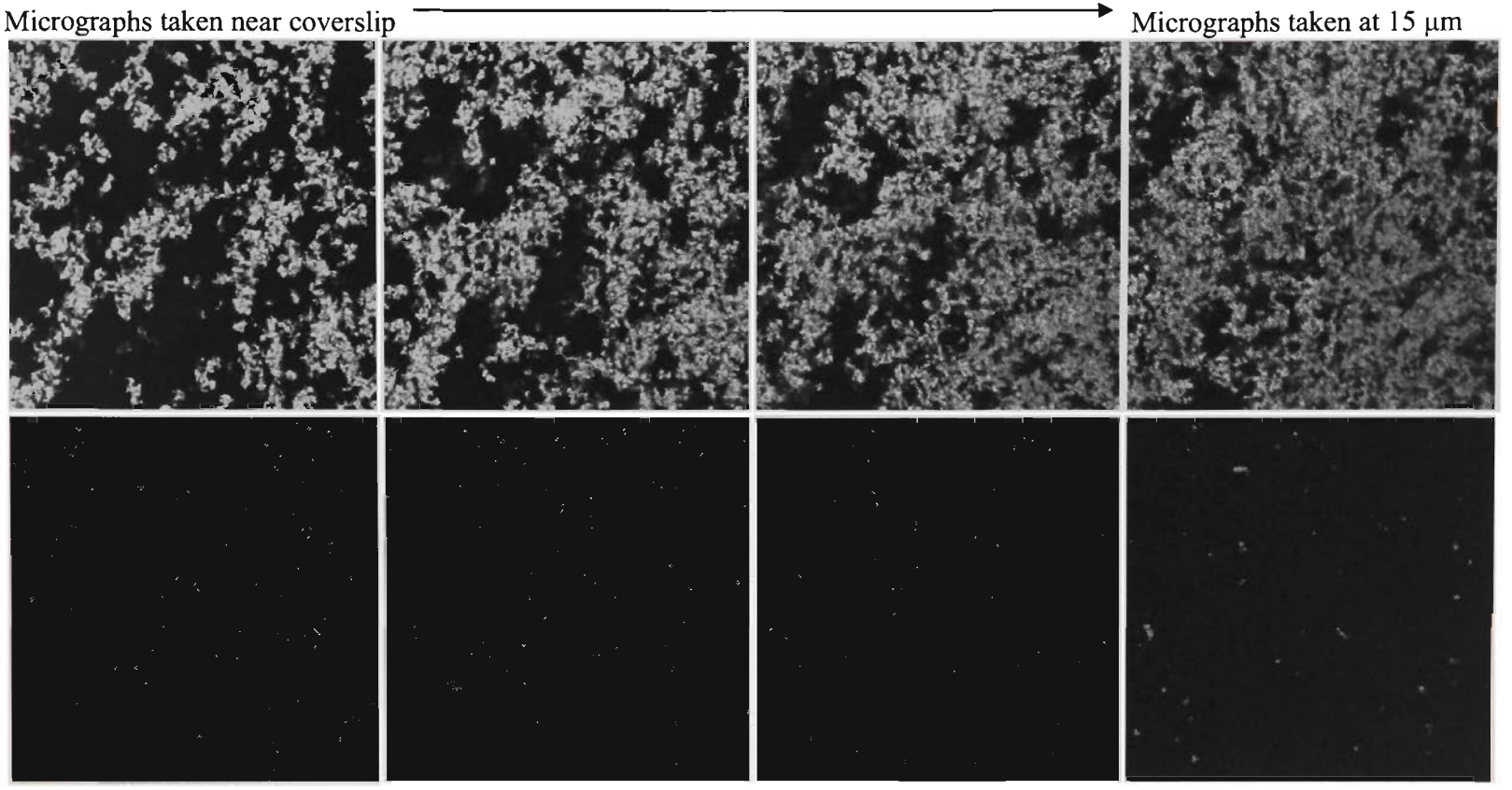

Figure 9.1: CSLM-micrographs taken through the depth of 15 µm of set yoghurt made at 9% solids using ropy EPS-producing starter cultures. The protein matrix (top) and EPS (bottom) are shown in green and red (tiny dot).

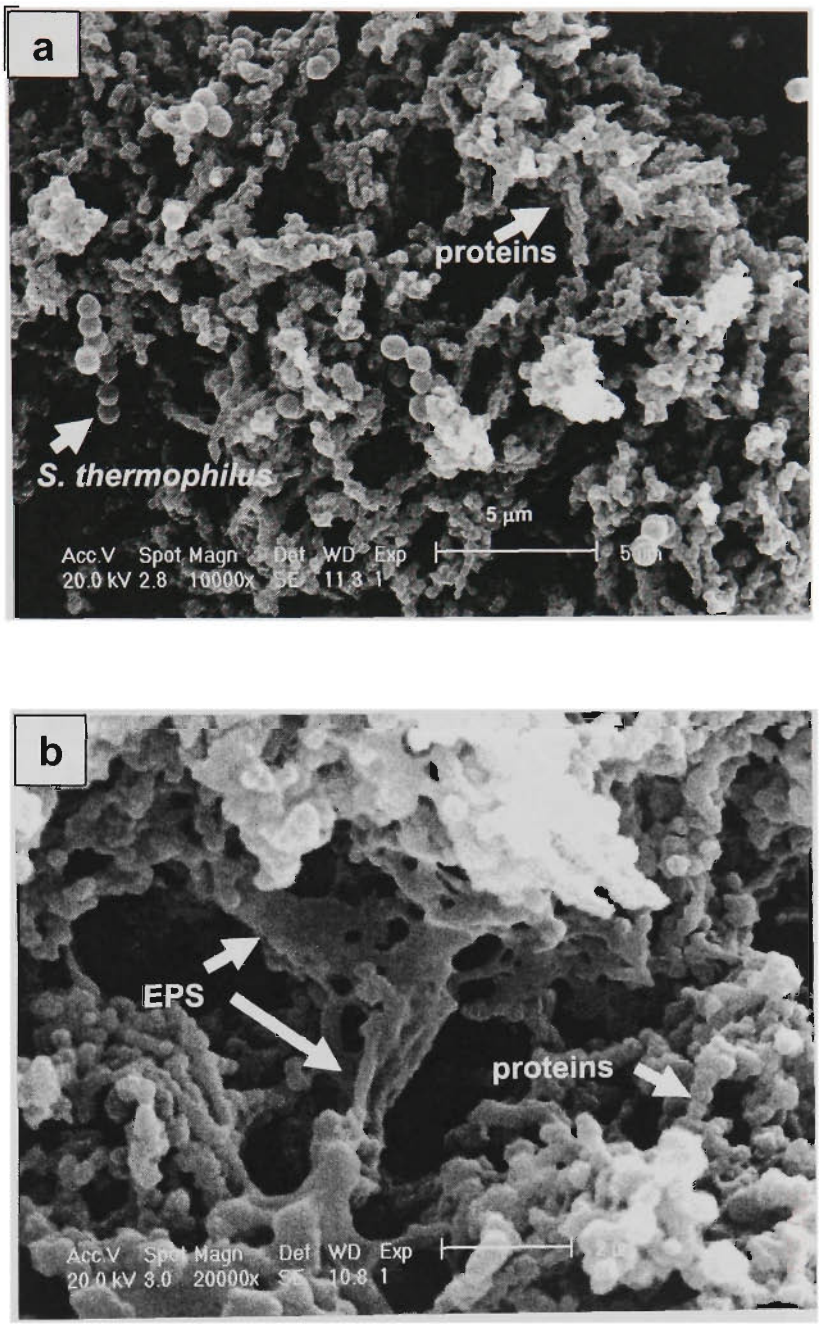

Figure 9.2: Conventional SEM-micrographs of set yoghurt made at 9% solids prepared at CN to WP ratio of 4:1 using (a) capsular EPS-producing starter cultures or (b) ropy EPS-producing starter cultures

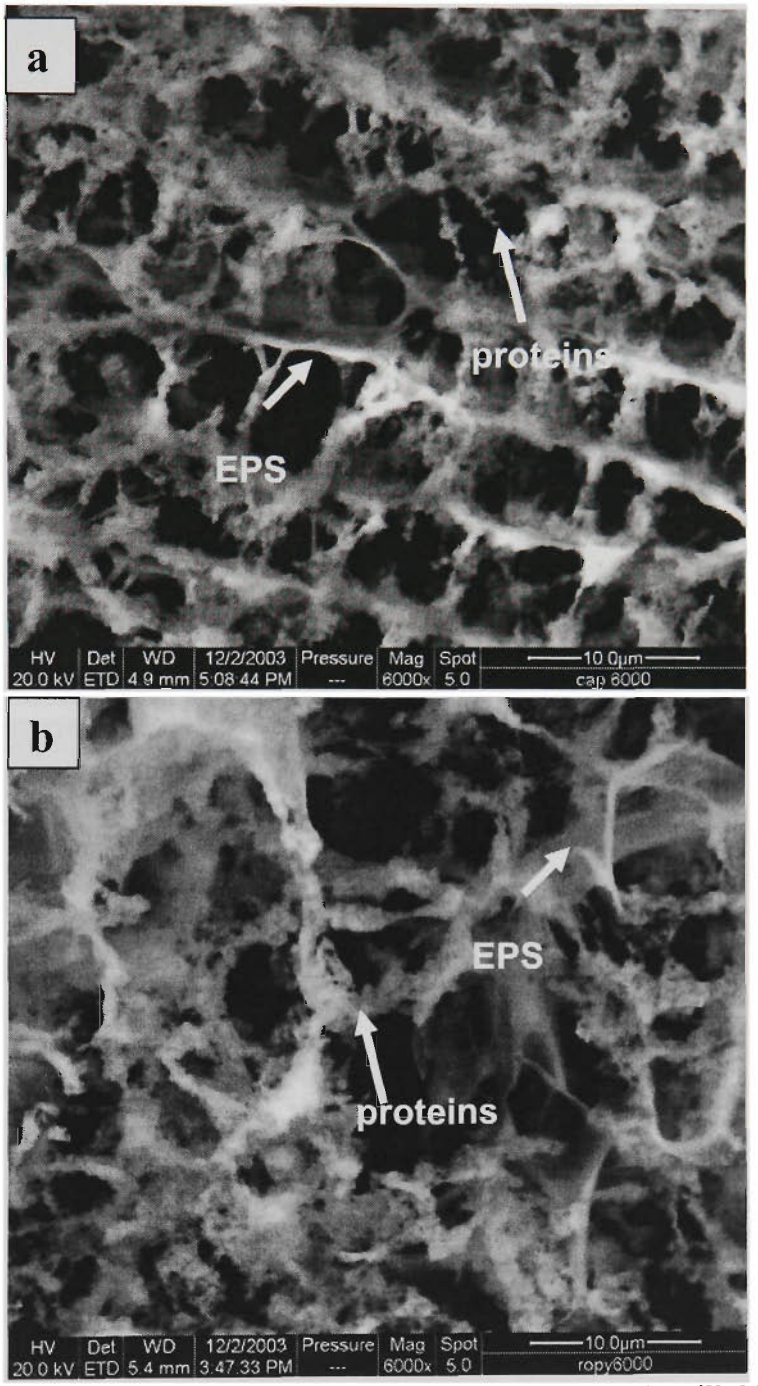

Figure 9.3: Cryo-SEM-micrographs of set yoghurt made with milk blend at 9% solids with CN to WP ratio of 4:1 using capsular EPS- (a) and ropy EPS- (b) producing starter cultures

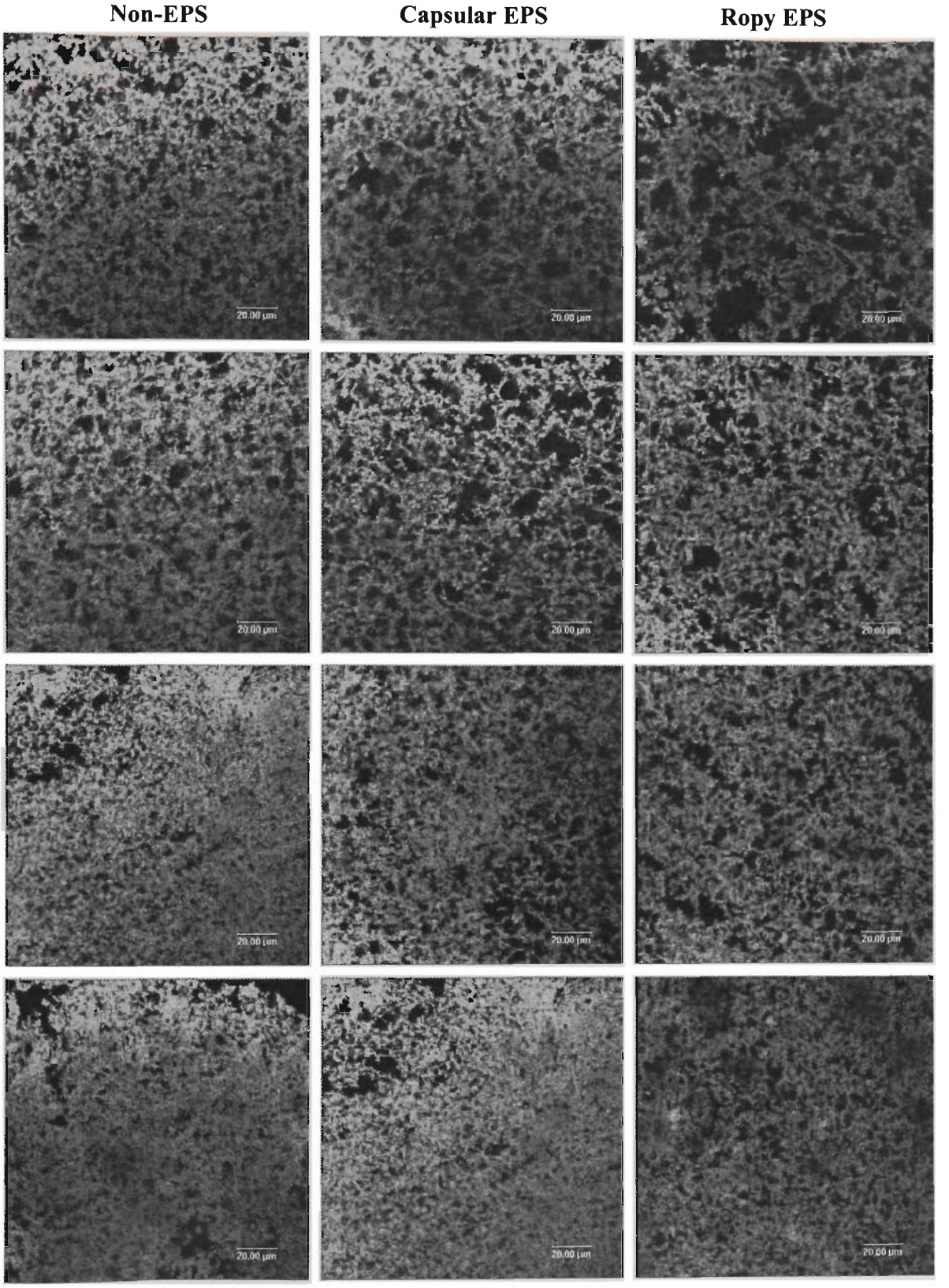

Figure 9.4: CSLM-micrographs of set yoghurts made at 9% solids using non-EPS, capsular EPS or ropy EPS-producing starter cultures with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at day 1 of storage. The protein matrix is presented in green and EPS in red (tiny dots)

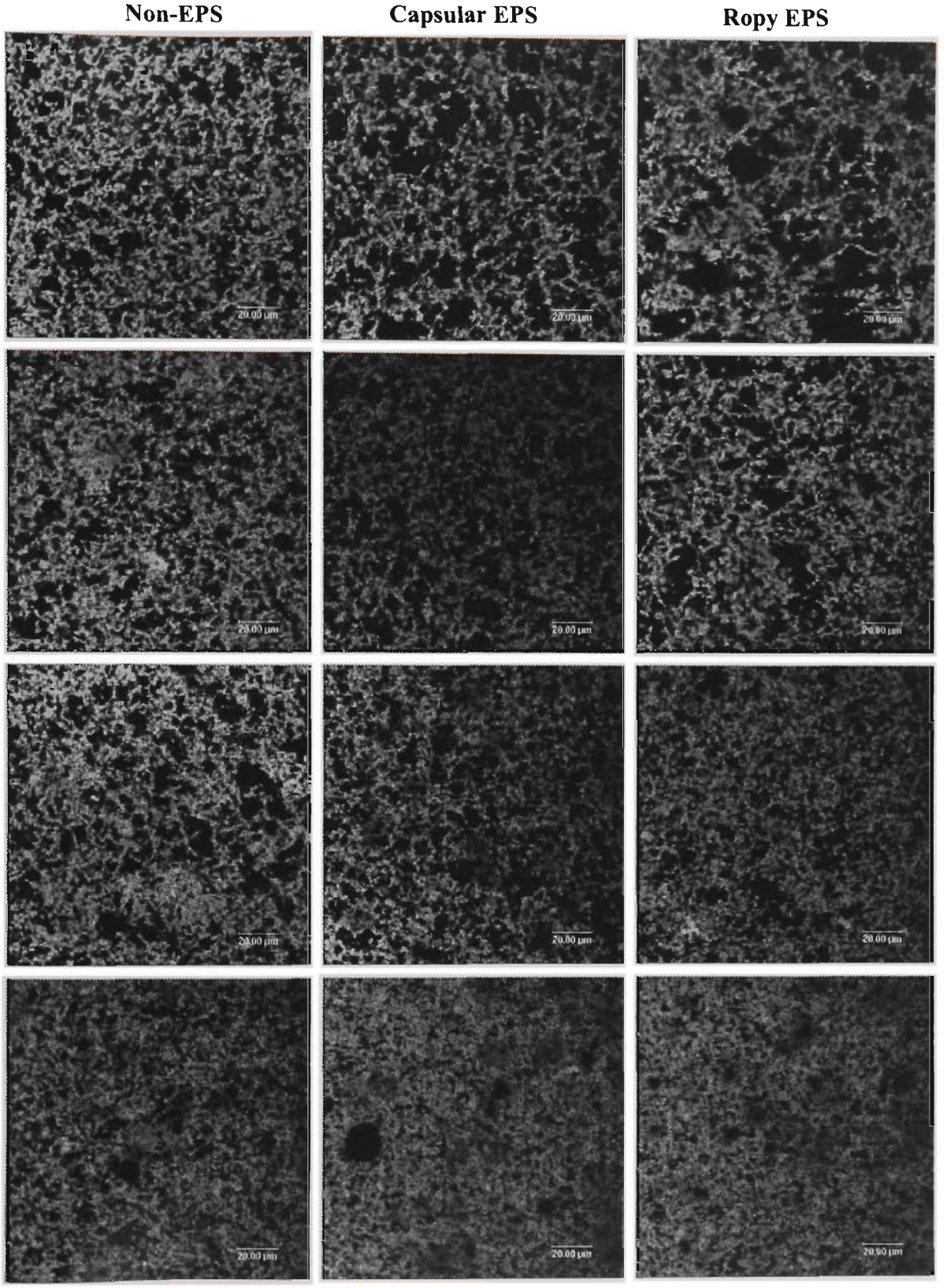

Figure 9.5: CSLM-micrographs of set yoghurts made at 9% solids using non-EPS, capsular EPS or ropy EPS-producing starter cultures with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at day 14 of storage. The protein matrix is presented in green and EPS in red (tiny dots)

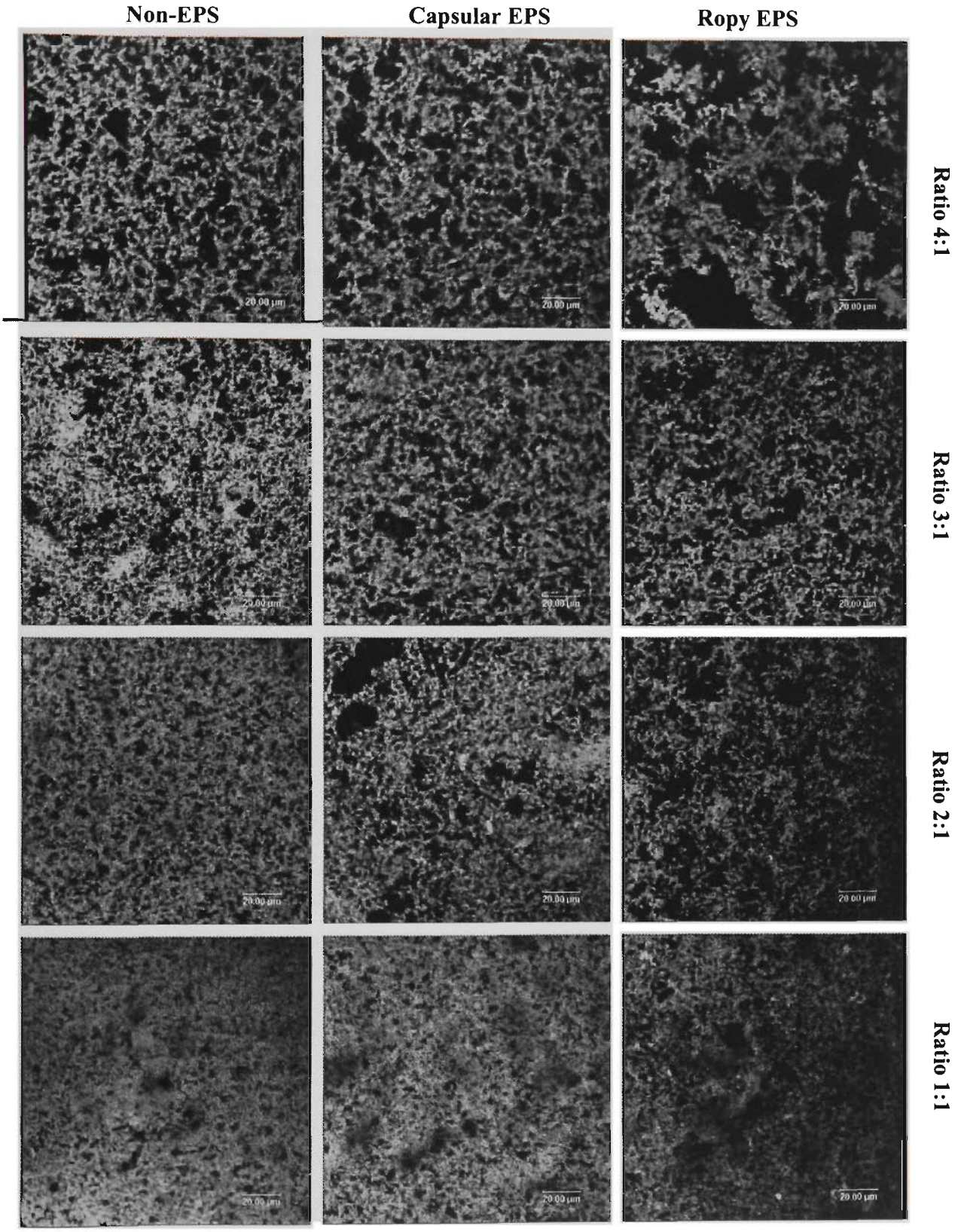

Figure 9.6: CSLM-micrographs of set yoghurts made at 9% solids using non-EPS, capsular EPS or ropy EPS-producing starter cultures with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at day 28 of storage. The protein matrix is presented in green and EPS in red (tiny dots)

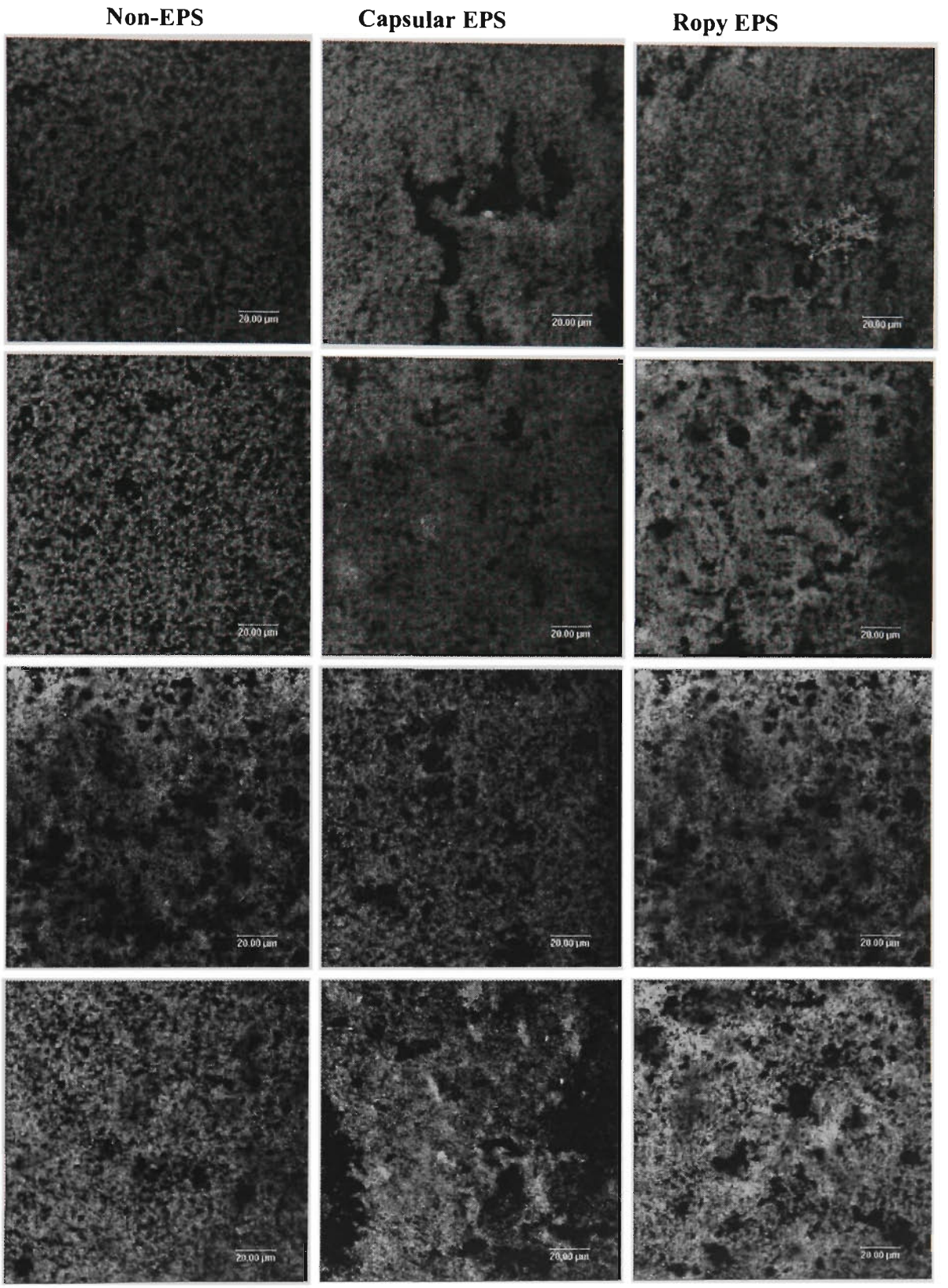

Figure 9.7: CSLM-micrographs of set yoghurts made at 14% solids using non-EPS, capsular EPS or ropy EPS-producing starter cultures with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at day 1 of storage. The protein matrix is presented in green and EPS in red (tiny dots)

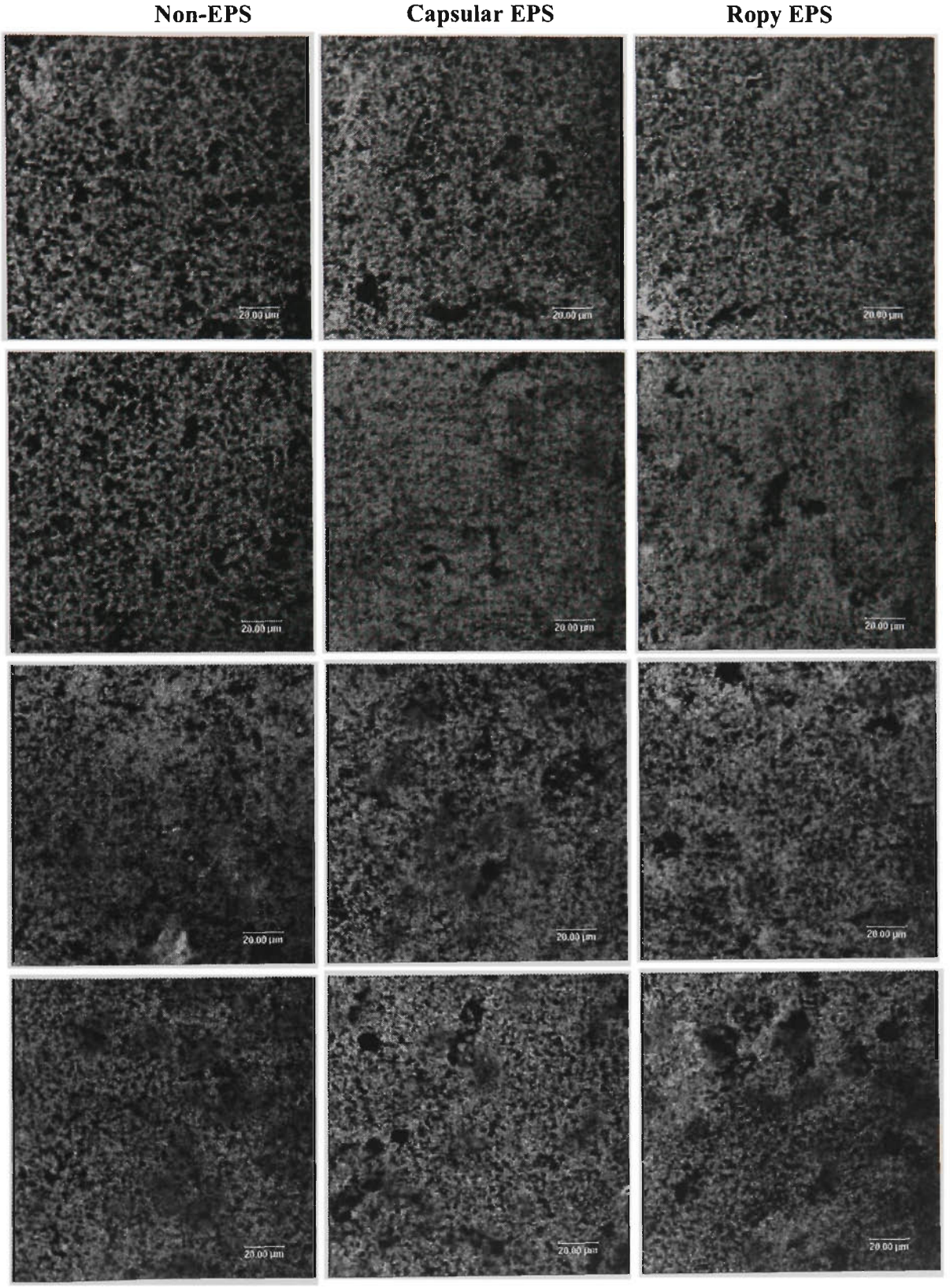

Figure 9.8: CSLM-micrographs of set yoghurts made at 14% solids using non-EPS, capsular EPS or ropy EPS-producing starter cultures with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at day 14 of storage. The protein matrix is presented in green and EPS in red (tiny dots)

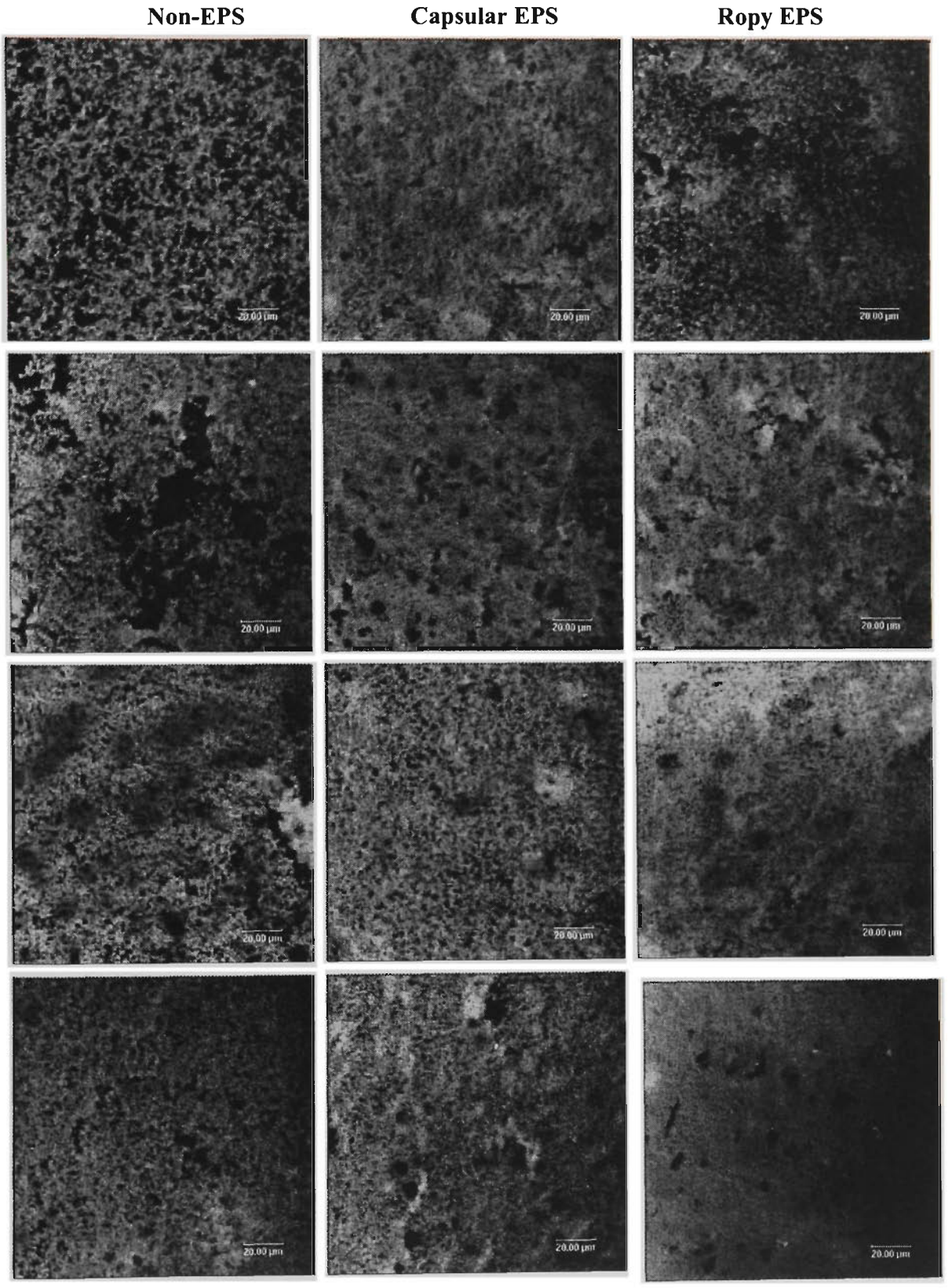

Figure 9.9: CSLM-micrographs of set yoghurts made at 14% solids using non-EPS, capsular EPS or ropy EPS-producing starter cultures with CN to WP ratios of 4:1, 3:1, 2:1 and 1:1 at day 28 of storage. The protein matrix is presented in green and EPS in red (tiny dots).

10.0 OVERALL DISCUSSIONS AND CONCLUSIONS

The results of this project showed that the total solids content, types of EPS-producing starter cultures, ratios of CN to WP and storage period affected population of starter cultures, concentration of lactic acid and EPS, and physical properties of yoghurts. The results from Chapter 5 showed that the siphon method was a good representative of spontaneous syneresis, although, the data showed high variation. This method was used in subsequent chapters. The level of syneresis determined by other methods (drainage and centrifugation) was affected by other influences such as gravity, curd breakage, collapsing from centrifugal force. The results from Chapters 4, 6, 7 and 8 showed that the increase in total solids from 9% to 14% as well as total proteins enhanced growth of starter cultures, the production of lactic acid and EPS. The increase in solids and protein contents also reduced syneresis as well as improved firmness in set yoghurts and apparent viscosity in stirred products. The firmness of set yoghurts and the apparent viscosity of stirred yoghurts made at 9% solids content were not much lower than those made at 14% solids level.

Increasing the proportion of WP in milk blends (decreasing CN to WP ratio), while keeping similar level of total solids and protein contents, resulted in a reduction in fermentation time, lactic acid concentration, syneresis and firmness in set yoghurts regardless of the type of EPS-producing starter cultures. More fracture phenomena

occurred during penetration test in set yoghurts as the ratios of CN to WP were reduced. According to van Vliet and Luyten, 1995, the results could be interpreted as an increase in weak points in the structure of protein network in set products. Moreover, the alteration in the CN to WP ratios affected EPS production. The highest EPS concentration was found in products made with ropy EPS-producing starter cultures at the CN to WP ratio of 3:1 at 9% solids level, and at the CN to WP ratio of 4:1 at 14% solids level. An increase in apparent viscosity and the area of hysteresis loop between upward and downward flow curves of stirred yoghurts made at 9 and 14% total solids correlated positively with the concentration of ropy EPS. The values of these two variables increased dramatically in products made with ropy EPS-producing starter cultures as compared to those made with non-EPS- and capsular EPS-producing starter cultures. As the area of hysteresis loop represents the damage of food structure during shearing, this may suggest that ropy EPS provided an additional structure in stirred yoghurt as a result of entanglement of EPS compared to those made using non-EPS- or capsular EPS-producing starter cultures. The SEM-micrographs in Chapter 9, Figure 9.2 and 9.3, confirm that ropy EPS exists as a minor structure in the protein matrix. Although the EPS in the micrographs may have been inevitably changed due to sample preparation, even in cryo-preparation procedure, the ropy EPS appeared as large sheets located in each pores of proteins matrix, while there was less capsular EPS.

The use of EPS-producing starter cultures helped reduce syneresis of the product made at 9% solids dramatically, however, it also decreased the firmness. The

incompatibility of EPS and milk proteins is believed to exist as the pH of milk decreased during fermentation resulting in EPS filled inside pores (Tolstoguzov, 1997, 1998, 2000). The presence of EPS may also interfere with protein network formation. However, the decrease in firmness as affected by EPS-producing starter cultures did not occur in the products made at 14% solids level. The results from microstructural study (Chapter 9) showed that the products made at 9% solids with ropy EPS-producing starter cultures had larger pore size than those made with non-EPS- and capsular EPS-producing starter cultures. There was much less difference in the protein structure between those products at 14%. This might suggest that the interfering effect of EPS on the 3-dimensional protein matrix is greater in set yoghurts made at 9% than those at 14%.

There was a decrease in the numbers of starter culture bacteria during storage, but there was an increase in the EPS concentration (particularly in products made using ropy EPS-producing starter cultures), firmness in set yoghurts, and the apparent viscosity in stirred yoghurts made with ropy EPS-producing starter cultures. The pore sizes in microstructure of set yoghurt made at 9% solids content using ropy EPS-producing starter cultures increased during storage; this correlated well with the increase in EPS concentration. Although the increase in the proportion of WP decreased firmness in set yoghurts, the approach carried out in this study is not common in commercialized yoghurt production. Generally, WP is added alone or in combination with other dairy ingredients to raise the solids level of yoghurts. This increases total protein content of yoghurts as well. As discussed in Chapter 4, the

firmness increased with the level of total protein, even though the total solids content was similar. This suggests that the protein content has the most profound influence on the firmness of set yoghurts as compared to the variation in CN to WP ratios and EPS-producing starter cultures.

The optimization of the combined use of non-EPS- or EPS-producing starter cultures with the variation in the ratio of CN to WP in yoghurt production could result in good quality product and the physical properties can be maintained at acceptable levels. For set yoghurts, the product should be made at 14% total solids or higher at a CN to WP ratio of 3:1 with ropy EPS-producing starter cultures with regard to obtaining acceptable syneresis and firmness. Decreasing total solids levels can result in much softer product when made with EPS-producing starter cultures. The use of ropy EPS-producing starter cultures is suitable in the production of stirred yoghurts. It may be possible to decrease solids content in stirred yoghurts from 14 to 11-12% without decreasing apparent viscosity of products by the combined use of ropy EPS-producing starter cultures and the CN to WP ratio of 3:1.

11.0 FUTURE WORK

It was found that the reduction in CN to WP ratios and the use of EPS-producing starter cultures had a negative influence on firmness, but a positive impact on syneresis of set yoghurt produced at 9% solids content. The use of EPS-producing starter cultures did not have any effect on firmness and syneresis when the solids content was elevated from 9 to 14%. However, the reduction in CN to WP ratios caused a decrease in firmness of products made at 14% solids. This may suggest the importance of protein content and structure over EPS on physical properties of set yoghurts. Therefore, supplementation with other sources of proteins, e.g. egg white or plant protein, other than WPC may help improve physical properties of set yoghurts. In addition, the physico-chemical characteristics of EPS produced from the two strains of EPS-producing *S. thermophilus* have not been studied due to the limitation of time. The characterisation of the structure, charge and molecular weight of EPS can be carried out by NMR, ion chromatography and size exclusion chromatography. The test on the compatibility between EPS and milk proteins should also be carried out at pH values ranging from 7.00 to 4.00 to cover the range of pH during yoghurt fermentation coupling with rheological measurement using a rheometer or diffused wave spectroscopy.

The use of higher solids content may also alter the EPS produced by starter cultures resulting in the changes in physical properties of yoghurts. Therefore, the

experiment should also be carried out in yoghurts made at high solids content, e.g. 18%. In stirred type products, ropy EPS-producing starter cultures were shown to have a significant influence on the apparent viscosity. This is due to an additional structure of ropy EPS as a result of entanglement in the structure of stirred yoghurts. Therefore, it is possible to produce stirred yoghurts at lower solids concentration than normal yoghurt by using ropy EPS-producing starter cultures. Another interesting approach that has not been explored in this study is to produce yoghurts containing high concentration of EPS (~1 to 4 g L^{-1}). This can be done by keeping the pH of milk at pH values ranging from 5.50 to 6.70 during fermentation until the desirable concentration of EPS is reached, followed by conventional yoghurt fermentation.

12.0 References

Amrane, A., & Prigent, Y. (1998). Lactic acid production rates during the different growth phases of *Lactobacillus helveticus* cultivated on whey supplemented with yeast extract. *Biotechnology Letter, 20*, 379-383.

Andrew, A. T. (1983). Protenases in normal bovine milk and their action on caseins. *Journal of Dairy Research*, 50, 45-55.

Anema, S. G., Lee, K. S., Lowe, E. K., & Klostermeyer, H. (2004). Rheological properties of acid gels prepared from heated pH-adjusted skim milk. *Journal of Agricultural and Food Chemistry, 52*, 337-343.

AOAC. (1995). *Official Methods of Analysis of AOAC International* (16 ed. Vol. 2). Arling, VA: AOAC international.

Ariga, H., Urashima, T., Michihata, E., Ito, M., Morizono, N., Kimura, T., Takahashi, S. (1992). Extracellular polysaccharide from encapsulated *Streptococcus salvarius* subsp. *thermophilus* OR 901 isolated from commercial yoghurt. *Journal of Food Science, 57*, 625-628.

Auty, M. A. E., Twomey, M., Guinee, T. P., & Mulvihill, D. M. (2001). Development and application of confocal scanning laser microscopy methods for studying the distribution of fat and protein in selected dairy products. *Journal of Dairy Research, 68*, 417-427.

Baig, M. I., & Prasad, V. (1996). Effect of incorporation of cottage cheese whey solids and *Bifidobacterium bifidum* in freshly made yogurt. *Journal of Dairy Research, 63*, 467-473.

Barbosa-Canovas, G. V., Kokini, J. L., Ma, L., & Ibarz, A. (1996). The rheology of semiliquid foods. *Advanced Food and Nutrition Research, 39*, 1-69.

Bautista, E. S., Dhahiya, R. S., & Speck, M. L. (1966). Identification of compounds causing symbiosis growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk. *Journal of Dairy Research, 33*, 299-307.

Benguigui, L., Emery, J., Durand, D., & Busnel, J. P. (1994). Ultrasonic study of milk clotting. *Lait, 74*, 197-206.

Bhattacharya, S. (1999). Yield-stress and time-dependent rheological properties of mango pulp. *Journal of Food Science, 64*, 1029-1033.

Bhullar, Y. S., Uddin, M. A., & Shah, N. P. (2002). Effects of ingredients supplementation on textural characteristics and microstructure of yoghurt. *Milchwissenschaft, 57*, 328-332.

Biliaderis, C. G., Khan, M. M., & Blank, G. (1992). Rheological and sensory properties of yoghurt from skim milk and ultrafiltered retentates. *International Dairy Journal, 2*, 311-323.

Bourne, M. C. (1982). *Food Texture and Viscosity: Concept and Measurement.* New York: Academic Press.

Bourne, M. C. (2002). *Food Texture and Viscosity: Concept and Measurement.* (2 ed.). New York: Academic Press.

Bouzar, F., Cerning, J., & Desmazeaud, M. (1996). Exopolysaccharide production in milk by *Lactobacillus delbrueckii* ssp. *bulgaricus* CNRZ 1187 and by two colonial variants. *Journal of Dairy Science, 79*, 205-211.

Bouzar, F., Cerning, J., & Desmazeaud, M. (1997). Exopolysaccharide production and texture-promoting ability of mixed-strain starter cultures in yoghurt production. *Journal of Dairy Science, 80*, 2310-2317.

Broadbent, J. R., McMahon, D. J., Welker, D. L., Oberg, C. J., & Moineau, S. (2003). Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: A review. *Journal of Dairy Science, 86*, 407-423.

Bury, D., Jelen, P., & Kimura, K. (1998). Whey protein concentrate as a nutrient supplementation for lactic acid bacteria. *International Dairy Journal, 8*, 149-151.

Carson, K., Meullenet, J. F. C., & Reische, D. W. (2002). Spectral stress strain analysis and partial least squares regression to predict sensory texture of yoghurt using a compression/penetration instrumental method. *Journal of Food Science, 67*, 1224-1228.

Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Reviews, 87*, 113-130.

Cerning, J., Bouillanne, C., Desmazeaud, M., & Landon, M. (1986). Isolation and characterization of exocellular polysaccharide produced by *Lactobacillus bulgaricus*. *Biotechnology Letters, 8*, 625-628.

Cerning, J., Bouillanne, C., Desmazeaud, M., & Landon, M. (1988). Exocellular polysaccharide production by *Streptococcus thermophilus*. *Biotechnology Letters, 10*, 255-260.

Cerning, J., Bouillanne, C., Landon, M., & Desmazeaud, M. (1992). Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. *Journal of Dairy Science, 75*, 692-699.

Cerning, J., Renard, C. M. G. C., Thibault, J. F., Bouillanne, C., Landon, M., Desmazeaud, M., *et al.* (1994). Carbon source requirements for exopolysaccharide production by *Lactobacillus casei* CG11 and partial

structure analysis of the polymer. *Applied and Environmental Microbiology, 60*, 3914-3919.

Cho, Y., Singh, H., & Creamer, L. K. (2003). Heat-induced interaction of α-lactoglobulin A and κ-casein B in a model system. *Journal of Dairy Research, 70*, 61-71.

Crang, R. F. E. (1991). Problems and techniques in biological scanning electron microscopy. In K. Kuo & J. Yao (Eds.), *International Symposium on Electron Microscopy*. Singapore: World Scientific Publishing Co. Pte. Ltd.

Dairy Australia (2005). Statistics of exported dairy products. Retrieved 6/25/2004, from http://www.dairyaustralia.com.au/Content/Markets_and_Trade/Australian_Dairy_Industry/Australian_Exports/Export_Summary.xls

Dalgleish, D. G., & Law, A. J. R. (1988). pH-induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. *Journal of Dairy Research, 55*, 529-538.

Dave, R. I., & Shah, N. P. (1998a). Ingredient supplementation effects on viability of probiotic bacteria in yogurt. *Journal of Dairy Science, 81*, 2804-2816.

Dave, R. I., & Shah, N. P. (1998b). The influence of ingredients supplementation on the textural characteristics of yogurt. *The Australian Journal of Dairy Technology, 53*, 180-184.

Davies, F. L., Shankar, P. A., Brooker, B. E., & Hobbs, D. G. (1978). A heat-induced change in the ultrastructure of milk and its effect on gel formation in yoghurt. *Journal of Dairy Research, 45*, 53-58.

de Kruif, C. G., & Tuinier, R. (2001). Polysaccharide protein interactions. *Food Hydrocolloids, 15*, 555-563.

De Vuyst, L., & Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews, 23*, 153-177.

De Vuyst, L., Vanderveken, F., Van de Ven, S., & Degeest, B. (1998). Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *Journal of Applied Microbiology, 84*, 1059-1068.

De Vuyst, L., Zamfir, M., Mozzi, F., Adriany, T., Marshall, V., Degeest, B., Vaningelgem, F. (2003). Exopolysaccharide-producing *Streptococcus thermophilus* strains as functional starter cultures in the production of fermented milks. *International Dairy Journal, 13*, 707-717.

Degeest, B., & De Vuyst, L. (1999). Indication that the nitrogen source influences both amount and size of exopolysaccharides produced by *Streptococcus thermophilus* LY03 and modelling of the bacterial growth and

exopolysaccharide production in a complex medium. *Applied and Environmental Microbiology*, *65*, 2863-2870.

Degeest, B., & De Vuyst, L. (2000). Correlation of activities of the enzyme α-phosphoglucomutase, UDP-galactose 4-epimerase, and UDP-glucose pyrophosphorylase with exopolysaccharide biosynthesis by *Streptococcus thermophilus* LY3. *Applied and Environmental Microbiology*, *66*, 3519-3527.

Degeest, B., Janssens, B., & De Vuyst, L. (2001). Exopolysaccharide (EPS) biosynthesis by *Lactobacillus sakei* 0-1: production kinetics, enzyme activities and EPS yields. *Journal of Applied Microbiology*, *91*, 470-477.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *38*, 350-356.

Duggan, E., & Waghorne, E. (2003). Long-term evaluation of production protocals for stirred yoghurts produced using two different cultures. *Milchwissenschaft*, *58*, 52-55.

Elfagm, A. A., & Wheelock, J. V. (1977). Heat interaction between α-lactalbumin, β-lactoglobulin and casein in bovine milk. *Journal of Dairy Science*, *61*, 159-163.

Esteves, C. L. C., Lucey, J. A., Wang, T., & Pirest, E. M. V. (2003). Effect of pH on the gelation properties of skim milk gels made from plant coagulants and chymosin. *Journal of Dairy Science*, *86*, 2558-2567.

Faber, E. J., van den Heek, M. J., Kamerling, J. P., & Vliegenthart, J. F. G. (2001). Structure of the exopolysaccharide produced by *Streptococcus thermophilus* S3. *Carbohydrate Research*, *331*, 173-182.

Famelart, M., Tomazewski, J., Piot, M., & Pezennec, S. (2004). Comprehensive study of acid gelation of heated milk with model protein systems. *International Dairy Journal*, *14*, 313-321.

Fox, P. F. (2001). Milk proteins as food ingredients. *International Journal of Dairy Technology*, *54*, 41-55.

Gancel, F., & Novel, G. (1994). Exopolysaccharide production by *Streptococcus salivarius* ssp. *thermophilus* cultures. 1. Conditions of production. *Journal of Dairy Science*, *77*, 685-688.

Gassem, M. A., Schmidt, K. A., & Frank, F. (1997). Exopolysaccharide production from whey lactose by fermentation with *Lactobacillus delbrueckii* ssp. *bulgaricus*. *Journal of Food Science*, *62*, 171-173, 207.

Glauert, A. M., and Lewis, P. R. (1998). *Biological Specimen Preparation for Transmission Electron Microscopy*. London: Portland Press.

Grobben, G. J., Boels, I. C., Sikkema, J., Smith, M. R., & de Bont, J. A. M. (2000). Influence of ions on growth and production of exopolysaccharide by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. *Journal of Dairy Research, 67*, 131-135.

Grobben, G. J., Chin-Joe, I., Kitzen, V. A., Boels, I. C., Boer, F., Sikkema, J., Smith, M. R., & de Bont, J. A. M. (1998). Enhancement of exopolysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 with a simplified defined medium. *Applied and Environmental Microbiology, 64*, 1333-1337.

Grobben, G. J., Smith, M. R., Sikkema, J., & de Bont, J. A. M. (1996). Influence of fructose and glucose on the production of exopolysaccharides and the activitites of enzymes involved in the sugar metabolism and the synthesis of sugar nucleotides in *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. *Applied and Microbiological Biotechnology, 46*, 274-284.

Grobben, G. J., van Casteren, W. H. M., Schols, H. A., Oosterveld, A., Sala, G., Smith, M. R., *et al.* (1997). Analysis of the exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continuous culture on glucose and fructose. *Applied and Microbiological Biotechnology, 48*, 516-521.

Gruter, M., Leeflang, B. R., Kuiper, J., Kamerling, J. P., & Vliegenthart, F. G. (1992). Structure of the exopolysaccharide produced by *Lactobacillus lactis* subspecies *cremoris* H414 grown in a defined medium or skimmed milk. *Carbohydrate Research, 231*, 273-291.

Gruter, M., Leeflang, B. R., Kuiper, J., Kamerling, J. P., & Vliegenthart, F. G. (1993). Structural characterization of the exopolysaccharide produced by *Lactobacillus delbrueckii* subspecies *bulgaricus* RR grown in skimmed milk. *Carbohydrate Research, 239*, 209-226.

Guinee, T. P., Feeney, E. P., Auty, M. A. E., & Fox, P. F. (2002). Effect of pH and calcium concentration on textural and functional properties of mozzarella cheese. *Journal of Dairy Science, 85*, 1655-1669.

Guyomarc'h, F., Queguiner, C., Law, A. J. R., Horne, D. S., & Dalgleish, D. G. (2003). Role of the soluble and micelle-bound heat-induced protein aggregates on network formation in acid skim milk gels. *Journal of Agricultural and Food Chemistry, 51*, 7743-7750.

Guzmán-González, M., Morais, F., & Amigo, L. (2000). Influence of skimmed milk concentrate replacement by dry dairy products in a low-fat set-type yoghurt model system. II: Use of caseinates, co-precipitate and blended dairy powders. *Journal of the Science of Food and Agriculture, 80*, 433-438.

Guzmán-González, M., Morais, F., Ramos, M., & Amigo, L. (1999). Influence of skimmed milk concentrate replacement by dry dairy products in a low fat

set-type yoghurt model system. I: Use of whey protein concentrates, milk protein concentrates and skimmed milk powder. *Journal of the Science of Food and Agriculture*, *79*, 1117-1122.

Halmos, A. L., & Tiu, C. (1981). Liquid foodstuffs exhibiting yield stress and shear-degradability. *Journal of Texture Studies*, *12*, 39-46.

Haque, A., Ridchardson, R. K., & Morris, E. R. (2001). Effect of fermentation temperature on the rheology of set and stirred yogurt. *Food Hydrocolloids*, *15*, 593-602.

Haque, Z., & Kinsella, J. E. (1988). Interaction between heated κ-casein and β-lactoglobulin: predominance of hydrophobic interactions in the initial stages of complex formation. *Journal of Dairy Research*, *55*, 67-80.

Harte, E., Amonte, M., Luedecke, L., Swanson, B. G., & Barbosa-Canovas, G. V. (2002). Yield stress and microstructure of set yoghurt made from high hydrostatic pressure-treated full fat milk. *Journal of Food Science*, *67*, 2245-2250.

Harwalkar, V. R., & Kalab, M. (1980). Milk gel structure. XI. Electron microscopy of glocono-delta-lactone-induced skim milk gels. *Journal of Texture Studies*, *11*, 35-49.

Harwalkar, V. R., & Kalab, M. (1986). Relationship between microstructure and susceptibility to syneresis in yoghurt made from reconstituted nonfat dry milk. *Food Microstructure*, *3*, 287-294.

Hashizume, K., & Sato, T. (1988). Gel-forming characteristics of milk proteins. I. Effect of heat treatment. *Journal of Dairy Science*, *71*, 1439-1446.

Hassan, A. N., & Frank, J. F. (1997). Modification of microstructure and texture of rennet curd by using a capsule-forming non-ropy lactic culture. *Journal of Dairy Research*, *64*, 115-121.

Hassan, A. N., Corredig, M., & Frank, J. F. (2002a). Capsule formation by nonropy starter cultures affects the viscoelastic properties of yogurt during structure formation. *Journal of Dairy Science*, *85*, 716-720.

Hassan, A. N., Frank, J. F., & Elsoda, M. (2003b). Observation of bacterial exopolysaccharide in dairy products using cryo-scanning electron microscopy. *International Dairy Journal*, *13*, 755-762.

Hassan, A. N., Frank, J. F., & Qvist, K. B. (2002b). Direct observation of bacterial exopolysaccharides in dairy products using confocal scanning laser microscopy. *Journal of Dairy Science*, *85*, 1705-1708.

Hassan, A. N., Frank, J. F., & Shalabi, S. I. (2001). Factor affecting capsule size and production by lactic acid bacteria used as dairy starter cultures. *International Journal of Food Microbiology*, *54*, 199-203.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A., & Shalabi, S. I. (1995a). Formation of yogurt microstructure and three-dimensional visualization as determining by confocal scanning laser microscopy. *Journal of Dairy Science*, *78*, 2629-2636.

Hassan, A. N., Frank, J. F., Farmer, M. A., Schmidt, K. A., & Shalabi, S. I. (1995b). Observation of encapsulated lactic acid bacteria using confocal scanning laser microscopy. *Journal of Dairy Science*, *78*, 2624-2628.

Hassan, A. N., Frank, J. F., Schmidt, K. A., & Shalabi, S. I. (1996a). Rheological properties of yogurt made with encapsulated nonropy lactic cultures. *Journal of Dairy Science*, *79*, 2091-2097.

Hassan, A. N., Frank, J. F., Schmid, K. A., & Shalabi, S. I. (1996b). Textural properties of yogurt made with encapsulated nonropy lactic cultures. *Journal of Dairy Science*, *79*, 2098-2103.

Hassan, A. N., Ipsen, R., Janzen, T., & Qvist, K. B. (2003a). Microstructure and rheology of yogurt made with culture differing only in their ability to produce exopolysaccharides. *Journal of Dairy Science*, *86*, 1632-1638.

Heertje, I., Visser, J., & Smits, P. (1985). Structure formation in acid milk gels. *Food Microstructure*, *4*, 267-277.

Herman, B. (1998). *Flurescence Microscopy* (2 ed.). Oxford, UK: BIOS Scientific Publishers Limited.

Hess, S. J., Roberts, R. F., & and Ziegler, G. R. (1997). Rheological properties of nonfact yoghurt stabilized using *Lactobacillus delbrueckii* ssp. *bulgaricus* producing exopolysaccharide or using commercial stabilizer systems. *Journal of Dairy Science*, *80*, 252-263.

Hinrichs, R., & Weisser, J. G. H. (2003). Water-holding capacity and structure of hydrocolloids-gels, WPC-gels and yogurts characterized by means of NMR. *Food Chemistry*, *82*, 155-160.

Hoppert, M. (2003). *Microstructure Techniques in Biotechnology*. Weinheim, Germany: Wiley -VCH GmbH & Co. KGaA.

Horne, D. S. (1998). Casein interactions: casting light on the black boxes, the structure in dairy products. *International Dairy Journal*, *8*, 171-177.

Horne, D. S., & Davidson, C. M. (1990). The use of dynamic light-scattering in monitoring rennet curd formation. *Milchwissenschaft*, *45*, 712-715.

Jaros, D., Rohm, H., Haque, A., Bonaparate, C., & Kneifel, W. (2002). Influence of the starter culture on the relationship between dry matter content and physical properties of set-style yogurt. *Milchwissenschaft*, *57*, 325-328.

Jones, D. S., Lawlor, M. S., & Woolfson, D. A. (2002). Examination of the flow rheological and textural properties of polymer gels compressed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone): rheological and mathematical interpretation of textural parameters. *Journal of Pharmaceutical Science, 91*, 2090-2101.

Kachlany, S. C., Levery, S. B., Kim, J. S., Reuhs, B. L., Lion, L. W., & Ghiorse, W. C. (2001). Structure and carbohydrate analysis of the exopolysaccharide capsule of *Pseudomonas putida* G7. *Environmental Microbiology, 3*, 774-784.

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

Kalab, M., Allan-Wojtas, P., & Phipps-Todd, B. E. (1983). Development of microstructure in set-style nonfat yoghurt - A review. *Food Microstructure, 2*, 51-66.

Kalab, M., Emmons, D. B., & Sargant, A. G. (1975). Milk-gel structure. IV. Microstructure of yoghurts in relation to the presence of thickening agents. *Journal of Dairy Research, 42*, 453-458.

Karleskind, D., Laye, I., Mei, F. I., & Morr, C. V. (1995a). Chemical pretreatment and microfiltration for making delipidized whey protein concentrate. *Journal of Food Science, 60*, 221-226.

Karleskind, D., Laye, I., Mei, F. I., & Morr, C. V. (1995b). Gelation properties of lipid-reduced, and calcium reduced whey protein concentrates. *Journal of Food Science, 60*, 731-737.

Kimmel, S. A., Roberts, R. F., & Ziegler, G. R. (1998). Optimization of exopolysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* RR grown in a semidefined medium. *Applied and Environmental Microbiology, 64*, 659-664.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2004). Comparison of texture of yoghurt made from conventionally treated milk and UHT milk fortified with low-heat skim milk powder. *Journal of Food Science, 69*, e276-e280.

Lillford, P. J. (2002). Mechanisms of fracture in foods. *Food Australia, 54*, 555-561.

Low, D., Ahlgren, J. A., Horne, D., McMahon, D. J., Oberg, C. J., & Broadbent, J. R. (1998). Role of *Streptococcus thermophilus* MR-1C capsular exopolysaccharide in cheese moisture retention. *Applied and Environmental Microbiology, 64*, 2147-2151.

Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids, 15*, 603-608.

Lucey, J. A. (2002). ADSA foundation scholar award: Formation and physical properties of milk protein gels. *Journal of Dairy Science*, *85*, 281-294.

Lucey, J. A. (2004). Cultured dairy products: an overview of their gelation and texture properties. *International Journal of Dairy Technology*, *57*, 77-84.

Lucey, J. A., & Singh, H. (1997). Formation and physical properties of acid milk gels. *Food Research International*, *30*, 529-542.

Lucey, J. A., Gorru, C., O'Kennedy, B., Kalab, M., Tan-Kinita, R., & Fox, P. F. (1996). Effect of acidification and neutralization of milk on some physico-chemical properties of casein micelles. *International Dairy Journal*, *6*, 257-272.

Lucey, J. A., Munro, P. A., & Singh, H. (1998c). Whey separation in acid milk gels made with glucono-delta-lactone: effects of heat treatment and gelation temperature. *Journal of Texture Studies*, *29*, 413-426.

Lucey, J. A., Munro, P. A., & Singh, H. (1998b). Rheological properties and microstructure of acid milk gels as affected by fat content and heat treatment. *Journal of Food Science*, *63*, 660-664.

Lucey, J. A., Tamehana, M., Singh, H., & Munro, P. A. (1998a). Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological properties of acid skim milk gels. *Journal of Dairy Research*, *65*, 555-567.

Lucey, J. A., Tamehana, M., Singh, H., & Munro, P. A. (2001). Effect of heat treatment on the physical properties of milk gels made with both rennet and acid. *International Dairy Journal*, *11*, 559-565.

Lucey, J. A., Teo, C. T., Munro, P. A., & Singh, H. (1997). Rheological properties at small (dynamic) and large (yield) deformations of acid gels made from heated milk. *Journal of Dairy Research*, *64*, 591-600.

Lum, L. S., Dovč, P., & Medrano, J. F. (1997). Polymorphisms of bovine β-lactoglobulin promoter and differences in the binding affinity of activator protein-2 transcription factor. *Journal of Dairy Science*, *80*, 1389-1397.

Lundin, L., Williams, M. A. K., & Foster, T. J. (2003). Phase separation in foods. In B. M. McKenna (Ed.), *Texture in Food Volume 1: Semi-solid foods*. Cambridge, England: Woodhead publishing limited.

Macedo, M. A., Laporte, M. F., & Lacroix, C. (2002). Quantification of exopolysarcharide, lactic acid and lactose concentrations in culture broth by near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, *50*, 1774-1779.

Malin, E. L., Basch, J. j., Shieh, J. J., Sullivan, B. C., & Holsinger, V. H. (1994). Detection of adulteration of buttermilk powder by gel electrophoresis. *Journal of Dairy Science*, 77, 2199-2206.

Marshall, V. M., Cowie, E. N., & Moreton, R. S. (1995). Analysis and production of two exopolysaccharides from *Lactobacillus lactis* subsp. *cremoris* LC330. *Journal of Dairy Research*, *62*, 621-628.

Marshall, V. M., Laws, A. P., Gu, Y., Levander, F., Rådström, P., De Vuyst, L., Degeest, B., Vaningelgem, F., Dunn, H., Elvin, M. (2001). Exopolysaccharide-producing strains of thermophilic lactic acid bacteria cluster into groups according to their EPS structure. *Letters in Applied Microbiology*, *32*, 433-437.

Marshall, V.M., & Rawson, H.L. (1999). Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. *International Journal of Food Science and Technology*, *34*, 137-143.

McKenna, B. M. (2003). Introduction. In B. M. McKenna (Ed.), *Texture in Food:Volume 1: Semi-solid Foods*. Cambridge, England: Woodhead Publishing Limited.

Modler, H. W., Larmond, M. E., Lin, C. S., Froehlich, D., & Emmons, D. B. (1983). Physical and sensory properties of yogurt stabilized with milk proteins. *Journal of Dairy Science*, *66*, 422-429.

Morr, C. V., & Foegeding, E. A. (1990). Composition and functionality of commercial whey and milk protein concentrates and isolates: A status report. *Food Technology, April*, 100, 102-104, 106, 108, 110, 112.

Morris, E.R. (1995). Polysaccharide rheology and in-mouth perception. In A.M. Stephen (Ed.), *Food Polysaccharides and their Applications* (pp. 517-546). New York, Marcel Dekker, Inc.

Mottar, J., Bassier, A., Joniau, M., & Baert, J. (1989). Effect of heat-induced association of whey proteins and casein micelles on yoghurt texture. *Journal of Dairy Science*, *72*, 2247-2256.

Mozzi, F., Rollan, G., Savoy, G., & Font de Valdez, G. (2001). Effect of galactose and glucose on the exopolysaccharide production and the activities of biosynthetic enzymes in *Lactobacillus casei* CRL 87. *Journal of Applied Microbiology*, *91*, 160-167.

Mozzi, F., Savoy de Giori, G., Oliver, G., & Font de Valdez, G. (1996). Exopolysaccharide production by *Lactobacillus casei* in milk under different growth conditions. *Milchwissenschaft*, *51*, 670-673.

Nassar, G., Nongaillard, B., & Noel, Y. (2001). Monitoring of milk gelation using a low-frequency ultrasonic technique. *Journal of Food Engineering*, *48*, 351-359.

Özer, B. H., Stenning, R. A., Grandison, A. S., & Robinson, R. K. (1999). Rheology and microstructure of labneh (concetrated yoghurt). *Journal of Dairy Science*, *82*, 682-689.

Özer, B. H., Grandison, A. S., Robinson, R. K., & Atamer, M. (2003). Effects of lactoperoxidase and hydrogen peroxide on rheological properties of yoghurt. *Journal of Dairy Research*, 70, 227-232.

Parnell-Clunies, E., Kakuda, Y., & Smith, A. K. (1987). Microstructure of yoghurt as affected by heat treatment of milk. *Milchwissenschaft*, *42*, 413-417.

Perry, D. B., McMahon, D. J., & Oberg, C. J. (1997). Effect of exopolysaccharide-producing cultures on moisture retention in low fat mozzarella cheese. *Journal of Dairy Science*, *80*, 799-805.

Petersen, B. L., Dave, R. I., McMahon, D. J., Oberg, C.J., & Broadbent, J. R. (2000). Influence of capsular and ropy exopolysaccharide-producing Streptococcus thermophilus on mozzarella cheese and whey. *Journal of Dairy Science, 83*, 1952-1956.

Petit, C., Grill, J. P., Maazouzi, N., & Marczak, R. (1991). Regulation of polysaccharide formation by *Streptococcus thermophilus* in batch and fed-batch cultures. *Applied and Microbiological Biotechnology*, *36*, 216-221.

Petry, S., Furlan, S., Crepeau, M. J., Cerning, J., & Desmazeaud, M. (2000). Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* grown in a chemically defined medium. *Applied and Environmental Microbiology*, *66*, 3427-3441.

Pham, P. L., Dupont, I., Roy, D., Lapointe, G., & Cerning, J. (2000). Production of exopolysaccharide by *Lactobacillus rhamnosus* R and analysis of its enzymatic degradation during prolonged fermentation. *Applied and Environmental Microbiology*, *66*, 2302-2310.

Pons, M., & Fiszman, A. M. (1996). Review paper: Instrumental texture profile analysis with particular reference to gelled systems. *Journal of Texture Studies*, *27*, 597-624.

Prentice, J. H. (1992). *Dairy rheology: a concise guide*. New York: VCH Publishers, Inc.

Puvanenthiran, A., Williams, R. P. W., & Augustin, M. A. (2002). Structure and visco-elastic properties of set yoghurt with altered casein to whey protein ratios. *International Dairy Journal*, *12*, 383-391.

Ramaswamy, H. S., & Basak, S. (1991). Rheology of stirred yogurts. *Journal of Texture Studies*, *22*, 231-241.

Rawson, H. L., & Marshall, V. M. (1997). Effect of ropy strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* on rheology

of stirred yogurt. *International Journal of Food Science and Technology, 32*, 213-220.

Remuef, F., Mohammed, S., Sodini, I., & Tissier, J. P. (2003). Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. *International Dairy Journal, 13*, 773-782.

Ricciardi, A., Parente, E., Crudele, M. A., Zanetti, F., Scolari, G., & Mannazzu, I. (2002). Exopolysacchride production by *Streptococcus thermophilus* SY: production and preliminary characterization of the polymer. *Journal of Applied Microbiology, 92*, 297-306.

Robijn, G. W., Gutiérrez Gallego, R., van den Berg, D. J. C., Haas, H., Kamerling, J. P., & Vliegenthart, J. F. G. (1996). Structural characterization of the exopolysaccharide produced by *Lactobacillus acidophilus* LMG9433. *Carbohydrate Research, 288*, 203-218.

Robinson, R. K. (1993). *Modern Dairy Technology: Advances in Milk Products* (Vol. 2). London: Elsevier Applied Science.

Rohm, H., & Kovac, A. (1994). Effects of starter cultures on linear viscoelastic and physical properties of yoghurt gels. *Journal of Texture Studies, 25*, 311-329.

Rohm, H., & Schmid, W. (1993). Influence of dry matter fortification on flow properties of yogurt. 1. Evaluation of flow curves. *Milchwissenschaft, 48*, 556-559.

Ruas-Madiedo, P., & Zoon, P. (2003). Effect of exopolysaccharide-producing *Lactococcus lactis* strains and temperature on the permeability of skim milk gels. *Colloids and Surfaces A: Physicochemical Engineering Aspects, 213*, 245-253.

Salvador, S., & Fiszman, S. M. (1998). Textural characteristics and dynamic oscillatory rheology of maturation of milk gelatin gels with low acidity. *Journal of Dairy Science, 81*, 1525-1531.

Savello, P. A., & Dargan, R. A. (1997). Reduced yogurt syneresis using ultrafiltration and very-high temperature heating. *Milchwissenschaft, 52*, 573-577.

Sawyer, W. H. (1969). Complex between β-lactoglobulin and κ-casein. A review. *Journal of Dairy Science, 52*, 1347-1355.

Schkoda, P., Hechler, A., & Hinrichs, J. (2001). Influence of the protein content on structural characteristics of stirred fermented milk. *Milchwissenschaft, 56*, 19-22.

Schorsch, C., Jones, M. G., & Norton, I. T. (1999). Thermodynamic incompatibility and microstructure of milk protein/locust bean gum/sucrose systems. *Food Hydrocolloids*, *13*, 89-99.

Schorsch, C., Wilkins, D. K., Jones, M. G., & Norton, I. T. (2001). Gelation of casein-whey protein mixtures: effects of heating whey proteins alone or in the presence of casein micelles. *Journal of Dairy Research*, *68*, 471-481.

Serp, D., Mueller, M., von Stockar, U., & Marison, I. W. (2002a). Low-temperature electron microscopy for the study of polysaccharide ultrastructure in hydrogels. I. Theoretical and technical considerations. *Biotechnology and Bioenginerring*, *79*, 243-252.

Serp, D., Mueller, M., von Stockar, U., & Marison, I. W. (2002b). Low-temperature electron microscopy for the study of polysaccharide ultrastructure in hydrogels. II. Effect of temperature on the structure of Ca^{2+}-alginate beads. *Biotechnology and Bioengineering*, *79*, 253-259.

Shah, N.P. (2003). Yogurt: the product and its manufacture. In B. Caballero, L. C. Trugo, and P. M. Finglas (Eds.), *Encyclopedia of Food Science and Nutrition*. Vol. 10, 2nd edn. (pp. 6252-6260). UK: Academic press.

Shin, H. S., Lee, J. H., Pestka, J. J., & Ustunol, Z. (2000). Growth and viability of commercial *Bifidobacterium* spp. in skim milk containing oligosaccharides and inulin. *Journal of Food Science*, *65*, 884-887.

Singh, H. (2004). Heat stability of milk. *International Journal of Dairy Technology*, *57*, 111-119.

Singh, H., & Creamer, L. K. (1991a). Aggregation and dissociation of milk protein complexes in heated reconstituted concentrated skim milks. *Journal of Food Science*, *56*, 238-246.

Singh, H., & Creamer, L. K. (1991b). Denaturation, aggregation and heat stability of milk protein during the manufacture of skim milk powder. *Journal of Dairy Research*, *58*, 269-283.

Singh, H., & Fox, P. F. (1985a). Heat stability of milk: the mechanism of stabilization by formaldehyde. *Journal of Dairy Research*, *52*, 65-76.

Singh, H., & Fox, P. F. (1985b). Heat stability of milk: pH-dependent dissociation of micellar κ-casein on heating milk at ultrahigh temperatures. *Journal of Dairy Research*, *52*, 529-538.

Singh, H., & Fox, P. F. (1986). Heat stability of milk: further studies of the pH-dependent dissociation of micellar κ-casein. *Journal of Dairy Research*, *53*, 237-248.

Singh, H., Roberts, M. S., Munro, P. A., & Tet Teo, C. (1996). Acid-induced dissociation of casein micelles in milk: effects of heat treatment. *Journal of Dairy Science*, *79*, 1340-1346.

Skriver, A., Holstborg, J., & Qvist, K. B. (1999). Relation between sensory texture analysis and rheological properties of stirred yogurt. *Journal of Dairy Research*, *66*, 609-618.

Steffe, J. F. (1992). *Rheological Methods in Food Processing Engineering.* Michigan: Freeman Press.

Sworn, G. (2004). Hydrocolloid thickeners and their application. In P.A. Williams, & G.O. Phillips (Eds.), *Gums and Stabilisers for the Food Industry 12* (pp. 12-22), UK, The Royal Society of Chemistry.

Szczesniak, A. S. (1963). Classification of textural characteristics. *Journal of Food Science*, *28*, 385-389.

Takeuchi, K., & Frank, J. F. (2001). Confocal microscopy and microbial viability detection for food research. *Journal of Food Protection*, *64*, 2088-2102.

Tamime, A. Y., & Deeth, H. C. (1980). Yogurt: technology and biochemistry. *Journal of Food Protection*, *43*, 939-977.

Tamime, A. Y., & Robinson, R. K. (1985). *Yoghurt: Science and Technology.* Oxford: Pergamon Press.

Tamime, A. Y., & Robinson, R. K. (1999). *Yoghurt: Science and Technology* (2 ed.): CRC Press.

Tamime, A. Y., Barantes, E., & Sword, A. (1996). The effect of starch based fat substitutes on the microstructure of set-style yoghurt made from reconstituted skimmed milk powder. *Journal of the Society of Dairy Technology*, *49*, 1-10.

Tamime, A. Y., Kalab, M., & Davies, G. (1984). Microstructure of set-style yoghurt manufactured from cow's milk fortified by various methods. *Food Microstructure*, *3*, 83-92.

Tamime, A. Y., Kalab, M., Davies, G., & Mahdi, H. A. (1991). Microstructure and firmness of labneh (high solids yoghurt) made from cow's, goat's and sheep's milks by a traditional method or ultrafiltration. *Food Structure*, *10*, 37-44.

Teggatz, J. A., & Morris, H. A. (1990). Changes in the rheology and microstructure of ropy yoghurt during shearing. *Food Structure*, *9*, 133-138.

Thaiudom, S., & Goff, H. D. (2003). Effect of κ-carageenan on milk protein polysaccharide mixtures. *International Dairy Journal*, *13*, 763-771.

The Australian Food Standard Code: *Standard 2.5.3: Fermented milk products.* (2004). Retrieved 1/11/2004, 2004, from http://www/foodstandards.gov.au/_srfiles/Standard_2_5_3_Fermented_milk_v64.pdf

Toba, T., Uemura, H., & Itoh, T. (1992). A new method for the quantitative determination of microbial extracellular polysaccharide production using a disposable ultrafilter membrane units. *Letters in Applied Microbiology, 14*, 30-32.

Toba, T., Uemura, H., Mukai, T., Fujii, T., Itoh, T., & Adachi, S. (1991). A new fermented milk using capsular polysaccharide-producing *Lactobacillus kefiranofaciens* isolated from kefir grains. *Journal of Dairy Research, 58*, 497-502.

Tolstoguzov, V. B. (1997). Protein-polysaccharide interactions. In S. Damodaran & A. Paraf (Eds.), *Food Proteins and their Applications*. USA: Marcel Dekker.

Tolstoguzov, V. B. (1998). Physico-chemical modification of food proteins:Food emulsions. *Nahrung, 42*, 205-209.

Tolstoguzov, V. B. (2000). Phase behaviour of molecular components in biological and food systems. *Nahrung, 44*, 299-308.

Torino, M. I., Taranto, M. P., Sesma, F., & Font de Valdez, G. (2001). Heterofermentative pattern and exopolysaccharide production by *Lactobacillus helveticus* ATCC 15807 in response to environmental pH. *Journal of Applied Microbiology, 91*, 846-852.

Tuinier, R., & de Kruif, C. G. (1999). Phase behavious of casein micelles/exocellular polysaccharide mixtures: experiment an theory. *Journal of Chemical Physics*, 110, 9296-9304.

Tunick, M. H. (2000). Rheology of dairy foods that gel, stretch, and fracture. *Journal of Dairy Science, 83*, 1892-1898.

van de Velde, F., van Riel, J., & Hans Tromp, R. (2002). Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). *Journal of the Science of Food and Agriculture, 82*, 1528-1536.

van de Velde, F., Weinbreck, F., Edelman, M. W., van der Linden, E., & Hans Tromp, R. (2003). Visulaisation of biopolymer mixtures using confocal scanning laser microscopy (CSLM) and covalent labeling techniques. *Colloids and Surface B: Biointerfaces, 31*, 159-168.

van den Berg, D. J. C., Robijn, G. W., Janssen, A. C., Giuseppin, M. L. F., Vreeker, R., Kamerling, J. P., Vliegenthart, J.F.G., Ledeboer, A.M., Verrips, C.T. (1995). Production of a novel extracellular polysaccharide by

Lactobacillus sake 0-1 and characterization of the polysaccharide. *Applied and Environmental Microbiology, 61*, 2840-2844.

Van Geel-Schutten, G. H., Faber, E. J., Smit, E., Bonting, K., Smith, M. R., Ten Brink, B., Kamerling, J.P., Vliegenthart, J.F.G., Dijkhuizen, L*et al.* (1999). Biochemical and structural characterization of the glucan and fructan exopolysaccharides synthesized by the *Lactobacillus reuteri* wild-type strain and by mutant strains. *Applied and Environmental Microbiology, 65*, 3008-3014.

Van Geel-Schutten, G. H., Flesch, F., ten Brink, B., Smith, M. R., & Dijkhuizen, L. (1998). Screening and characterization of *Lactobacillus* strains producing large amounts of exopolysaccharides. *Applied and Microbiological Biotechnology, 50*, 697-703.

van Vliet, T., & Luyten, H. (1995). Fracture Machanics of Solid Foods. In E. Dickinson (Ed.), *New Physico-chemical Techniques for the Characterization of Complex Food Systems*. London: Blackie Academic & Professional.

Vasbinder, A. J., & de Kruif, C. G. (2003). Casein-whey protein interactions in heated milk: the influence of pH. *International Dairy Journal, 13*, 669-677.

Vasbinder, A. J., van de Velde, F., & de Kruif, C. G. (2004). Gelation of casein-whey protein mixtures. *Journal of Dairy Science, 87*, 1167-1176.

Vodovotz, Y., Vittadini, E., Coupland, J., McClements, D. J., & Chinachoti, P. (1996). Bridging the gap: Use of confocal microscopy in food research. *Food Technology*, 74, 76-82.

Wacher-Rodate, C., Galvan, M. V., Farres, A., Gallardo, F., Marshall, V. M., & Garcia-Garlibay, M. (1993). Yoghurt production from reconstituted skim milk powders using different polymer and non-polymer forming starter cultures. *Journal of Dairy Research, 60*, 247-254.

Walstra, P., & Jenness, R. (1984). *Dairy Chemistry and Physics*. New York: John Wiley & Sons, Inc.

Walstra, P., Geurts, T. J., Noomen, A., Jellema, A., & van Boekel, M. A. J. S. (1999). *Dairy Technology : Principles of Milk Properties and Processes*: Marcel Dekker, Inc.

Walther, P. (2003). Recent progress in freeze-fracturing of high-pressure frozen samples. *Journal of Microscopy, 212*, 34-43.

Walzem, R. L., Dillard, C. J., & German, J. B. (2002). Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking. *Critical Reviews in Food Science and Nutrition, 42*, 354-375.

Yang, Z., Staaf, M., Huttunen, E., & Widmalm, G. (2000). Structure of a viscous exopolysaccharide produced by *Lactobacillus helveticus* K16. *Carbohydrate Research*, *329*, 465-469.

Zadow, J. G. (1992). *Whey and Lactose Processing*. London: Elsevier Applied Science.

Zisu, B., & Shah, N. P. (2003). Effects of pH, temperature, supplementation with whey protein concentrate, and adjunct cultures on the production of exopolysacccharides by *Streptococcus thermophilus* 1275. *Journal of Dairy Science*, *86*, 3405-3415.

Zisu, B., & Shah, N. P. (2004). Textural and functional changes in low fat mozzarella cheeses in relation to proteolysis and microstructure as influenced by the use of fat replacers, pre-acidification and EPS starter. *International Dairy Journal*. (in press).

Appendix

Appendix A: The reference profiles of milk proteins in native-PAGE gel from Özer *et al.* (2003)

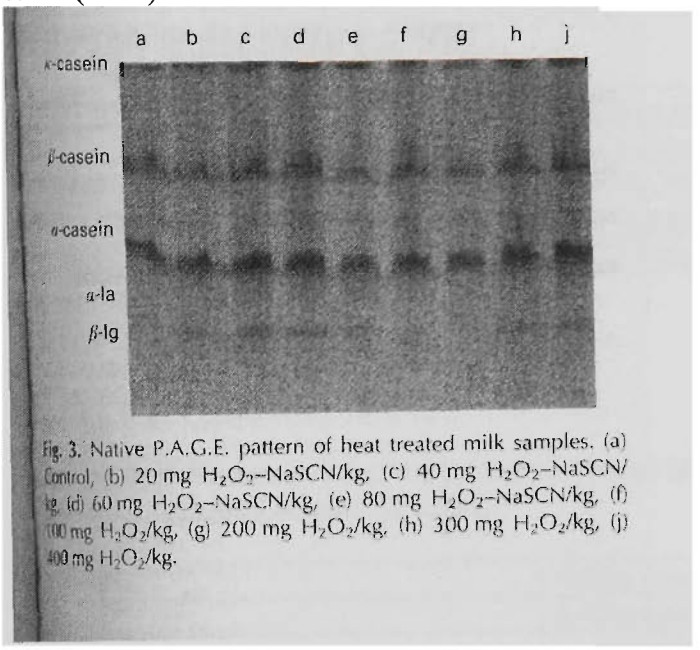

Fig. 3. Native P.A.G.E. pattern of heat treated milk samples. (a) Control, (b) 20 mg H_2O_2–NaSCN/kg, (c) 40 mg H_2O_2–NaSCN/kg, (d) 60 mg H_2O_2–NaSCN/kg, (e) 80 mg H_2O_2–NaSCN/kg, (f) 100 mg H_2O_2/kg, (g) 200 mg H_2O_2/kg, (h) 300 mg H_2O_2/kg, (j) 400 mg H_2O_2/kg.

Appendix B: The reference profiles of milk proteins in SDS-PAGE gel from Malin *et al.* (1994)

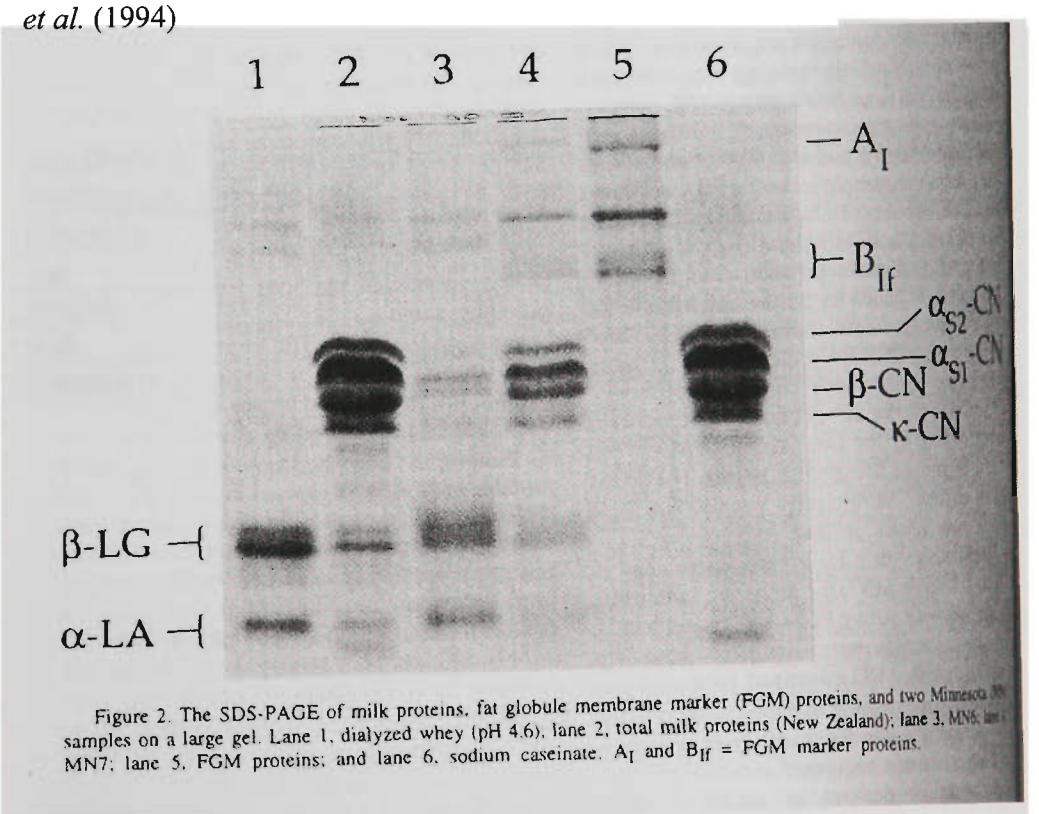

Figure 2. The SDS-PAGE of milk proteins, fat globule membrane marker (FGM) proteins, and two Minnesota [illegible] samples on a large gel. Lane 1, dialyzed whey (pH 4.6), lane 2, total milk proteins (New Zealand); lane 3, MN6; lane 4, MN7; lane 5, FGM proteins; and lane 6, sodium caseinate. A_I and B_{If} = FGM marker proteins.

Appendix C: Composition of SMP from Murray Goulbourn Co-operative Co. Ltd., Brunswick, Australia (January, 2003)

Moisture	4.00% (maximum)
Fat	1.25% (maximum)
Protein	34.00% (minimum)
Ash	8.20% (maximum)
Lactose	66.80% (maximum)

Appendix D: Composition of WPC 80 from United Milk Tasmania Ltd., Spreyton, Tasmania, Australia (December, 2002)

Moisture	4.00% (maximum)
Fat	6.00% (maximum)
Protein	76.00% (maximum)
Ash	3.00% (maximum)
Lactose	11.00% (maximum)

Appendix E: Composition of WPC 392 from New Zealand Milk Product Ltd., Wellingtion, New Zealand

Moisture	4.40% (maximum)
Fat	6.50% (maximum)
Protein	80.40% (maximum)
Ash	3.00% (maximum)
Lactose	5.50% (maximum)