Polysaccharide and exopolysaccharide utilisation in processed and natural cheese systems

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Nilesh Saija

Fonterra Innovation, Palmerston North,

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Appendix D

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Abstract

Several polysaccharides are of interest in dairy products because of their ability to bind water and other components of the food systems, often leading to major changes in their functional properties. This work aimed to measure and understand the effects of specific polysaccharides in cheeses on the rheological properties related to functionality. The following polysaccharide–cheese systems were used: the microbial polysaccharides xanthan gum (xanthan), high acyl gellan gum (Gellan-H) and low acyl gellan gum (Gellan-L) in processed cheese and an exopolysaccharide (EPS) from a lactic acid bacteria (LAB) in very low fat Mozzarella cheese. Locust bean gum (LBG) was also used with xanthan.

Model processed cheese using rennet casein and soya oil was developed on a small scale (30 g) using a controlled temperature, stirrer speed and time of mixing. Initially, lactose content, moisture losses and sample preparation were standardised to lower the variability in rheological measurements. The effects of xanthan, xanthan+LBG, Gellan-H and Gellan-L on the rheology of the processed cheese were studied. As the polysaccharide concentration increased from 0.0 to 2.0% (wt/wt), the fracture stress (firmness) increased whereas the fracture strain (longness) decreased for gellan gum and the effect depended on the polysaccharide. The crossover temperature (where $G' = G''$), an indicator of viscoelasticity, was increased dramatically by these polysaccharides. Confocal laser scanning microscopy showed polysaccharide clusters in the protein matrix for Gellan-H, xanthan and xanthan+LBG but not very distinct clusters for Gellan-L.

The effect of Gellan-H and Gellan-L on the water mobility and spreading properties of model processed cheese was investigated. Spreading properties were measured by elongational viscosity, and water mobility was measured by nuclear magnetic resonance (NMR) relaxometry. The NMR data revealed that both polysaccharides significantly reduced the water mobility in the cheese but that the reduction was greater for Gellan-H. The rheology data showed that the addition of polysaccharide increased the elongational viscosity for processed cheese containing both Gellan-H and Gellan-L.

In situ exopolysaccharide (EPS)-producing cultures are widely used to improve moisture retention and texture in low fat cheese manufacture but are limited by a low level of EPS production. The aim of this study was to develop an "all-dairy" ingredient with an increased content of EPS and greater functionality of the EPS for dairy applications such as Mozzarella cheese. An EPS-producing *Streptococcus thermophilus* was chosen and its growth was optimised for the development of the bioingredient. The fermented biomass was harvested at the end of the exponential phase and freeze dried. The reduced viable cell count and the retention of ropiness of the powder from the drying process enabled a higher level of EPS inoculation in a preliminary Mozzarella cheese manufacturing trial.

Pilot-scale very low fat model Mozzarella cheese was manufactured with and without added EPS powder and in situ EPS culture (EPS-C). Large strain rheology, elongational viscosity, melt and NMR relaxometry were used to determine the effects of the in situ and added EPS on the functionality of the cheeses. Cheeses made with the EPS ingredient (EPS-P) retained the highest moisture content (66.0%) without any visible

serum exudation. The cheeses made with non-EPS-producing cultures (CTR) and EPS-C had lower moisture contents of 57.5 and 60.2% respectively. Such higher moisture retention of the cheeses made with EPS-P was reflected in the rheological properties of the final cheeses. The cheeses made with EPS-P exhibited greater meltability, lower elongational viscosity and lower modulus of deformability (stiffness) and fracture stress than those made with EPS-C and CTR.

Future work to develop this area of the functional effects of the addition of polysaccharides to cheese would include protein–polysaccharide interactions and better definition of the water affinity of polysaccharide compared with that of protein.

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Chapter 1 Introduction

1.1 Problem definition

Fonterra companies make more than 30,000 tonnes of processed cheese and 300,000 tonnes of natural cheese per annum. In addition, they make more than 200,000 tonnes of raw materials for processing in the form of cheese, butter, anhydrous milkfat (AMF) and powders. Thus, both processed cheese and natural cheese are a very important part of the business, with significant research and development effort devoted to gaining a better understanding and therefore control of their manufacture.

Cheese can be regarded as a complex emulsion comprising water, protein, fat and minerals. As protein is often the most expensive major component in cheese, a reduction in the protein content of cheese can offer economic benefit to the manufacture, as well as potentially lower cost to the consumer. However, as protein provides the structural backbone of cheese, reducing the protein content can seriously compromise the product quality through softening and loss of some functional properties. The protein and moisture contents of cheese are important in terms of flavour delivery and textural attributes (such as hardness) and other attributes including spreadability and meltability. Thus, removing protein must be offset by adding another agent that is capable of contributing to the structure of the product. Polysaccharides (and exopolysaccharide-secreting cultures) can be used in this capacity.

1.2 Proposed solution

The application of microbial polysaccharides, such as xanthan gum, gellan gum and lactic acid bacteria (LAB) exopolysaccharide (EPS) produced from waste dairy stream media, is a recent area of research in the New Zealand dairy industry. Processed cheese and natural cheese have recently been identified as potential areas of application for such polysaccharides, and the research has been more focused on gellan gum. To date, only limited research has focused on the use of such polysaccharides in New Zealand dairy products. Moreover, many technological and functional problems related to the use of such polysaccharides in cheese have yet to be resolved.

Therefore, the aim of this project was to contribute to the understanding of the behaviour of polysaccharides such as xanthan gum and gellan gum in processed cheese formulations and of LAB EPS in Mozzarella cheese. This project investigated the use of commercially available polysaccharides in processed cheese and in-house-produced LAB microbial polysaccharides in Mozzarella cheese. Because of their unique moisture retention properties and their effects on cheese rheology, these polysaccharides have the potential to improve some functional properties of lower protein cheese and dairy products, including their textural and slicing properties.

1.3 Commercial significance

Polysaccharides have the potential to reduce syneresis in the cheese manufacturing process by retaining more moisture. Another focus in this manufacturing process is reducing the cost of a formulation. Fat and protein are the most expensive ingredients (protein being more expensive than fat) and moisture is the cheapest ingredient in cheese. Incorporation of more water has the potential to increase yield (reduce the

protein concentration). Thus polysaccharides may offer significant benefits in terms of reductions in the processing time and the formulation cost. In some cases, improved functionality may be obtained by the addition of polysaccharide to cheese with no significant reduction in the protein content, e.g. the common practice of adding polysaccharide to cream cheese to reduce syneresis.

1.4 Thesis objectives

The first goal was to study selected commercially available polysaccharides for their potential use in a model processed cheese. This included microstructural analysis (confocal laser microscopy and transmission electron microscopy) to characterise structural changes. Rheological and fracture properties related to functional properties were analysed to characterise functional changes (textural, spreading and melting properties). The analysis helped to establish an understanding of the behaviour of polysaccharides in making processed cheese.

The second phase aimed to study the effect of LAB EPS on model Mozzarella cheese. This involved producing a functional EPS ingredient for application in Mozzarella cheese and investigating the effect of the addition of LAB EPS on the textural and functional characteristics of a targeted model Mozzarella cheese.

The specific objectives of this research were as follows.

1. Establish a model processed cheese formulation for the study.
2. Using the model cheese system, analyse the specific interactions between the rheological and microstructural properties of formulations containing

polysaccharides, to determine the associations of the individual components: polysaccharide, fat, protein and water.

3. Include in this analysis the relative water affinities of polysaccharides and proteins and the basic concepts of phase separation. To limit the scope to be appropriate for this size of work, exclude the large area of protein–polysaccharide bonding chemistry.
4. Develop a LAB EPS ingredient for application in a model Mozzarella cheese.
5. Determine the influence of the developed LAB EPS ingredient on the texture of Mozzarella cheese.

1.5 Thesis structure

The thesis is structured in the traditional manner, with a literature review, followed by a series of chapters to address each of the above objectives. Each chapter also contains literature relevant to the area of investigation; some of this literature may not be addressed in the initial literature review. Each chapter is outlined briefly below.

Chapter 1 introduces the topic of study, by defining the problem and explaining the thesis objectives, as well as the economic benefit, and provides a brief outline of the thesis.

Chapter 2 gives a general review of the literature and discusses processed cheese, xanthan gum, gellan gum and LAB EPS.

Chapter 3 outlines the common materials and methods used in the individual chapters. Each chapter also contains materials and methods relevant to the area of work done.

Chapter 4 establishes a model processed cheese for further experimental work. It defines the experimental and analytical limits of the developed model cheese containing polysaccharides.

Chapter 5 uses the model cheese developed in Chapter 4 to study the relevant functional properties of a model processed cheese containing a blend of xanthan gum and locus bean gum (LBG). It compares xanthan gum and a blend of xanthan gum and LBG and measures the synergistic effect of the blend.

Chapter 6 studies the effect of two forms of gellan gum on the functional properties of the model processed cheese. It quantifies the effects of high acyl gellan gum (Gellan-H) and low acyl gellan gum (Gellan-L) on the emulsion, microstructural and rheological properties of the model processed cheese.

Chapter 7 describes the effects of Gellan-L and Gellan-H on the rheological properties related to spreadability, including elongational viscosity, and also the water mobility and the microstructure of the model processed cheese.

Chapter 8 describes the development of a bioingredient with a high level of functional EPS for inclusion in cheese, using dairy protein sources and a high EPS-yielding *Streptococcus thermophilus* strain. It optimises the yield of EPS by manipulating the medium composition and process control, with the medium subsequently being freeze dried to convert it into the ingredient.

Chapter 9 describes a pilot-scale study on the manufacture of Mozzarella cheese containing the developed bioingredient. It also studies the effect of such bioingredients on the functionality of Mozzarella cheeses.

Chapter 10 summarises the key conclusions of the thesis. It also discusses practical findings that have application to industry and includes recommendations for further work.

Chapter 2 Literature review

2.1 Processed cheese

Processed cheese is a dairy emulsion produced by blending natural cheese, an emulsifying agent and other dairy and non-dairy ingredients followed by heating with continuous mixing (Caric & Kaláb, 1993; Meyer, 1973). Processed cheese was initially manufactured without an emulsifying agent; the first attempt was as early as 1895 (Caric & Kaláb, 1993). However, production of processed cheese with citrate emulsifying salts was invented in 1911, in Switzerland, by Walter Gerber and Fritz Stettler of Gerber and Co. (Kapoor & Metzger, 2008). A few years later in 1917, processed cheese was developed independently by Kraft, who processed Cheddar cheese with citrates and orthophosphates to increase its shelf life (Caric & Kaláb, 1993; Kapoor & Metzger, 2008). When processed cheese was first invented, no-one was able to foresee the significance of the breakthrough, or the many facets of its potential for further development. The advantages associated with processed cheese production fall into four major categories (Berger, Klostermeyer, Merkenich, & Uhlmann, 1993; Caric & Kaláb, 1993).

(1) Advantages from the point of view of the dairy industry and in economic terms such as better keeping quality, with fewer apparent changes during prolonged storage.

(2) Advantages from the point of view of production technology and in terms of transport and storage, such as reduced refrigeration costs during storage and transport, which are especially important in hot climates.

(3) Versatile properties and a wide range of potential uses.

a. Great diversity of type and intensity of flavour, e.g. from mild to sharp, native cheese flavour or specific spices.
b. Suitability for home use as well as for snack restaurants, e.g. in cheese burgers, hot sandwiches, spreads and dips, for fast foods.

(4) Virtually unlimited possibilities for combinations of raw materials and added ingredients in the development of new varieties of processed cheese.

Initial successes in processed cheese were followed by numerous patents for different dairy ingredients and later for the inclusion of food ingredients other than cheese (Guinee, Caric, & Kaláb, 2004). As shown in Table 1, in addition to natural cheeses, other dairy and non-dairy ingredients may be included in the blend. The inclusion of dairy and non-dairy components makes it possible to produce processed cheeses differing in consistency, flavour, size and shape (Caric & Kaláb, 1993).

Table 1 Main functions and effects of dairy and non-dairy ingredients in processed cheese products { adapted from (Guinee et al., 2004)}

Ingredient type	Main function/effect	Examples
Milk fat	-Standardisation of composition -Contributes to flavour, texture and cooking characteristics	Cream, anhydrous milk fat, dehydrated cream, butter
Milk proteins	-Standardisation of composition -Assist in "creaming" (thickening of blend during manufacture) and formation of product -Contribute to texture and rheological (e.g. fracturability, hardness) and cooking properties	Caseins, caseinates, whey proteins, milk protein concentrate (ultrafiltered milk and microfiltered milk preparations), co-precipitates, skim milk powder
Lactose	-Low cost filler; may affect texture	Whey powder, skim milk powder, whey permeate powder
Cheese base	-Substitute for young cheese -Similar in behaviour to milk proteins, it contributes to thickening during manufacture, texture and cooking properties	Typically, high dry matter milk solids (~ 60% w/w) prepared by evaporation of milk ultrafiltrates to which starter culture and rennet have been added
Stabilisers	-Assist control of the pH of the final product -Impart desired texture and cooking characteristics	-Emulsifying salts: sodium phosphates and sodium citrates -Hydrocolloids: carob bean gum, guar gum, xanthan gum, sodium carboxymethylcellulose, carrageenan
Acidifying agents	-Assist control of the pH of the final product	Food-grade organic acids, e.g. lactic, acetic, citric, phosphoric
Flavourings	-Impart flavour to processed cheese foods and spreads, especially those in which significant proportions of young cheese, cheese base or milk proteins are used	Enzyme-modified cheese, starter distillate, wood smoke extracts, spices
Flavour enhancers Condiments	-Accentuate flavour -Affect appearance, flavour and texture, and product differentiation	-NaCl, yeast extract -Sterile preparation of meat, fish, vegetables, nuts and/or fruits
Sweetening agents	-Increase sweetness, especially in products targeted for young children	Sucrose, dextrose, corn syrup, hydrolysed lactose
Colours	-Impart desired colour	Annato, paprika, artificial colours
Preservatives	-Retard mould growth; prolong shelf life	Nisin, potassium sorbate, calcium or sodium propionate

2.1.1 Main groups

Characterisation of processed cheese can be done essentially by composition, water content and consistency. According to these criteria, four main groups may be distinguished, as shown in Table 2.

Table 2 Some characteristics of processed cheese types {adapted from (Caric & Kaláb, 1993)}

Type of product	Ingredients	Cooking temperature (°C)	Composition	pH
Processed cheese block	Natural cheese, emulsifiers, NaCl, colouring	71–80	Moisture and fat contents correspond to the legal limits for natural cheese	5.6–5.8
Processed cheese food	Same as above plus optional ingredients such as milk, skim milk, whey, cream, albumin, skim milk cheese, organic acids	74–85	⩽ 44% moisture, < 23% fat	5.2–5.7
Processed cheese spread	Same as processed cheese food plus gums for water retention	88–91	⩾ 44% and ⩽ 60% moisture	5.2
Processed cheese analogue	Sodium caseinate, calcium caseinate, suitable vegetable fats, emulsifying agent, salt, artificial flavour	85–95 As for processed cheese food	⩽ 55% moisture As for processed cheese food	5.7–6.0

2.2 Legislation in Australia and New Zealand

The present Food Standards Australia New Zealand (FSANZ) code addresses cheese, including processed cheese briefly, in Standard 2.5.4. The joint FSANZ standard (2011) defines processed cheese as a product that is manufactured from cheese and products obtained from milk; it is heated and melted, with or without added emulsifying salts, to form a homogeneous mass. No other compositional requirements are given in the present standard. Section 1.3.1 of the Food Code, Schedule 5, addresses food additives, as shown in Table 3. Therefore, wide ranges for the polysaccharide levels were used for experimental work in this study. Polysaccharides fit as thickeners and stabilisers.

Table 3 The 21 additive functional classes listed in the FSANZ Food Code, Section 1.3.1, Schedule 5: adapted from FSANZ (2011)

Acidity regulator [a]	Foaming agent
Anti-caking agent	Gelling agent
Antioxidant	Glazing agent
Bulking agent	Humectant
Colour	Intense sweetener
Colour fixative	Preservative
Emulsifier [b]	Propellent
Firming agent	Raising agent
Flavour enhancer	Sequestrant
Flavouring	Stabilisers
	Thickener

[a] Acid is a subcategory.

[b] Emulsifying salt is a subcategory.

Table 4 Typical compositions of some New Zealand commercial processed cheeses (adapted from Fonterra Product Bulletins)

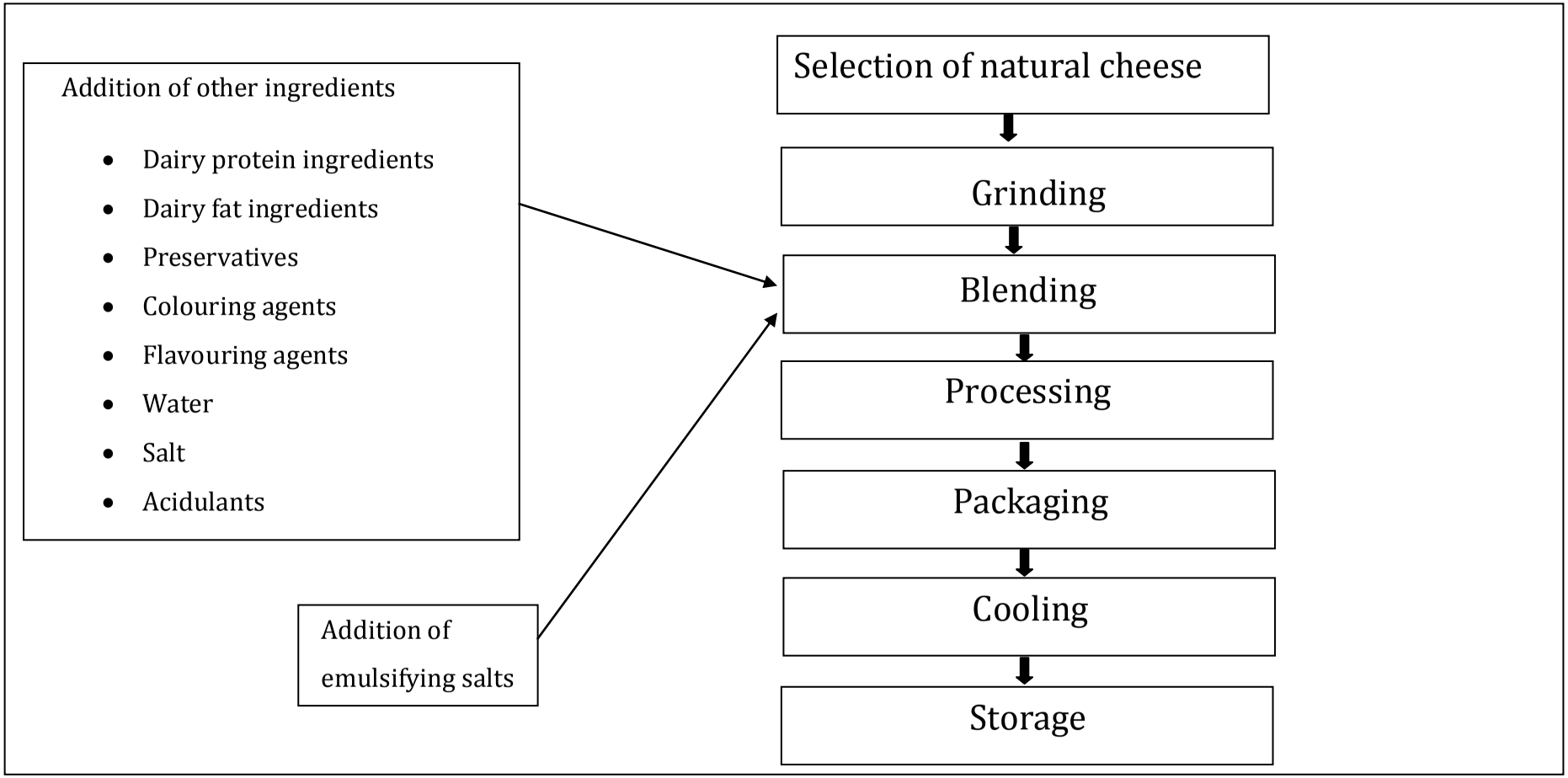
Type of commercial processed cheese	Typical composition (%)			
	Fat	Protein	Moisture	Intact casein [a]
Spreads	25–30	10–15	52–55	< 5.0–5.5
Slice on slice/ Individually wrapped slices	26–28	20–21	41–43	6.0–9.0
Blocks	30–38	20–27	34–44	9.0–11.0

[a] From New Zealand Dairy Research Institute technical reports.

2.3 Processed cheese principles and manufacture

As shown in Figure 1, the processed cheese manufacturing procedure involves the operations performed in sequence. Caric & Kaláb (1993), Berger et al. (1993) and Zehren & Nusbaum (1992a) have described the steps of processed cheese manufacture extensively.

Figure 1 The manufacturing procedure for processed cheese consists of operations performed in the following order {adapted from (Fox, O'Connor, McSweeney, Guinee, & O'Brien, 1996; Guinee, 1987; Kapoor & Metzger, 2008)}



2.3.1 Selection of natural cheese

Selection of the right natural cheese is a very important part of successful processed cheese manufacture. The characteristics of the natural cheese used to manufacture processed cheese have a major influence on its characteristics. In selecting natural cheeses on the basis of their suitability for a required processed cheese, two major aspects need to be considered: (1) type of natural cheese; (2) maturity of the natural cheese.

2.3.1.1 Type of natural cheese

Any type of cheese can easily be used for processed cheese manufacture. The quality of processed cheese is largely determined by the quality of the natural cheese used. Therefore, the natural cheeses should be selected with the final quality of the processed cheese in mind (Caric & Kaláb, 1993; Zehren & Nusbaum, 1992a). However, in practice, the selection of the natural cheese is largely based on economic considerations, plant technology and consistent supply of good quality cheese that can be processed without any great difficulty (Berger et al., 1993).

"Cheddar cheese" can be processed without any complications and causes hardly any problems during processing and in the resultant final quality of the processed cheese (Berger et al., 1993). Therefore, in New Zealand, Cheddar cheese is one of the most popular natural cheeses that is used as a raw material in processed cheese manufacture. However, other popular cheese varieties such as Emmental, Gruyere, Gouda, Edam, Tilsit and Provolone are widely used in processed cheese manufacture worldwide (Berger et al., 1993).

2.3.1.2 Maturity of the natural cheese

Selection of the maturity of the natural cheese is determined by the intended use of the processed cheese and consumer preference. For example, a highly flavoured processed cheese would require a larger portion of mature cheese in the blend than a lower flavoured processed cheese (Zehren & Nusbaum, 1992a).

The other concept of maturity is aligned with the body and texture profile of the final product, which is governed mainly by the relative casein content. As noted by (Berger et al., 1993), each type of processed cheese is characterised by a characteristic texture, which is heavily dependent on the level of intact protein needed to stabilise the dispersion and the emulsion. Therefore, selection of the maturity of the natural cheese for blend preparation is critical in defining the final chemical, textural and functional characteristics of processed cheeses such as block processed cheeses, slices, spreads, processed cheese preparations and canned processed cheeses. Combinations of young, medium mature and mature natural cheeses are shown in Table 5.

Table 5 A general guideline for selection of young, medium mature and mature natural cheeses in processed cheese manufacture {adapted from (Berger et al., 1993)}

Processed cheese	Young (%)	Medium mature (%)	Mature (%)
Block processed cheese, long structure	60	30	10
Block processed cheese, short structure	40	40	20
Toasting blocks (approximately 45% fat in dry matter, 53% dry matter)	80	20	–
Toasting slices (approximately 45% fat in dry matter, 53% dry matter)	70	30	–
Toasting slices (approximately 50% fat in dry matter, 60% dry matter)	100	–	–
Processed cheese slices (to be consumed cold)	60	30	10
Small blocks, smoked-sausage-type cheeses	50	40	10
Sliceable portion	50	40	10
Preserved processed cheese in cans	40	50	10
Spreadable processed cheese (20–45% fat in dry matter)	30	50	20
Processed cheese preparations (50–65% fat in dry matter)	60	40	–
Processed cheese preparations with flavourings	60	40	–

2.3.2 Grinding

This operation enables better contact between the emulsifying agent and the blend ingredients during processing (Zehren & Nusbaum, 1992a).

2.3.3 Blending

According to Zehren & Nusbaum (1992a), blending is "bringing together in a blend or batch cheese from two or more vat lots". The major objective of blending is to produce uniform flavour, body, texture, composition, colour and other attributes of processed cheese, which is accomplished by the skilful blending of the cheese ingredients. The requirements of the blend are determined by the intended use of the processed cheese and consumer preference.

2.3.3.1 Addition of emulsifying salts

Adding an emulsifying agent is the last step in preparing the blend for processing (Caric & Kaláb, 1993). The emulsifying agent is a major ingredient that is responsible for the unique features of processed cheese. More detail about its role is given in Section 2.4.3.

2.3.4 Processing

Processing means heat treatment of the blend by direct or indirect steam, under a partial vacuum and with constant agitation (Caric & Kaláb, 1993). Processed cheese manufacturers use various types of cooking devices that differ on the basis of their mode of production (batch or continuous), the type of mixing and agitating system involved, and the type and mechanism of heating (Berger et al., 1993; Meyer, 1973;

Zehren & Nusbaum, 1992a). Two common types of processing devices used are: (1) batch cookers; (2) continuous cookers.

2.3.4.1 Batch cookers

There are two basic types of batch cooking device: Blentech cookers use single/twin screw augers; the Stephan cooker is a high speed cutting blade device. The single/twin screw cookers operate at low mixing speeds, ranging from 50 to 150 rev/min, with product temperatures ranging from 70 to 90°C and with manufacturing times from 3 to 7 min. The high speed cutting blade type cookers operate at 1500–3000 rev/min at temperatures of above 95°C for 2–5 min (Kapoor & Metzger, 2008).

2.3.4.2 Continuous cookers

Continuous cookers are being used extensively to manufacture ultra high temperature (UHT)-treated processed cheeses. They are commonly known as Rota Therm or Gold Peg (Gold Peg International Pty Ltd, Victoria, Australia) cookers. In this method, a blend of cheese and ingredients is brought to a pre-determined standard composition prior to cooking. The mixture is then transferred to a jacketed hopper and is processed at high mixing speed (600–1000 rev/min) at temperatures above 130–145°C and with a residence time of approximately 3–4 s (Fonterra Research Centre process).

Processing conditions such as cooking time, temperature of cooking, extent of agitation (mixing) during cooking and the rate at which the cooked processed cheese is cooled have a significant effect on the functional properties of the processed cheese (Kapoor &

Metzger, 2008). Many chemical, mechanical and thermal parameters act as regulating factors in various types of processed cheese manufacture, as shown in Table 6.

Table 6 Chemical, mechanical and thermal parameters commonly used in cheese processing {adapted from(Caric & Kaláb, 1993)}

Process conditions	Processed cheese block	Processed cheese slice	Processed cheese spread
Raw material			
1. Average of cheese	Young to medium ripe, predominantly young	Predominantly young	Combination of young, medium ripe, overripe
2. Water-insoluble nitrogen as a % of total nitrogen	75–90	80–90	60–75
3. Structure			
	Predominantly long	Long	Short to long
Emulsifying salt	Structure-building, not creaming, e.g. high molecular weight polyphosphate, citrate	Structure-building, not creaming, e.g. phosphate/citrate mixtures	Creaming, e.g. low and medium molecular weight polyphosphate
Water addition (%)	10–25 (all at once)	5–15 (all at once)	20–45 (in portions)
Processing temperature (°C)	80–85	78–85	85–98
Duration of processing (min)	4–8	4–6	8–15
pH	5.4–5.7	5.6–5.9	5.6–6.0
Agitation	Slow	Slow	Rapid
Reworked cheese (%)	0–2.0	0	5–20
Homogenisation	None	None	Advantageous
Filling (min)	5–15	The quickest possible	10–30
Cooling	Slow (10–12 h)	Very rapid	Rapidly (15–30 min) in cool air

2.3.5 Packaging

Hot processed cheese can be transported to film-forming rotors (for slices) or filling machines by pumping, or can be transferred in containers. Processed cheese is usually packed and wrapped in plastic film (individually wrapped slices), plastic wrap, lacquered foil, tubes, cups, cans, cardboard or plastic cartons and occasionally in glass jars (Caric & Kaláb, 1993).

2.3.6 Cooling

As mentioned in Table 6, the cooling rate is different for different types of processed cheese. Cooling should be as fast as possible for processed cheese spreads and relatively slow for processed cheese blocks (Caric & Kaláb, 1993).

2.3.7 Storage

The final product should be stored at temperatures at or below 10°C (Berger et al., 1993; Caric & Kaláb, 1993). Temperatures between 12 and 15°C are cool enough to prevent any creaming during storage (Berger et al., 1993). However, storage at very low temperatures may induce crystal formation in the stored product (Caric & Kaláb, 1993).

2.4 Factors affecting the functional properties of processed cheese

Various chemical and compositional factors affect the functional properties of processed cheese. These factors are mainly the fat content, moisture content, pH, intact casein content, lactose content and whey protein content (Berger et al., 1993; Gupta & Reuter, 1992, 1993; Lee, Anema, & Klostermeyer, 2004; Lee & Klostermeyer, 2001; Marchesseau & Cuq, 1995; Marchesseau, Gastaldi, Lagaude, & Cuq, 1997; Meyer, 1973;

Templeton & Sommer, 1930). In commercial processed cheese manufacture, because of regulatory requirements, every effort is made to keep the moisture and fat contents constant in the final product. However, different sources and ages of natural cheese tend to lead to day-to-day variations in the pH and the intact casein content of the final cheese (Berger et al., 1993). The other ingredients, such as rennet casein and milk/whey powders, influence the whey protein and lactose contents of the final product (Berger et al., 1993; Meyer, 1973). These variations in chemical properties ultimately lead to inconsistency in the final functional properties of the product. Therefore, controlling the formulation parameters is very important for the production of a processed cheese with consistent functional properties.

The influence of these formulation parameters is discussed below.

2.4.1 Intact casein

As mentioned in Section 2.3.1, the type and the age of the cheese used in the formulation have major impacts on the quality of the processed cheese. The main contributing factor in such changes in quality is intact casein. The intact casein content of natural cheese is inversely related to its age. The intact casein content decreases as the natural cheese becomes more mature (Berger et al., 1993; Meyer, 1973; Purna, Pollard, & Metzger, 2006). Hydrolysis of the cheese protein is responsible for the decay in the intact casein content of natural cheese. The enzymes and starter/non-starter bacteria present in natural cheese hydrolyse intact casein (non-hydrolysed protein) to small peptides and eventually to free amino acids (Desmazeaud & Gripon, 1977; Farkye & Fox, 1990; Farkye, Madkor, & Atkins, 1995; van den Berg & Exterkate, 1993). Use of such natural

cheeses with different ages (intact casein) affects the textural and rheological properties of the resulting processed cheese (Purna et al., 2006). Purna et al. (2006) found that a decrease in the intact casein content of the natural cheese resulted in decreased viscosity and firmness and increased meltability of the resultant processed cheese.

2.4.2 pH

The stability of the processed cheese emulsion and therefore the final functional properties of the processed cheese are governed by the final pH of the product (Marchesseau et al., 1997). Three major factors that can modify the pH value are: (1) the maturity and buffering capacity of the cheese and other raw materials; (2) the acidity/alkalinity and buffering capacity of the emulsifying salt; (3) the fat content of the processed cheese (Berger et al., 1993). The pH range of good quality processed cheeses – regardless of whether they are blocks or spreads – ranges between 5.3 and 6.2 (Berger et al., 1993). However, the stability of the processed cheese emulsion decreases when the pH values are below 5.4 or above 5.8 (Marchesseau et al., 1997). Marchesseau et al. (1997) indicated that a lower pH (5.2) resulted in increased protein–protein interactions, and thereby increased aggregation of the proteins, leading to a weaker emulsification of the fat phase in the processed cheese, whereas a higher pH (6.1) created an open structure in the processed cheese and a resultant weaker emulsion. They also noted that pH 5.7 produced a uniform fat emulsion and a well-knit protein structure in experimental processed cheese.

2.4.3 Emulsifying salts

In the processed cheese industry, emulsifying salts are used to provide a uniform structure during the melting process, and also of the products (Caric & Kaláb, 1993). The most common emulsifying salts used for processed cheese manufacture are trisodium citrate and disodium phosphate. Trisodium citrate is the preferred emulsifying salt for slice-on-slice processed cheese varieties, whereas disodium phosphates or combinations of di- and trisodium phosphates are used in loaf-type block processed cheeses and processed cheese spreads (Kapoor & Metzger, 2008). The essential role of emulsifying agents in the manufacture of processed cheese is to supplement the emulsifying capability of the cheese proteins. This is accomplished by: (1) removing calcium from the protein system; (2) hydrolysing, solubilising and dispersing the proteins; (3) hydrating and swelling the proteins; (4) emulsifying the fat and stabilising the emulsion; (5) controlling and stabilising the pH; (6) forming an appropriate structure of the product after cooling (Caric & Kaláb, 1993).

The ability to sequester calcium is one of the most important functions of emulsifying salts. The principal caseins in cheese (α_{s1}-, α_{s2}-, β-) have non-polar, lipophilic C-terminal segments, whereas the N-terminal regions, which contain calcium phosphate, are hydrophilic (Caric & Kaláb, 1993). As described by Caric & Kaláb (1993) in a graphical presentation (Figure 2), calcium in the calcium paracaseinate complex of melting salts, insoluble paracaseinate, is solubilised, usually as sodium caseinate. During heat treatment with agitation, polyvalent anions from the emulsifying agent become attached

to the protein molecules, which increase their hydrophilic properties. The binding of additional quantities of water increases the viscosity of the blend, causing “creaming”.

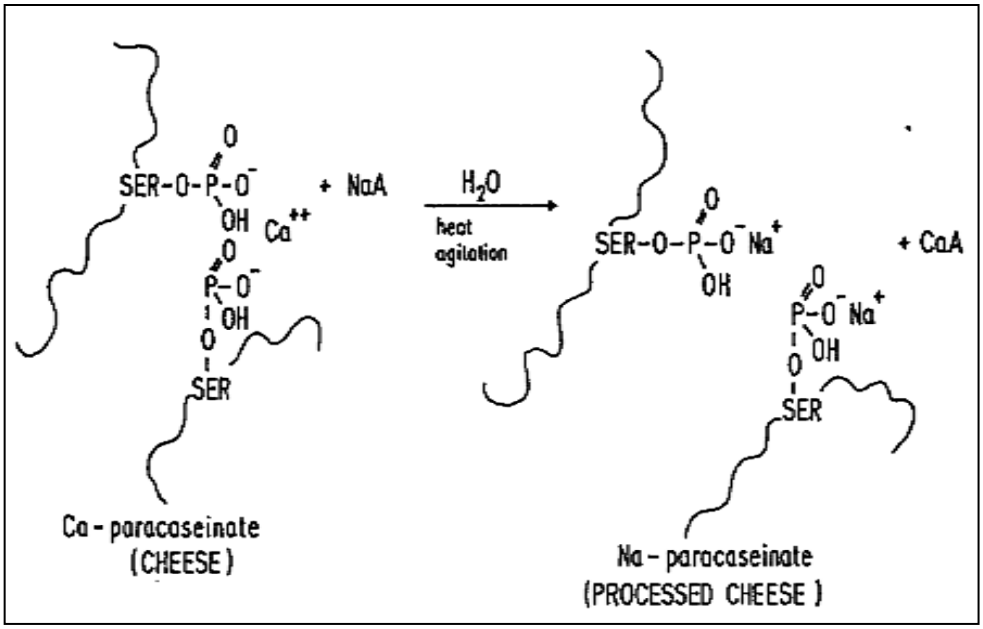


Figure 2 Chemical reactions during cheese processing. NaA – calcium sequestering agent; A – anion: phosphate, polyphosphate, citrate etc.: adapted from(Caric & Kaláb, 1993).

2.4.3.1 Effect of emulsifier on processed cheese functionality

Much literature on the effects of the type and the amount of emulsifying salts on the functional properties of processed cheese is available (Berger et al., 1993; Gupta, Karahadian, & Lindsay, 1984; Meyer, 1973; Rayan, Kaláb, & Ernstrom, 1980; Shirashoji,

Jaeggi, & Lucey, 2005; Shirashoji, Jaeggi, & Lucey, 2004, 2006, 2010; Templeton & Sommer, 1932a; Templeton & Sommer, 1932b, 1936; Zehren & Nusbaum, 1992a). However, deriving any interpretation as a rule of thumb from these publications is difficult because of the different experimental conditions used in these studies. As shown in Table 7, Kapoor and Metzger (2008), in their recent publication, have compiled some relevant information on selected emulsifying salts and their effect on some of the functional properties of processed cheese.

Table 7 Physicochemical properties of some emulsifying salts and their influence on processed cheese properties: adapted from (Kapoor & Metzger, 2008)

Emulsifying salt	Physicochemical properties				Influence on processed cheese properties		
	Chemical formula	Formula weight (g/mol)	Solubility (g/100 g water)	pH, 1% solution	pH of processed cheese	Hardness (kg)	Meltability (mm)
Trisodium citrate(dihydrate)	$NaH_2C_6H_5O_7.2H_2O$	294	75	8.6	5.9	32	131
Monosodium phosphate (monohydrate)	NaH_2PO_4,H_2O	138	85	4.5	5.1	27	No Melt
Disodium phosphate (dihydrate)	$Na_2HPO_4,2H_2O$	178	80	9.1	5.8	32	70
Trisodium phosphate (dodecahydrate)	$Na_3PO_4.12H_2O$	380	11	11.9	7.3	26	70
Dipotassium phosphate	K_2HPO_4	174	160	8.9	5.9	29	76
Sodium hexametaphosphate	$(NaPO_3)_n$ (n = 10 to 15)	$(102)_n$	157	6.6	5.2	33	No Melt
Sodium aluminium phosphate				9.2	5.9	33	101

2.4.3.2 Amount of emulsifying salts

The amount of emulsifying salts used is calculated according to the nature of the raw material and of the desired finished product and the "effectiveness" of the salts in terms of their ion exchange and protein breakdown properties. Berger et al. (1993) gave a guideline for the amount of emulsifying salt required to emulsify relative and intact casein contents. Table 8 shows that the quantity of emulsifying salts changes as the relative casein content and/or the amount of intact casein changes. If the relative casein content decreases, the quantity of emulsifying salts should be reduced proportionally under the identical production conditions (Berger et al., 1993).

Table 8 Relationships between intact and relative casein contents and quantity of emulsifying salts (polyphosphate with an average 60% P_2O_5: adapted from (Berger et al., 1993)

Total protein (%)	Intact casein (%)	Relative casein content (%)	Quantity of emulsifying salt (%)
30	30	100	3.6
30	27	90	3.3
30	24	80	3.0
30	21	70	2.7
30	18	60	2.4
30	15	50	2.1
30	12	40	1.8
30	9	30	1.5
30	6	20	1.2

This guideline is generally used for emulsifying salts containing polyphosphates. However, salts that are based on monophosphates and citrates need to be added at slightly higher dosages because polyphosphates have a better capacity for ion exchange and the breakdown of protein than monophosphates and citrates (Berger et al., 1993).

2.4.4 Lactose content

Milk powders and whey powders are the major sources of ingredients that contribute to the lactose content in processed cheese. The presence of lactose at a high level can lead to the formation of lactose crystals or Maillard browning in processed cheese (Lee et al., 1999; Templeton & Sommer, 1934). Therefore, it is critical to maintain the lactose content in a processed cheese formulation below its critical crystallisation level. Lactose crystal growth can usually be avoided by ensuring that the lactose concentration is less than its saturation concentration at the appropriate temperature; for example, the saturation concentration is 16.9 g lactose/100 g water at 15°C and 11.0 g lactose/100 g water at 0°C (Lee et al., 1999). Templeton and Sommer (1932a) also indicated a maximum soluble level of lactose of 17% at 20°C. Lactose crystallisation and Maillard browning can be avoided by maintaining the maximum lactose level below such limits.

2.5 Processing conditions

2.5.1 Melting temperature

The processing temperature is widely known as the cook temperature or the melting temperature in the processed cheese manufacturing industry. The melting temperature

has an important role to play in processed cheese processing for mainly three reasons (Berger et al., 1993).

(1) The physicochemical process, i.e. the creation of a homogeneous melt, can be brought about only in the presence of heat – temperatures of 70–75°C.

(2) The phenomenon known as "creaming", without which a spreadable processed cheese would not acquire the desired structure, intensifies with increasing temperature and is optimum between 80 and 90°C.

(3) A wide range of melting temperatures, from 70 to 145°C, is used with the aim of giving the processed cheese product a long shelf life.

2.5.2 Melting time

The time required for the melting process is determined by a number of factors (Berger et al., 1993) as indicated below.

(1) The properties of the raw material to be processed such as type, structure, degree of maturity and tendency to swell.

(2) Other added ingredients.

(3) Type of end-use product (spreadable cheese to block type).

(4) Size and design of the processing cookers.

(5) Processing temperature.

(6) Temperature, and amount and pressure of steam.

(7) Mode of heating, i.e. direct or indirect.

(8) Mechanical handling.

(9) Type and level of emulsifying salts.

(10) Desired shelf life.

Generally, it takes about 3–4 min to produce a stable emulsion in the traditional processed cheese manufacturing process. However, with the advances in technology and the use of the right equipment, it is now possible to obtain perfect melts within 2–3 min (Berger et al., 1993).

Block processed cheese, which needs to be handled as gently as possible and which has to have a long consistency, is melted for as short a time as possible, usually for 4–6 min. In contrast, for processed cheese spread and other processed cheese preparations, a heating time of 8–15 min is given to obtain the necessary creaming effect (Berger et al., 1993).

2.5.3 Mixing speed

Mixing is an essential step in processed cheese manufacture to achieve a stable emulsion and creaming. Mixing also ensures that the pieces of cheese are processed and that the added ingredients are mixed thoroughly with the emulsifying salts and water. Purna et al. (2006) noted significant changes in the functional properties of a processed cheese food when measured at two mixing speeds (450 and 1050 rev/min) at a constant temperature of 85°C for 6 min. They observed that the processed cheese food manufactured at higher speed had an immediate increase in viscosity after manufacture and higher firmness, whereas the melting and flow properties were decreased significantly. Because of this effect of mixing on the final cheese properties, in commercial processed cheese manufacture, it is possible to obtain products of any

consistency by controlling the mechanical action exerted on the material by the mechanical stirrer (Berger et al., 1993).

2.5.4 Cooling the finished product

Processed cheese undergoes creaming through intensive heating in the processing cookers. Therefore, it is important to stop this conversion process by rapid cooling. The two main ways of cooling the final product (Berger et al., 1993) are:

(1) storing the packed product on open shelves, where they can be exposed to cold air;

(2) conveying the product on a belt through a cooling tunnel.

Piska and Stetina (2004), Zhong and Daubert (2004) and Zhong, Daubert, and Velev (2004) demonstrated the role of the rate of cooling on the rheological properties of processed cheese. They observed that a slow cooling rate produces processed cheese with significantly higher firmness and significantly higher adhesiveness and gumminess.

2.6 Rheology and functional properties of processed cheese

Rheology is defined as “the study of the deformation and flow of matter”. Deformation pertains to matter that is solid; flow pertains to matter that is liquid. In the simplest case, the rheological properties of interest are elasticity in solids and viscosity in liquids (Borwankar, 1992; Gunasekaran & Mehmet, 2003). Rheology is used to provide a better understanding of the structure of food products, because the rheological behaviour of any material is largely dependent on its internal architecture (Prentice, Langley, &

Marshall, 1993). From a materials science point of view, processed cheese can be defined as a viscoelastic material because it is neither truly elastic nor truly viscous (Gunasekaran & Mehmet, 2003). However, from a rheology point of view, the functional properties of processed cheese can be defined as the properties that control its deformation and flow behaviour when subjected to a stress or strain (Visser, 1991).

Guinee (2002), based on the end-use applications of processed cheeses, grouped their functional properties into two major categories: (1) melted rheological properties; (2) unmelted rheological properties.

2.6.1 Rheology related to unmelted cheese

The most common types of rheological measurements in cheese involve linear (uniaxial) displacement, e.g. using an Instron Universal Testing machine (Creamer & Olson, 1982; Kapoor & Metzger, 2005; van Vliet, 1991; Visser, 1991). This is a widely used technique (Drake, Gerard, Truong, & Daubert, 1999; Gupta et al., 1984) for measuring the texture of (unmelted) processed cheese, such as hardness, fracturability, cohesiveness, adhesiveness, gumminess, chewiness, slicing ability, and elastic and viscous properties at low temperatures (Drake et al., 1999).The terms commonly applied to the rheology of cheese and sensory texture are described in Table 9.

Table 9 Rheological and fracture properties of cheese and their common sensory terms: partly adapted from (van Vliet, 1991; Zoon, 1991) and (Fox, Guinee, Cogan, & McSweeney, 2000)

Textural sensory descriptors	Definition
Firmness	Force at fracture or at large deformation
Softness (opposite of firmness)	Low resistance to deformation by applied force
Longness	Relative compression at fracture
Shortness (opposite of longness)	The tendency to plastic fracture at a small deformation; low resistance to breakdown upon mastication
Crumbliness	The tendency to break down easily into small, irregularly shaped particles (e.g. by rubbing)
Toughness	Work up to fracture
Adhesion	The tendency to resist the separation of two bodies (materials) in contact
Rigidity (stiffness)	Modulus(resistance to denting a surface)

Gunasekaran and Mehmet (2003) have provided an extensive description of basic concepts and the terminology used to describe the rheology of cheese. They state that rheology deals with the relationship between three variables: strain, stress and time.

Strain and stress are related to deformation and force respectively. Strain accounts for the size effect on the material deformation as a result of the difference in length of specimens, whereas stress accounts for the size effect on the applied force as a result of the difference in cross-sectional area of specimens. Using strain and stress, rheologists are able to obtain true material properties independent of the sample size and geometry, and are able to compare test results for samples of different sizes and geometries (Gunasekaran & Mehmet, 2003). Some of the terms used in this thesis have been very well defined and discussed by van Vliet (1991), O'Callaghan and Guinee (2004) and Gunasekaran and Mehmet (2003).

2.6.1.1 Strain

As described by O'Callaghan and Guinee (2004), the displacement in response to force at the point of application is known as deformation. The term "deformation" as used in this sense does not imply permanent deformation but rather a change in shape that may be temporary, permanent or partly recoverable. The conditions that affect the force–displacement response include temperature, type of deformation (compression, extension, shear or pressure), level of deformation in relation to the elastic limit and the fracture point of the material, and rate of deformation. Strain may be defined as the fractional displacement that occurs under an applied stress (O'Callaghan & Guinee, 2004).

2.6.1.2 Stress

Stress is defined as the distribution of force over an area of a material. The "area" over which a force is distributed may be a surface (e.g. the surface of a cylindrical sample

exposed to a compression plate) or an imaginary section within a material (e.g. an internal fracture plane). The force applied at a source is distributed throughout the material and is borne by the structural elements (O'Callaghan & Guinee, 2004).

The rheological properties that are derived from stress/strain curves obtained from small to large strain deformation of cheese in compression are shown in Table 10. In addition, if a tension stroke is added to this compressor, the negative area under the force–time curve in tension or the maximum negative force is often used to measure stickiness or adhesiveness (Zoon, 1991).

Table 10 Rheological properties obtained from a large strain fracture deformation {adapted from O'Callaghan & Guinee (2004)}

Rheological property	**Abbreviation**	**Interpretation**	**Textural characteristic to which the parameter is related**
Fracture stress	σ_f	Stress required for fracture and collapse of the cheese mass beyond the point of recovery	Firmness
Fracture strain	ε_f	Strain required to induce fracture	Longness (opposite of brittleness or shortness)
Modulus of deformability	E_d	Stress per strain (at low enough strain to have negligible irreversible change of structure)	Stiffness (rigidity)

2.6.2 Rheology related to melted cheese

As described by Gunasekaran and Mehmet (2003), the melted properties or meltabilities of cheese refer to the "ease and extent to which the cheese will melt and spread/flow upon heating". The most popular empirical melt test for processed cheese is the Schreiber melt test that was developed by L. D. Schreiber Co. (Kosikowski & Mistry, 1997a). In this test, a cheese sample of specific dimensions is heated in an oven at a specific temperature for a specific time and the final diameter or area of the processed cheese after melting is reported as its melt (Muthukumarappan, Wang, & Gunasekaran, 1999). Over the years, the test has been modified to overcome some shortcomings in terms of sample dimensions and testing conditions (Gunasekaran & Mehmet, 2003; Muthukumarappan et al., 1999). A dynamic rheological analysis technique called dynamic stress rheology (DSR) was developed by Sutheerawattananonda and Bastian (1998) to measure and evaluate the melted viscoelastic properties of processed cheese. This method heats the processed cheese samples and measures the storage modulus (G'), the loss modulus (G'') and the melting temperature(the temperature at which $G' = G''$ or $\tan \delta = 1$). The ratio of G' and G'' is the tangent of the phase angle shift δ between stress and strain vectors. Thus, $G'/G'' = \tan \delta$. This measures the damping ability of the cheese. Prow and Metzger (2005) found a good correlation between G' from DSR and the extent of flow as measured by the tube melt test.

Squeeze flow or squeezing flow is often used to determine the flow properties of highly viscous materials such as cream cheese and processed cheese (Campanella, Popplewell,

Rosenau, & Peleg, 1987; Gunasekaran & Mehmet, 2003). One of the most common examples of the squeezing flow rheological technique is the UW melt meter, which was developed at the University of Wisconsin, Madison, Wisconsin, USA (Wang, Muthukumarappan, Ak, & Gunasekaran, 1998). This method is discussed in detail in Chapter 3.

In recent publications, a Rapid Visco Analyser (RVA) has been used extensively to measure the textural properties of processed cheeses and processed cheese spreads in the laboratory (Kapoor & Metzger, 2005; Metzger, Kapoor, Rosenberg, & Upreti, 2002; Prow & Metzger, 2004, 2005). The RVA was originally developed by Newport Scientific (Warriewood, NSW, Australia) to measure the viscous properties of cooked starches, grains, batters and other food (Kapoor & Metzger, 2008). This instrument can measure apparent viscosity over variable conditions of shear and temperature as defined by the operator.

2.7 Microstructure measurement of processed cheese

Processing markedly changes the structure of natural cheese and results in the development of new structures in processed cheese (Guinee et al., 2004). Low resolution techniques, such as light microscopy, confocal microscopy, confocal laser scanning microscopy and fluorescence microscopy (Bowland & Foegeding, 2001; Lee, Buwalda, Euston, Foegeding, & McKenna, 2003; Sutheerawattananonda & Bastian, 1998), and high resolution techniques, such as transmission electron microscopy and scanning electron microscopy (Kaláb, 1993; Lee et al., 2003; Rayan et al., 1980; Savello, Ernstrom, & Kaláb, 1989), are well known for studying the effect of pH, emulsifying salts

and processing conditions on the microstructure of processed cheese. Microscopy is the only method by which visual presentations of particles are observed and measured. Some of the published literature describes changes in the distribution in fat globules (Rayan et al., 1980) and the paracaseinate structure (Heertje & Lewis, 1993; Lee et al., 2003) that occur when natural cheese is converted into processed cheese. Microscopy techniques have also been used to study the characteristics of the protein-based structural network of processed cheese.

2.7.1 Confocal laser scanning microscopy

This microscopy technique is a well known in the food scientist community for its versatility, its ease of use and relative ease of sample preparation. In this technique, sample observations are done at atmospheric pressure and do not require dehydration process(Blonk & van Aalst, 1993). This techniques allows the investigation of the specimen without any prior requirements such as embedding, and fixing (Vodovotz, Vittadini, Coupland, McClements, & Chinachoti, 1996). More detail about the principle and working (multiple labeling) is provided in appendix A.

2.7.2 Transmission electron microscopy

Transmission electron microscopy (TEM) is being used widely to study the microstructure of a wide range of food products and ingredients. (Holgate & Webb, 2003). For TEM, resolution strongly depends on the thickness of the prepared sample and the acceleration voltage for the electron beam-the higher the voltage, the better the theoretical resolution. However, for imaging food samples, especially those containing

structures prone to electron damage, the optimal accelerating voltage of TEM would be limited to up to 100kV for most food samples(Dudkiewicz et al., 2011).

A significant limitation of all TEM studies is that the specimen must be transparent to the electron beam and this means that accurate thinning processes are often necessary to produce suitable specimens. Working principle and other operating detail are explained in appendix C

2.8 Fat globule size determination in processed cheese

Light scattering techniques have been used in a number of studies to measure the fat globule size of dairy products (Lee et al., 2004; Michalski, Briard, & Michel, 2001; Trivedi, 2006). Walstra (1965) developed a method for diluting a milk sample for analysis of the fat globule size using a light scattering technique. This method was developed to obtain an emulsion without clusters of fat globules. Over time, this technique has been modified to make it more efficient in terms of dissolution and dispersion of the samples. Michalski et al. (2001) used a Malvern Mastersizer 2000 to measure the fat globule size in milk. Lee et al. (2004) investigated the effect of moisture on the pH, rheological properties and fat particle size of a model processed cheese spread system. They measured the fat globule size using a Beckman Coulter laser scattering particle sizer (similar to a Malvern Mastersizer) and they stored their samples for 24 h in a refrigerator. The same sample preparation method and a Malvern Mastersizer were also used successfully by Trivedi (2006) to measure the fat globule size in processed cheese containing different starches. As the processed cheese samples in the present study had higher solids contents than the spreads used in the study by

Lee et al. (2004), S. K. Lee (personal communication) suggested that a sample size of 0.2 g of processed cheese for dispersion would be more appropriate. The principles and other relevent operating details are provided in appendix B

2.9 Hydrocolloids and processed cheese

The pasteurised processed cheese industry uses the term stabiliser for gums that are used as an optional ingredient in processed cheese spreads and related products (Zehren & Nusbaum, 1992b). FSANZ allows the addition of polysaccharides in processed cheese as a stabiliser or gelling agent (Table 3). The addition limit in the finished product is not mentioned anywhere in the standards.

Meyer (1973) referred to gums as binders and thickening agents that serve to cement together finely dispersed casein particles. They also described that gums consist of hydrophilic carbohydrates or polymers of sugars that have an affinity for water and exhibit binding properties with water and other organic/inorganic materials. Traditionally, they have been used to improve consistency and stability, the main aim being to ensure a “smooth” mouthfeel and to avoid exudation of water (syneresis) in cheeses (Zehren & Nusbaum, 1992b). Because of their water-binding and/or gelation properties (Phillips, Wedlock, & Williams, 1986), they impart viscosity, especially in instances of high water content or low creaming action (thin consistency) as affected by, for example, the use of overripe cheese.

However, the available literature describing the effect of various microbial polysaccharides on the functional properties of processed cheese is limited. Moreover,

recently, gums, along with polysaccharides/polysaccharide derivatives, are finding increasing applications, as fillers and texturisers, in the manufacture of low fat processed cheese products (Brummel & Lee, 1990).

2.9.1 Method of incorporation

The practice of weighing out the polysaccharide in production rooms is common in many factories in New Zealand. If the processing of the cheese is to take less time than the hydration time of the polysaccharides, it is advisable to add the hydrocolloids in solution rather than directly in powder form.

Zehren & Nusbaum (1992b) described the sequence of polysaccharide addition in processed cheese. They described that the ground cheese is added to the cooker with a dry mix of the stabilizer and emulsifying salts. The required amount of water is added and the mix cooked to around 75 °C in 3 minutes. Acid is added at the end of cooking. The hot cheese is discharged in about 5 minutes and packaged.

Gums may be dispersed by sifting slowly while water is vigorously agitated. The speed should be sufficient to produce a vortex, with the gum added into the vortex. In the case of gums soluble in hot water, dispersion may be facilitated by first wetting with cold water(Klose & Glicksman ,1969 as cited in (Zehren & Nusbaum, 1992b). Some polysaccharides are barely soluble, particularly in cold water. However, the presence of certain ions in the product, with which they may react, may make them hydrated and solubilise.

2.9.2 Characteristics of microbial polysaccharides

2.9.2.1 Xanthan gum

Xanthan gum is a water-soluble extracellular hydrophilic polysaccharide that is produced by *Xanthomonas campestris* in a well-aerated fermentation medium containing glucose, a nitrogen source and needed trace elements (Slodki & Cadmus, 1978). The xanthan gum is recovered by precipitation in ethanol, purified by separation with isopropyl alcohol and dried (Garcia-Ochoa, Santos, & Alcon, 1993).

Xanthan gum was discovered in 1963 at the Northern Regional Research Center of the United States Department of Agriculture (USDA) (Margaritis & Zajic, 1978). By 1964, commercial production of xanthan gum had begun and it was commercialised by several companies, including Kelco Co. (now CP Kelco). Authorisation for the use of xanthan gum in food was granted by the Food and Drug Administration in 1969, following extensive animal feeding trials; since then, the functional properties of xanthan gum have been widely utilised in a range of food products (Pettitt, 1982). The properties of xanthan gum are well described in CP Kelco product bulletins.

2.9.2.1.1 Rheological properties

Xanthan gum solutions are highly pseudoplastic (Kawakami, Okabe, & Norisuye, 1991; Whitcomb & Macosko, 1978).when shear stress is increased, viscosity is progressively reduced. Upon the removal of shear, the initial viscosity is recovered almost instantaneously(Kawakami et al., 1991; Urlacher & Noble, 1999). This behaviour can have various advantages in process cheese making process; such as viscosity decrease

with the increasing shear rate, the product become easy to pour, mix and pump (Zehren & Nusbaum, 1992b). Pseudoplasticity is also important in contributing good sensory qualities and flavour release to foods, as well as long term shelf stability of emulsifying and suspending.

Another feature of xanthan gum solution is its viscoplasticity, which gives a high yield value even at a low concentration. The yield value is the minimum shear stress required for a solution to flow. The yield value results from the formation of a weak network, which is a result of interactions between xanthan molecules. However, the network formed is not a true gel as these interactions are not permanent and are totally shear reversible (Sanderson, 1981).

2.9.2.1.2 Stability and compatibility

Xanthan gum shows a very slight change in the viscosity with increase in temperature from 10 °C-90 °C (Sanderson, 1981; Sworn, 2000b). As with temperature, pH has little effect on the viscosity. Uniform and high viscosity is maintained over the pH range 2-12 (Sanderson, 1981; Sworn, 2000b). Salt concentration below 0.08 % causes a decrease in viscosity, however salt concentration is always greater than this level in processed cheese (Zehren & Nusbaum, 1992b).

Xanthan gum solutions are compatible with alcohol. Moreover, enzymes such as proteases, cellulases, pectinases and amylases do not degrade the xanthan gum molecules (Sworn, 2000b).

2.9.2.1.3 Gum association

Many combinations of gums have proved to be interesting because their rheological and functional properties are complementary. In this area, gum combinations containing xanthan gum have not yet been fully exploited by the cheese manufacturing industry. In addition, many specific and strong interactions between xanthan gum and different hydrocolloids occur. These interactions generally give a positive synergistic effect, such as enhancement of the viscosity or gelation, which is especially important and useful with galactomannans such as locust bean gum(LBG) (Sworn, 2000b; Zhan, Ridout, Brownsey, & Morris, 1993). Xanthan/LBG gels are thermally reversible setting and melting at approximately 55-60 °C (Sworn, 2000b). Moreover, mixtures of xanthan and LBG require heating to approximately 90 °C to fully hydrate the LBG and maximize the synergistic interaction (Sworn, 2000b).

The interaction of xanthan gum with galactomannans is dependent on the ratio of the mixture, pH and ionic environment. Optimum ratio is 50:50 for LBG : xanthan gum (Sanderson, 1981). Generally, the synergistic interaction with galactomannans is maximum in deionised water at neutral pH (Sanderson, 1981).

2.9.2.2 Locust bean gum (LBG)

LBG is a galactomannan polysaccharide with a molecular weight of about 330,000 Daltons. It is a straight chain D-manno-pyranose polymer with a side chain branch of D-galacto-pyranose on every fourth or fifth mannose. The viscosity of a 1.0% solution is approximately 3500 cP. LBG partially swells in cold water and increases in viscosity when heated to about 82–85°C. Maximum hydration occurs when LBG is held at that

temperature for about 15 min. The viscosity is then further increased on cooling. pH has little effect on viscosity in the pH range from 3 to 11. LBG is compatible with carbohydrates, proteins and other gums (Zehren & Nusbaum, 1992b). Glicksman (1982) indicated that LBG improves the textural quality of soft and spoonable jar cheese spreads and that LBG can be added to such soft cheese spreads to eliminate the thin, watery consistency of the blend.

2.9.2.3 Gellan gum

Gellan gum is a bacterial exopolysaccharide that was discovered through the screening of thousands of bacteria. It is prepared commercially by aerobic submerged fermentation of a carbon source in the presence of nitrogen from *Sphingomonas elodea* (previously called *Pseudomonas elodea*), in a manner similar to xanthan gum. It is an extracellular high molecular weight ($0.5–1 \times 10^6$) polysaccharide. Gellan gum is currently under consideration as a permitted food additive.

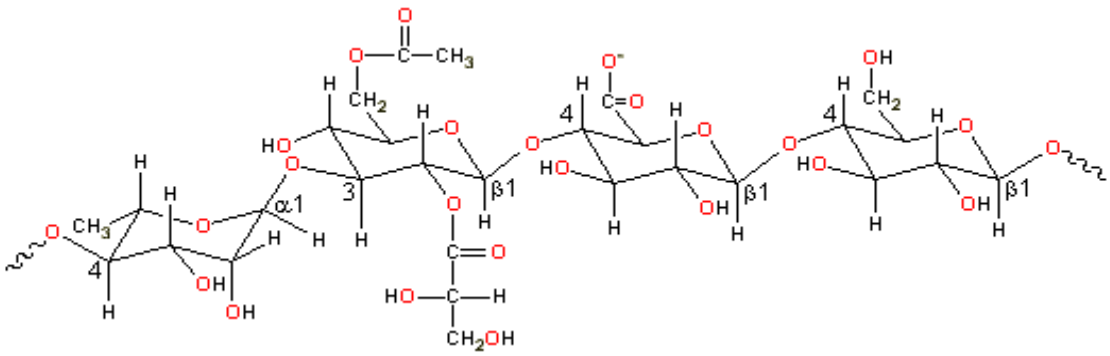


Figure 3 Structural unit of gellan gum: →4)-L-rhamnopyranosyl-(α-1→3)-D-glucopyranosyl-(ß-1→4)-D-glucuronopyranosyl-(ß-1→4)-D-glucopyranosyl-(ß-1→ with O(2) L-glyceryl and O(6) acetyl substituents on the 3-linked glucose.

Gellan gum is a linear tetrasaccharide. It has high molecular weight, consists of about 50,000 residues and is normally de-esterified by alkali treatment before use in food.

The molecular structure of gellan gum is a straight chain that is based on repeating glucose, rhamnose and glucuronic acid units (Jansson, Lindberg, & Sandford, 1983; O'Neill, Selvendran, & Morris, 1983). O'Neill et al. (1983) determined the structure of the acetylated polysaccharide (containing approximately one O-acetyl group for every eight sugar units), which has a tetrasaccharide repeating unit consisting of two β-D-glucose residues, one β-D-glucuronic acid residue and one α-L-rhamnose residue linked together. This form of gellan gum is commonly known as low acyl gellan gum (Figure 4). In low acyl gellan gum, the acyl groups are removed completely.

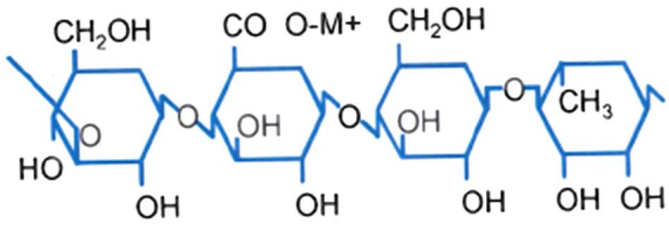


Figure 4 The repeating unit of low acyl gellan gum.

Two acyl substituents – acetate and glycerate – are present in the native or high acyl form of gellan gum (Figure 5). Both substituents are located on the same glucose residue, and, on average, there is one glycerate per repeat and one acetate per every two repeats (Kuo, Mort, & Dell, 1986).

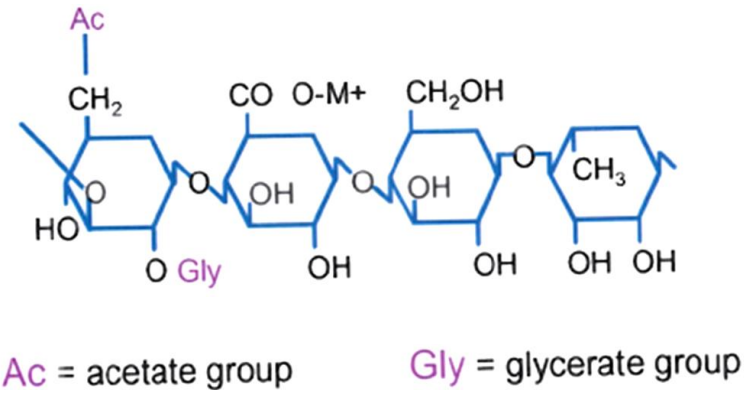


Figure 5 The repeating unit of high acyl gellan gum.

It has been revealed that gellan gum is not just a gelling agent; in fact, it is also a texturising, stabilising, film-forming, viscosifying and flavour-releasing polymer. Its multi-functional properties and its synergy with other polysaccharides, such xanthan gum and LBG, allow the production of a wide range of textures (Chandrasekaran & Radha, 1995). Today, gellan gum is approved for food use in the USA and several other countries including Argentina, Australia, Canada, Chile, Colombia, Costa Rica, Hong Kong, Indonesia, Israel, Japan, Mexico, Philippines, Singapore, South Africa, South Korea, Sri Lanka, Taiwan, Thailand and the European Union.

2.9.2.4 Texture formation by Gellan gum

It has been reported in the literature that the textural properties of gellan gum are depended on its acyl content.(Morris, 1996). Recent studies have shown that both the levels of glycerate and acetate substitutents in gellan gum can be controlled independently (Morrison, Sworn, Clark, Talashek, & Chen, 2002). Changes in the glycerate and acetate parameters have a significant effect on the structural and rheological characteristics of gellan gum gels (Kasapis et al., 1999). Therefore, a wide

range of gel textures can be produced by manipulating the blends of high and low acyl gellan gum.

When hot solution of gellan gum are cooled in the presence of gel promoting cations, gels ranging in texture, from elastic (high acyl gellan) to brittle (low acyl gellan), are formed through cations mediated helix-helix aggregation. Sworn, Sanderson, & Gibson(1995) reported that low acyl gellan gum gels are formed by cooling a hot solution through its setting temperature at about 40 °C to 50°C. Morris (1996) observed a significant thermal hysteresis between the setting point and the melting point for low acyl gellan gum i.e. the gels melt at a higher temperature than that at which they set. Whereas, high acyl gellan gum gels are typically formed at around 80°C and does not show any hysteresis between the setting point and the melting point i.e. they melt at the same temperature at which they set (Morrison et al., 2002). The ability to reduce setting temperature whilst retaining many of the elastic properties of the high acyl gellan has many advantages in high solid applications (Morrison et al., 2002).

2.9.2.5 Hydration

The temperature at which low acyl gellan hydrates is dependent on the type and concentration of ions in solution. The presence of ions such as sodium, and calcium will inhibit the hydration of low acyl gellan in solution. Whereas, sequestrants such as sodium citrate (commonly used in processed cheese making) between 0.1% to 0.3% to allow complete hydration at about 65 °C even in water with hardness of 600ppm of $CaCO_3$ (Sworn, 2000a). Incomplete hydration will result if the sodium ion concentration exceeds 0.5% (about 1.3% sodium chloride).(Sworn, 2000a).

Addition of cations is not necessary for the formation of high acyl gellan gels and their properties are much less dependent on the concentration of ions in solution (Morrison et al., 2002).

2.9.2.6 Limitations in complex emulsions

The functionality of xanthan gum and gellan gum is dependent on the behaviour of the polysaccharide in the complex processed cheese environment. The presence of salt, emulsifying salt and citric acid makes processed cheese a complex system. Therefore, based on the published literature (mainly for solutions), it is difficult to determine the properties of the polysaccharides when present individually in processed cheese because they are influenced heavily by such additives.

2.10 Mozzarella cheese

Mozzarella cheese is a pasta filata cheese that originated in Italy. Plasticising and kneading of fresh curd in hot water imparts a unique fibrous structure, melting and stretching properties to Mozzarella cheese. After World War II, Mozzarella cheese began its rise in popularity, which continues to the present day (Kindstedt, 2004). High moisture Mozzarella and part-skim Mozzarella cheeses have moisture contents > 52% and are often used fresh as table cheeses. In contrast, low moisture Mozzarella and part-skim Mozzarella cheeses have much lower water content, typically 47–48%, and are used as ingredients for pizzas and related foods (Kindstedt, 1993; Kowsikowski & Mistry, 1997b). High moisture and low moisture Mozzarella cheeses differ in terms of their manufacturing protocol.

2.10.1 Manufacture of low moisture Mozzarella cheese

As shown in Figure 6, the manufacture of low moisture Mozzarella cheese is similar to that of Cheddar cheese. Traditional Mozzarella cheese is produced by adding small amounts of starters to milk, followed by rennet (milk coagulator) (Kowsikowski & Mistry, 1997b). *Streptococcus salavarious* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgariculs* or *Lactobacillus helveticus* are usually used for the manufacture of low moisture Mozzarella cheese (Kindstedt, 1993; Kowsikowski & Mistry, 1997b). As these starters grow at higher temperature (e.g. 42°C), the scalding and cheddaring temperature used for low moisture Mozzarella cheese is usually higher than that used for Cheddar cheese. The kneading and plasticising process in hot water is carried out at about 65–70°C and the curd is cheddared to about pH 5.2.

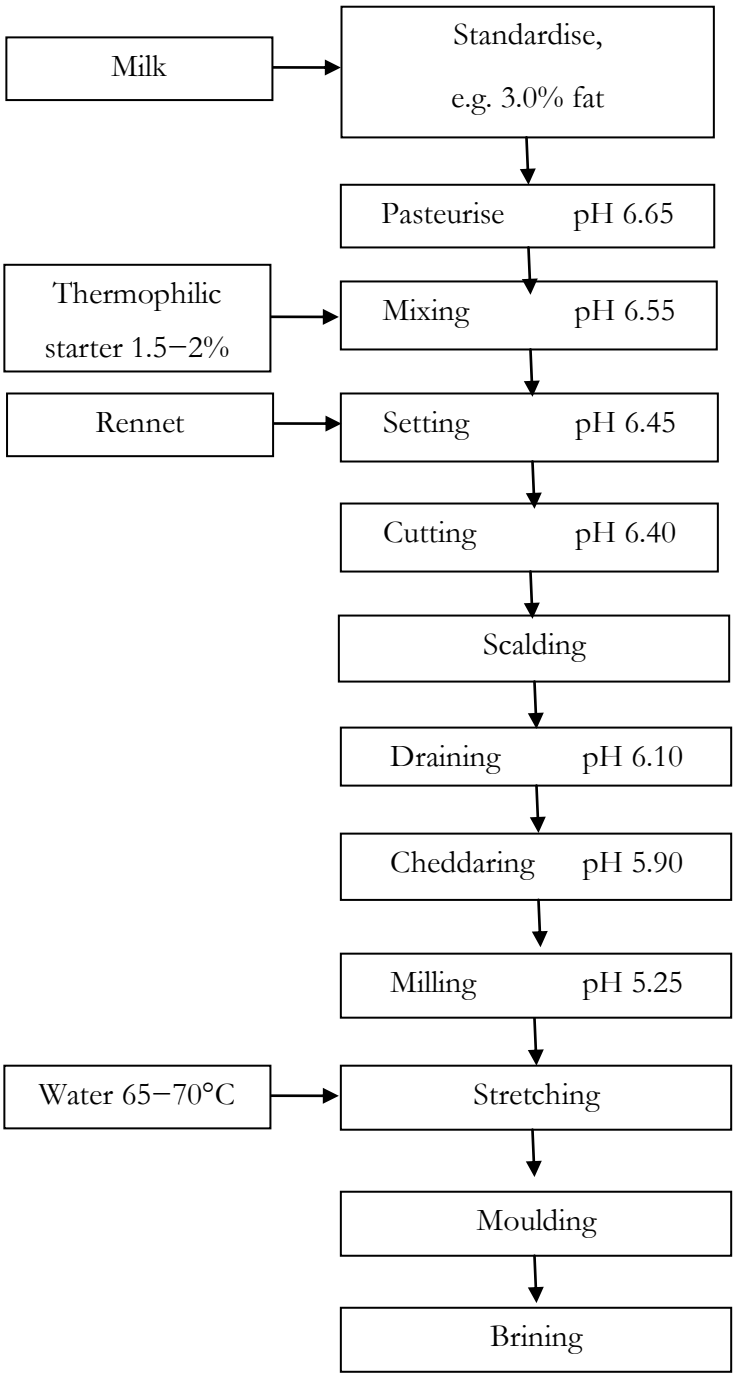


Figure 6 Example of flow diagram for the manufacture of low moisture Mozzarella cheese by a traditional process {adapted from (Kindstedt, 1993)}.

2.11 Exopolysaccharides (EPSs) in cheese

2.11.1 Lactic acid bacteria (LAB) EPS

LAB is a diverse group of industrially important, Gram-positive, non-spore-forming microbes that produce lactic acid as a major product of carbohydrate fermentation. LAB have been used around the world to improve the preservation, sensorial characteristics, functional characteristics and nutritional value of a large variety of products including fermented products such as cheese. The lactic fermentation of milk products is by far the best studied and known use of LAB. A great variety of dairy products with different flavour, texture and health-promoting properties can be obtained from milk using different technologies and starter cultures. Important features of the starter are rapid acidification, microbial preservation, formation of specific flavours, texturising capabilities and health benefits. The fermentation of lactose during bacterial growth causes acidification and thereby protects the product against spoilage microorganisms and the proliferation of pathogens. Another effect of acidification is the neutralisation of the negative charges on the milk proteins, resulting in coagulation. The acid also gives fermented products, such as yoghurt and cheese, their pleasant fresh and milk acid taste. Furthermore, LAB convert sugar, organic acids, proteins and fats into the typical aroma and flavour components of fermented products. Several LAB strains can also contribute to improving the texture and viscosity of fermented products by synthesising EPSs (Ruas-Madiedo, Hugenholtz, & Zoon, 2002).

The term EPS is commonly used to refer to extracellular polysaccharides. EPSs may be homopolysaccharides, composed of a single type of sugar monomer, or

heteropolysaccharides, containing several types of sugar monomers (Sutherland, 1972). LAB that produce EPSs play an important role in the dairy industry because of their contribution to the consistency and rheology of fermented milk products. The EPS polymers can be considered to be natural biothickeners because they are produced in situ by LAB starters that have General Recognised as Safe (GRAS) status (Ruas-Madiedo, Hugenholtz, et al., 2002). As many LAB are food-grade microorganisms with GRAS applications, the use of their EPSs in foods has an obvious advantage over the use of polysaccharides produced by non-food-grade (can not be used without purification) bacteria, such as dextran, gellan gum, pullulan, xanthan gum and bacterial alginates (Dabour, Kheadr, Fliss, & LaPointe, 2005).

2.11.2 EPS-producing cultures in cheese manufacture

As the major defects of reduced-fat cheeses are related to texture and body problems, the suggestion has been made to increase their moisture content beyond that of full-fat cheese to correct these problems (Mistry, 2001). An increase in the moisture content of reduced-fat cheese could be obtained by several means, including

(1) modification to the cheese-making procedure,
(2) the addition of moisture-binding agents, and
(3) EPS-producing cultures.

Modifying the cheese-making procedure may not be applicable for all cheese plants and would require additional financial cost; the use of emulsifying thickening agents may adversely affect the flavour of the cheese. Thus, the use of EPS-producing LAB could be a

potential means of increasing the moisture content and improving the textural attributes of reduced-fat cheese.

EPS-producing cultures have been used to improve the rheological and textural characteristics of fermented dairy products (Perry, McMohan, & Oberg, 1998). Different types of EPS with varied functional properties are produced by lactic acid starter cultures (Broadbent, McMahon, Welker, Oberg, & Moineau, 2003). EPSs increase the water-holding capacity of a milk gel and interfere with protein–protein interactions, two functions that are required in low fat cheeses (Mistry, 2001). Both bacterial capsules and milk fat produce milk gels with a spongy structure (Mistry & Anderson, 1993). Some strains of LAB produce capsular polysaccharides, which are a polymeric matrix of high water content surrounding the cells (Hassan, Frank, Farmer, Schmidt, & Shalabi, 1995). They can be distinguished from ropy polysaccharides because they are of a discrete size and are not liberated into the growth medium. Ropy cultures may produce capsular polysaccharides as well as ropy polysaccharides, but capsule-producing cells do not necessarily produce ropy polysaccharides (Hassan et al., 1995).

2.11.3 EPS starters and its effect on functional properties of Mozzarella cheese

Several studies have indicated the improved physical and functional properties of low fat cheeses by using EPS producing cultures (Awad, Hassan, & Muthukumarappan, 2005; Hassan, Awad, & Muthukumarappan, 2005; Perry et al., 1998; Zisu & Shah, 2005a; Zisu & Shah, 2006, 2007). Excellent water-binding properties, and moisture retention is a key strategy to improving the functionality of low fat cheese (McMahon, Oberg, & McManus, 1993). Perry et al (1997)investigated the influence of an EPS producing

starters on the moisture and melt properties of low fat (6%) Mozzarella cheese was investigated. The bacteria used in these studies, *Streptococcus thermophilus*(MR-1C) and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (MR-1R), produce EPS (Perry et al., 1997; Perry et al., 1998). As shown in Table 11, low fat Mozzarella cheese manufactured with MR-1C and MR-1R contained significantly ($P < 0.05$) more moisture and had better melt properties than cheese made with a commercial non-EPS producing starter pair (*S. thermophilus* TA061 and *L. helveticus* LH100).

Table 11 Influence of starter culture pair on the moisture and melt properties of low fat Mozzarella cheese {adapted from (Perry et al., 1997; 1998)}

Starter pair	**Moisture (%)**	**Melt at day 1 (cm)**
***S. thermophilus* MR-1C plus *L. delbrueckii* subsp. *bulgaricus* MR-1R**		
10 kg vats, hand stretched	61.0 ± 0.47	12.1 ± 0.6
363 kg vats, mechanically stretched	57.1 ± 0.35	10.6 ± 0.5
***S. thermophilus* TA061 plus *L. helveticus* LH100**		
10 kg vats, hand stretched	58.2 ± 0.50	10.7 ± 0.4
363 kg vats, mechanically stretched	55.3 ± 0.15	9.5 ± 0.1

To determine whether MR-1C, MR-1R or both cultures were required for the increased moisture content of the cheese, Low et al. (1998) performed cheese-making trials using different combinations of MR-1C, MR-1R, TA061 and LH100. Table 12 indicates that these trials also showed that cheeses made with MR-1R and MR-1C had significantly higher moisture contents and established that this effect was due exclusively to *S. thermophilus* MR-1C.

Table 12 Influence of individual capsular-producing and EPS-producing starter cultures on the moisture content of low fat Mozzarella cheese { adapted from Low et al. (1998)}

Starter pair	Moisture (% ± SE)
***S. thermophilus* MR-1C with *L. bulgaricus* MR-1R**	61.9 ± 0.2
***S. thermophilus* MR-1C with *L. bulgaricus* LH100**	61.6 ± 0.1
***S. thermophilus* TA061 with *L. helveticus* MR-1R**	60.0 ± 0.1
***S. thermophilus* TA061 with *L. helveticus* LH100**	60.0 ± 0.2

Commercial-scale (22,500 L vat) cheese-making trials with an industrial partner have also confirmed that *S. thermophilus* MR-1C (paired with an EPS-negative rod) can increase the moisture content of part-skim Mozzarella cheese by 1.5% (Broadbent, McMahon, Oberg, & Welker, 2001).

Petersen, Dave, McMahon, Oberg, & Broadbent (2000) also investigated the effect of capsular and ropy EPS-producing *S. thermophilus* starter bacteria on Mozzarella cheese functionality and whey viscosity. They found that the moisture contents were significantly higher in Mozzarella cheese made with EPS-positive streptococci than in

cheese made with EPS-negative streptococci, and the melt properties were better in the higher moisture cheeses.

Zisu and Shah (2005a) studied the influence of various treatments such as microbial EPS, pre-acidification and whey protein concentrate (WPC) on low fat Mozzarella cheeses containing 6% fat. The impact of these treatments on moisture retention, changes in texture profile analysis, cheese melt, stretch and pizza bake performance were investigated over 45 days of storage at 4°C. The pre-acidified cheeses without EPS (control) had the lowest moisture content (53.75%), were the hardest and exhibited the greatest springiness and chewiness. The meltability and stretchability of these cheeses increased most during the first 28 days of storage. The moisture content in the cheeses increased to 55.08, 54.79 and 55.82% with EPS starter (containing 41.18 mg/g of EPS), co-culturing (containing 28.61 mg/g of EPS) and WPC (containing 44.23 mg/g of EPS) respectively. EPS reduced the hardness, springiness and chewiness and increased the cohesiveness and meltability of low fat cheese. Although the stretch distance was similar in all cheeses, those containing EPS were softer than the control.

Exopolysaccharide producing cultures can be used to improve the physical properties of dairy products. Therefore, in situ EPS production by lactic acid bacterial has been recommended. However, no studies have been found on use of dried ingredient containing EPS as a conventional stabilizer in cheese making systems. Production of such polysaccharides commercially would be rewarding opportunities to the dairy industry. However, cost of production and purification would be a major challenge.

Chapter 3 Common materials and methods

3.1 Manufacture of model processed cheese

3.1.1 Ingredients

Model processed cheese samples were prepared from rennet casein (80.8% protein, ALAREN 779, NZMP, New Zealand), soya oil (AMCO, Goodman Fielder, New Zealand), trisodium citrate (TSC) (Jungbunzlauer, Basel, Switzerland), sodium chloride (NaCl) (BDH Laboratory Supplies, Poole, England), citric acid (CA) (BDH Laboratory Supplies) and water (Milli-Q, Millipore Corporation, MA, USA).

3.1.1.1 Polysaccharides

The following polysacchairdes were procured from CP Kelco for the experimentation work.

1) Kelcogel F: Low acyl gellan gum: CP Kelco, US., Inc. Chicago, USA .Lot no 6J7657A.
2) Kelcogel LT100: High acyl gellan gum: CP Kelco, US., Inc. Chicago, USA .Lot no 6J7656A
3) Keltrol: Xanthan gum: CP Kelco, US., Inc. Chicago, USA .Lot no 6I7565A
4) Genu gum: Locust bean gum: CP Kelco, US., Inc. Chicago, USA .Lot no 6H7556A

3.1.2 Processing in the Rapid Visco Analyser

In the preparation of 30 g batches of processed cheese, the dry ingredients, i.e. rennet casein, lactose, trisodium citrate and polysaccharide, were weighed out into a Rapid Visco Analyser (RVA-4; Newport Scientific Pty Ltd, Warriewood, NSW, Australia)

canister. Water was added to the dry ingredients at ~ 20°C and the mixture was stirred manually using a thin spatula until the ingredients were properly dispersed. This mixture was allowed to hydrate for 40 min at ~ 20°C in the covered aluminium RVA canister. Oil, NaCl and citric acid were added to the hydrated mixture. The sample was processed for 10 min in the RVA from 0 to 1500 rev/min in five increasing speed steps and in two heating steps, as shown in Table 13.

Table 13 RVA profile in terms of time, temperature and speed; the time between readings was 4 sec.

Step	Time (hh.mm.ss)	Type	Value (°C or rev/min)
1	00:00:00	Temperature	25°C
2	00:00:00	Speed	0 rev/min
3	00:00:30	Speed	200 rev/min
4	00:01:00	Speed	500 rev/min
5	00:02:00	Speed	1000 rev/min
6	00:04:00	Speed	1500 rev/min
7	00:04:00	Temperature	85°C
8	00:10:00	Temperature	85°C

3.2 Uniaxial compression

3.2.1 Sample preparation

The molten processed cheese was carefully poured from the RVA canister into two stainless steel cylinders (50 mm in height x 19 mm in diameter) to the brim to avoid the inclusion of air bubbles. The inner surface of the cylinders was lined with polypropylene film (Figure 7). The bottom and upper ends of the cylinders were closed using aluminium foil and tied with a rubber band. The samples were kept at 5 ± 1°C for 24 h prior to physical testing. The cheese samples were removed from the cylinders using an 18 mm diameter plastic rod. The cylinder-shaped samples were then trimmed to a height of 25 mm using a wire cutter.

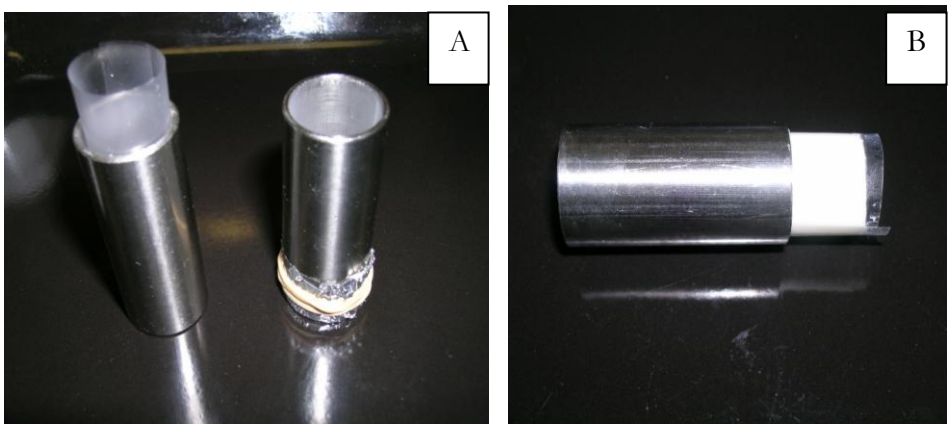


Figure 7 Sample preparation for double compression testing: (A) cylinders lined with polypropylene film and tied with a rubber band at one end; (B) a set cylinder used for double compression testing after trimming.

3.2.2 Uniaxial compression

A uniaxial compression method with a TA-HD compression–tension test instrument (Stable Micro Systems, Haslemere, UK) was used to measure the fracture properties at 5°C. The uniaxial compression was performed on cylinder-shaped samples of 19 mm

diameter and 25 mm height at a constant cross-head speed of 0.83 mm/s. Stepwise procedure is explained in appendix D

The TA-HD instrument was used with a 50 kg load cell with a resolution of 1 g and an accuracy of 0.025%. The distance measurement had a resolution of 0.001 mm. The TA-HD was connected to a personal computer with a data transfer rate of force, displacement and time data triplets of 50 Hz. Temperature was controlled by placement of the instrument and sample in a controlled temperature cabinet. Two parallel Teflon plates were used. Samples were placed between plates that had been lubricated with paraffin oil and were compressed to 80% Cauchy strain, and then a tension stroke was applied so that the top plate moved back to its original position (refer to Figure 8).

The experimental data were initially analysed using Texture Expert Exceed 2.6 software (Stable Micro Systems) and the appropriate data were exported into software (Master Work Software, Tawa, New Zealand) written in J, a functional programming language (McIntyre, 1991; Watkinson et al., 1997). This software (using J) calculated Hencky strain, as suggested by Peleg (1977), which hereafter is abbreviated to strain. The software calculated stress assuming a constant volume during compression. The assumption of a negligible decrease in sample volume upon compression was reasonable for processed cheese (Calzada & Peleg, 1978). The equations were as follows.

Equation 1

$$\varepsilon_0 = \nu \,/\, h_0$$

Equation 2

$$\varepsilon_c = \Delta h_t$$

Equation 3

$$\varepsilon_h = -In\,(1 - \varepsilon_c)$$

Equation 4

$$\sigma = (1000F_t / \pi r_0^2)\,(1 - \varepsilon_c)$$

v = speed of compression (mm/s);

h_0= initial sample height (mm);

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

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

r_0 = initial radius of sample (mm);

ε_0 = initial strain rate (s^{-1});

ε_c = Cauchy strain (−);

ε_h = Hencky strain (−);

σ = stress from lubricated compression test (kPa).

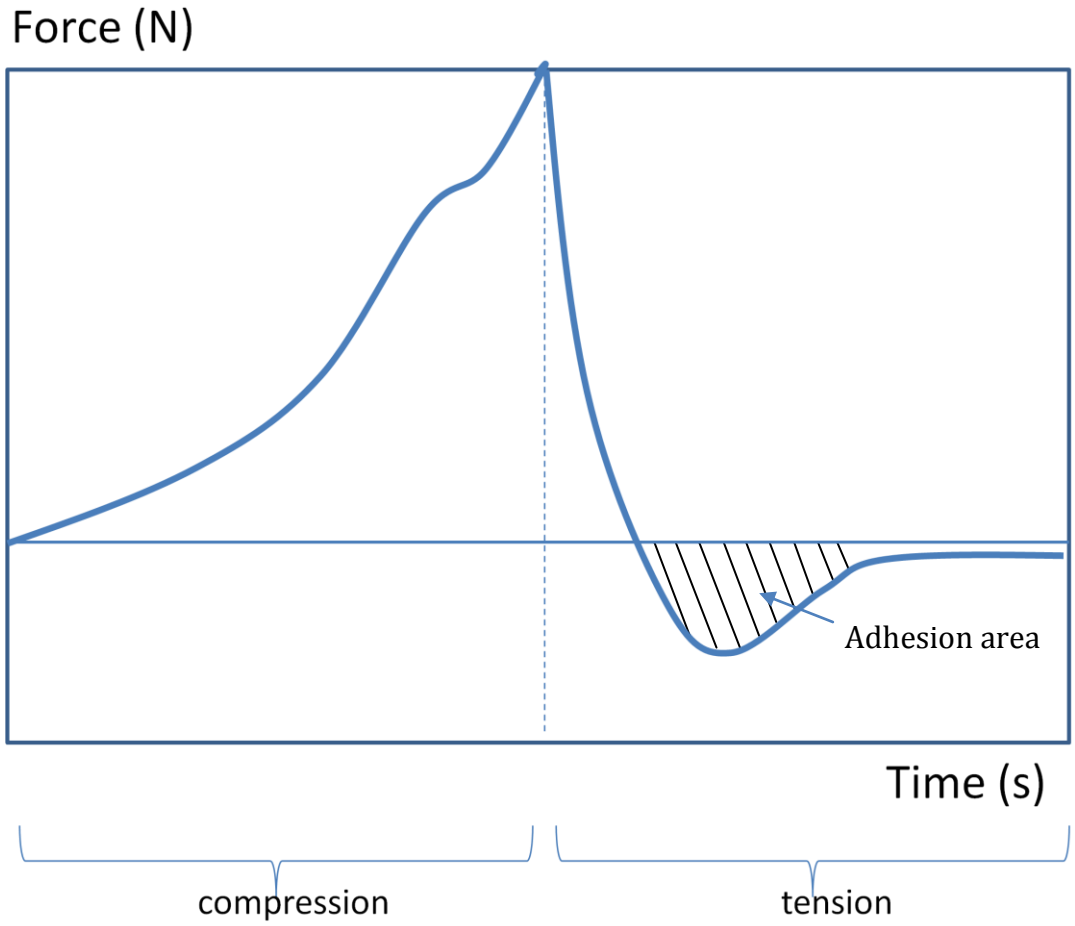


Figure 8 Illustrative force versus time curve for compression and tension stroke.

The apparent fracture stress (σ_f) was the local maximum in stress. The apparent fracture strain (ε_f) was the strain at the local maximum for stress in the stress versus strain curve. The apparent fracture area (A_f) was the area under the curve to fracture. The apparent modulus of deformability (E_d) was the maximum slope of the stress versus strain curve at low strain (typically below 0.03), when the curve was close to a straight line(Figure 9). Although rheological and fracture properties are dependent on ε, the term apparent, used to denote this dependence, is omitted for the sake of brevity;

that is, in simple terms, as shown in Figure 9, fracture stress, fracture strain and modulus of deformability.

This method was used to indicate textural properties as follows: fracture stress indicates firmness (the force to crack cheese); fracture strain indicates longness (the resistance to crumbling); modulus of deformability indicates stiffness (the resistance to dent a surface) (Watkinson et al., 2001).

Adhesion area, "adhesiveness", (Friedman, Whitney, & Szczesniak, 1963; Watkinson et al., 2001) was the area under the negative force peak in the force versus time curve during the tension stroke (see Figure 8).

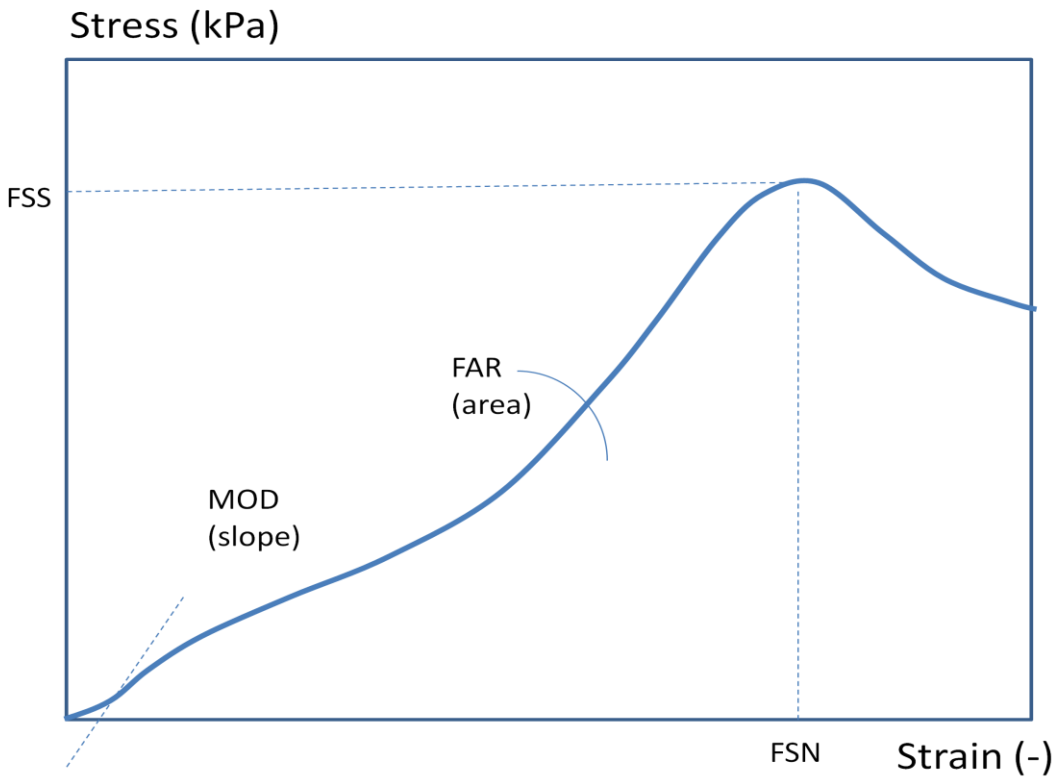


Figure 9 Illustrative stress versus strain curve for compression: FSN = fracture strain; FSS = fracture stress; FAR = fracture area; MOD = modulus of deformability.

3.3 Dynamic oscillatory measurement

The molten processed cheese from the RVA was poured on to a piece of polypropylene film on a flat surface and placed between two 2 mm thick metal strips. It was rolled between two polypropylene film layers using a ceramic rolling pin to achieve a 2 mm thick layer. The formed slice was then packed into a plastic zip lock bag and stored immediately in a refrigerator at 5°C for 24 h. The sample was cut to a 20 mm diameter circle using a sharp-edged cylindrical cutter. The cut samples were placed on the parallel plate geometry (20 mm diameter flat plate) of a 2000 Paar-Physica rheometer (Physica Messtechnik GmbH, Stuttgart, Germany). The upper flat plate was lowered until a 2 mm gap was achieved. The sample was then coated with a thin layer of mineral oil to prevent moisture loss during measurement.

3.4 Sample sections and confocal laser scanning microscopy (CLSM)

A processed cheese sample was cut to 5 mm x 5 mm x 2 mm and allowed to freeze at –20°C for 10–15 min in a cryocut machine (Leica Jung Frigocut 2800 E Cryo-Microtome, Leica Instruments, Nussloch, Germany). Sectioning of the frozen sample to achieve a thickness of about 50 µm was carried out. The cut sections were placed on a glass microscope slide and stained with fluorescent dyes. In order to observe the protein, fat and polysaccharide, three different dyes were used: Fast green (0.2% solution) for protein; Nile blue (0.2% solution) for fat; wheat germ agglutinin (WGA) Alexa Fluor 488 (0.2%) for polysaccharide. The samples were left for 1 h at ~ 20°C to allow the dye to penetrate. A confocal laser scanning microscope (CLSM) Leica (Lasertechnik GmbH, Heidelberg, Germany) was used to capture the microstructure. All samples were

observed at a depth of 15 µm. The emission wavelengths for Nile blue, Fast green and WGA were 488, 580 and 488 nm respectively.

3.5 Fat globule size determination

A Malvern Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK) was used to determine the fat particle size distribution in the processed cheese samples. A sample of processed cheese (0.2 g) was dispersed in a solution (50 mL) containing EDTA (0.375% wt/wt) and Tween 20 (0.125% vol/vol) and the pH was adjusted to 10 using sodium hydroxide (Walstra, 1965). After standing overnight in a refrigerator (5°C), the samples were allowed to equilibrate to room temperature for 1 h. About 3 mL of suspension was loaded into the cell of a Malvern Mastersizer 2000. The fat particle size obtained, $D(3,2)$, was the volume-to-surface average diameter of the particles, and was used as a measure of the relative size of the fat particles in the processed cheese samples.

3.6 Squeezing flow test

Squeezing flow has been used to describe cheese melting phenomena (Campanella & Peleg, 2002; Wang et al., 1998). A schematic diagram of a device that performs squeezing flow on cheese, called a UW melt meter (Wang et al., 1998), is shown in Figures 10, 11 and 12. It was made at the University of Wisconsin-Madison, Madison, WI, USA. This device consists of an aluminium body with a doughnut-shaped heater inside, and a temperature controller unit (CN 4400, Omega Engineering Inc., Stanford, CT, USA) to control the heater. The heater is in contact with a stationary piston. The outer ring can be moved up and down around the stationary piston using the lever arm. A circular plate is attached to an LVTD (linear variable differential transformer) to

monitor the flow of cheese upon melting. Data acquisition software supplied with the UW melt meter (DAS 16G High Speed Analog 110 Board, Metrabyte Corp., Taunton, MA, USA; Easyest LX Software, Asyst Technologies, Inc., Rochester, NY, USA) was used for data collection and analysis.

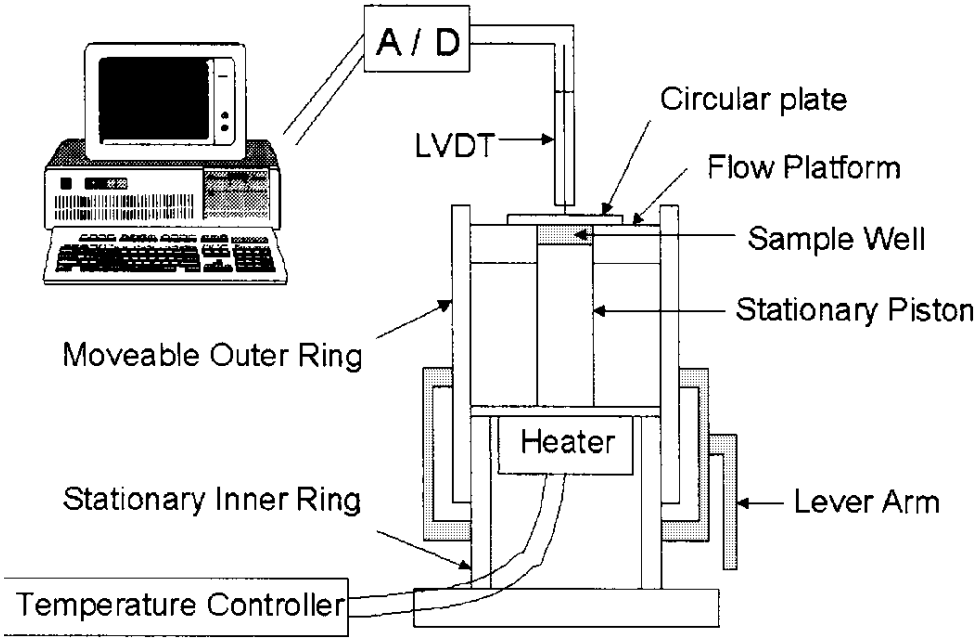


Figure 10 Schematic diagram of the UW melt meter, a modified squeezing flow test device: A/D = analog-to-digital converter; LVDT = linear variable differential transformer(Wang et al., 1998).

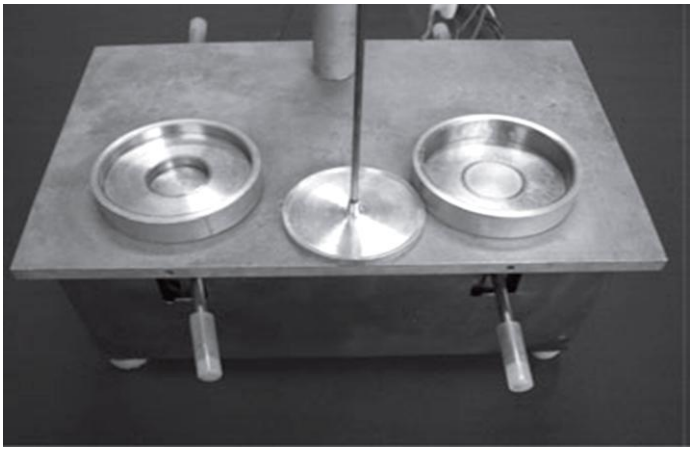


Figure 11 Photograph of the modified squeezing flow device, the UW melt meter, with two sample wells and the LVDT.

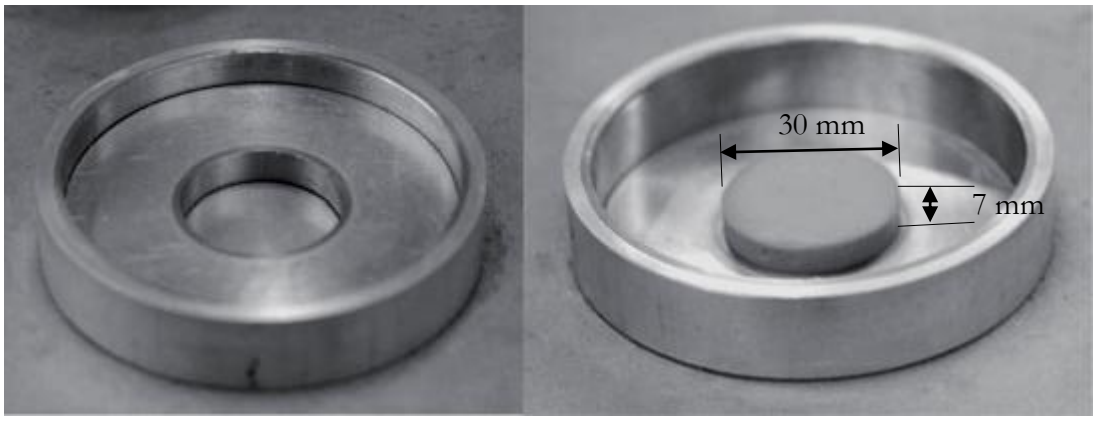


Figure 12 **(Left) Sample well in the UW melt meter when the outer ring is in the up position. (Right) Sample specimen formed when the outer ring is in the down position.**

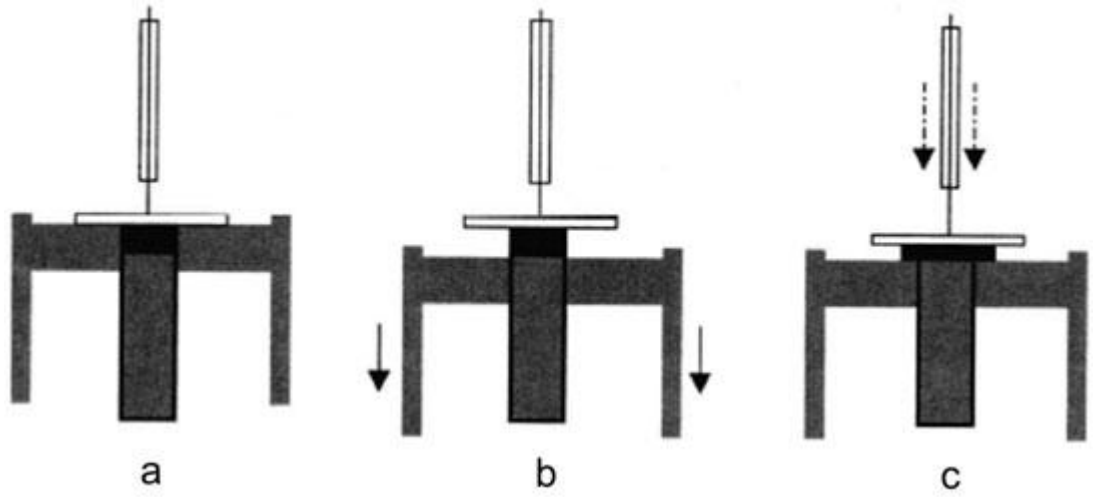


Figure 13 **(a) Starting position while the sample is resting; (b) the lever is lowered and the upper plate is in contact with the sample; (c) the load governed by the upper plate squeezes the sample (Wang et al., 1998).**

The temperature of the melt meter was set before the specimen was placed on it. Both the top surface of the melt meter and the circular plate were lubricated with mineral oil to ensure lubricated squeezing flow. The cheese sample was heated while covered with

a circular plate to prevent moisture loss. The temperature of the cheese that was in the sample well was initially monitored by a digital thermocouple thermometer inserted into the sample. After the time to reach the target temperatures of 25°C (in Chapter 7) and 60°C (in Chapter 9) had been determined (7 min), the thermocouple was no longer used. Once the sample reached the test temperature, the data acquisition system was started; the lever arm was then raised to lower the outer ring and allow the sample to flow under the constant force (as shown in Figure 13). Different forces were used for model processed cheese (Chapter 7) and very low fat Mozzarella cheese (Chapter 9). The height of the specimen was recorded as a function of time.

Empirical meltability was calculated as the ratio of the height of the melted cheese at 240 s to the initial height of the cheese. During the test, the software monitored data points rather than time. Thus the number of data points collected was 1200, calculated from the data acquisition rate of 5 data points per second. Biaxial elongational viscosity and biaxial strain rate calculations were performed using macros in Excel (Microsoft Corp).

3.6.1 Squeezing flow using constant force test

This squeezing flow test was used in a constant force (creep) mode (although it can also be used with a uniaxial test instrument in a constant speed mode). The kinematics of biaxial elongational flow can be described by the following set of equations (Dealy, 1995).

Equation 5

$$V_z = -2\dot{\epsilon}_B z$$

$V_r = \dot{\epsilon}_B r$

$V_\theta = 0$

where V_z, V_r and V_θ are the vertical, radial and angular velocity components, z is the axial distance, r is the radial distance and $\dot{\epsilon}_B$ is the biaxial strain rate.

The net stretching stress, σ_B, (Dealy, 1995) can be calculated from the squeezing force (F) divided by the momentary cross-sectional area of the specimen ($A(t)$).

Equation 6

$$\sigma_B \equiv \sigma_{rr} - \sigma_{zz} = \frac{F}{A(t)}$$

where $A(t) = H_0 A_0 / H(t)$, assuming a constant specimen volume, $H(t)$ is the height at time t, H_0 is the height at time zero and A_0 is the initial cross-sectional area of the specimen.

The biaxial stress growth coefficient (BSGC), η_B, is defined as (Dealy, 1995):

Equation 7

$$\overset{+}{\eta}_B(t, \dot{\varepsilon}_B) \equiv \frac{\sigma_B}{\dot{\varepsilon}_B}$$

Stress growth has units of viscosity and, under steady conditions, is called biaxial extensional viscosity (Dealy, 1995). As steady conditions were not achieved in our tests, we used the biaxial stress growth coefficient (BSGC), which describes the resistance of the melted cheese to flow.

The following formula was obtained from Equations (5), (6) and (7):

Equation 8

$$\eta_B^+ = \frac{2FH^2(t)}{H_0 A_0 (-\frac{dH(t)}{dt})}$$

3.7 T_2 relaxation measurements

Nuclear magnetic resonance (NMR) experiments were undertaken using a Bruker AMX 200 MHz horizontal wide-bore magnet (Rheinstetten, Germany). The proton transverse (T_2) relaxation was measured using the Carr–Purcell–Meiboom–Gill (CPMG) spin-echo pulse sequence. A CPMG sequence containing 70 echoes was sampled, the echo time ranged from 1 to 150 ms and each sample was analysed at 25°C.

The MRI/NMR software Proposa (Wellington, New Zealand) was used to perform the spectrum processing and analysis. The T_2 relaxation constant distributions were fitted to the relaxation properties of the water and fat components using the Non-Negative Least Squares Inverse Laplace Transform Method in Proposa.

3.8 Chemical analysis of cheese

3.8.1 Fat , protein and moisture content for processed cheese

The FoodScan™ analyser was used to determine the fat content, protein content and moisture content of all cheese products. This analyzer is based on NIT (Near Infrared Transmittance) technology, which can be used for determination of moisture. From a tungsten-halogen lamp, light is guided through an optical fibre into the monochromator inside the instrument that provides monochromatic light in the spectrum from 850nm to 1050nm. Through an optical fibre, light is guided to the collimator lens system, which

is mounted over the sample cup in the sample cup room. After the light is transmitted through the sample, the unabsorbed light reaches the detector. The detector measures the amount of light, and sends the result to the digital signal processor (DSP), that communicates with the PC, also calculating the result. Rotation of the sample cup between sample scans (called sub-samples) allows analysis of various parts of the sample. The sub-samples are chosen from one or two concentric circles in the sample cup, giving a more representative result from an inhomogeneous sample.

3.8.2 Fat and protein content for Mozzarella cheese

The amount of fat in mozzarella cheese was determined by modified Babcock test (Kosikowski & Mistry, 1999). In this test, protein is digested by adding sulfuric acid, and the heat generated melts the butterfat. Centrifugation forces the clear, liquid fat into the graduated columns of the test bottles where it is measured after added hot water raises its level above the stem.

The total protein content in Mozzarella cheese was determined by Kjeldahl method (Kosikowski & Mistry, 1999). In this test, a fixed weight of cheese is digested by heating in sulfuric acid. The solution is made alkaline and distilled, using a Kjeldahl apparatus, into a standard-strength hydrochloric acid of fixed volume. The liberated ammonia coming over neutralizes a portion of the hydrochloric acid. The acid which remains is determined by back-titrating with a standard sodium hydroxide solution. Protein for milk products is calculated by converting the ammonia (NH3) using a standard conversion factor. 6.38.

3.8.3 Lactose content: LactPS

This test specifies a photometric method for the determination of the lactose content of cottage cheese, processed cheese, natural and cream cheese. In this method, the sample is dissolved in an appropriate reagent. The protein and fat is then removed by precipitation and filtration. The lactose in the filtrate is determined calorimetrically by reaction with phenol and sulphuric acid. The lactose content is given as a percentage for the original sample.

3.8.4 Determination of pH

pH is a way of expressing acidity or alkalinity. The acidity is caused by hydrogen ions (H^+), and the concentration of these hydrogen ions is a measure of the acidity. In water - and in practice pH is nearly always measured in water solutions – the following equilibrium exists:

$$H_2O \rightleftharpoons H^+ + OH^-$$

The H^+ concentration in pure water is equal to 10^{-7} mol/L – corresponding to pH = 7. A pH value of 7 therefore signifies a neutral solution. A solution having a pH below 7 is said to be acidic, and a solution having a pH above 7 is aid to be alkaline or basic.

Chapter 4 Manufacture of model processed cheese in a Rapid Visco Analyser (RVA)

4.1 Introduction

The processed cheese used in this research project was a key part of the experimental work. Most small scale or pilot-scale research and development is carried out on equipment such as a twin-blade cooker/blender or a lower shear twin-screw cooker/blender. These cookers are labour intensive and require significant quantities of raw material. Moreover, the processes are highly dependent on the skill of the operator, whose manual control of each batch is more important than the operating guidelines. Thus, such equipment is not ideal for multiple replicate trials.

The RVA provides an alternative. It is a device that can mix and heat 30 g of material in a canister while measuring its apparent viscosity. The published literature (Kapoor, Lehtola, & Metzger, 2004; Kapoor & Metzger, 2005) on the manufacture of processed cheese using the RVA indicates that it can be used for small scale production and as a tool for evaluating the effects of formulation and processing parameters on the functional properties of processed cheese. This published research indicates that the functional properties of processed cheese manufactured using the RVA correlate with those of processed cheese produced on a pilot scale (Kapoor & Metzger, 2005). In addition, the final apparent viscosity during manufacture using the RVA correlates well with the functional properties of the processed cheese.

For replicate studies of processed cheese, the composition of each cheese must be tightly controlled; otherwise any measurement of its functional properties may be invalid. One of the biggest challenges is controlling the moisture content, especially with small scale cookers that use direct steam injection (DSI). Although the RVA eliminates the variability associated with DSI by using indirect heating, its canister is not sealed and moisture can escape by evaporation during cooking. Such moisture losses could lead to a deviation from the targeted composition, which, in turn, could affect the physicochemical properties of the processed cheese (Kapoor & Metzger, 2008). One of the objectives of this study was to measure the evaporative losses and then to adjust the formulations to allow for the moisture loss.

For this study, a model processed cheese in which the polysaccharide component could be systematically varied while holding the other components constant was required. To create some "space" in these formulations, lactose was included. In the commercial manufacture of processed cheese, lactose is usually added in the form of whey powder or skim milk powder. In this study, the only protein source used in the manufacture of the processed cheeses was rennet casein, which is essentially lactose free. Therefore, it was possible to use lactose as an ingredient to create some room to allow polysaccharide to be incorporated in the selected model processed cheese formulation.

The problems that are most commonly associated with the inclusion of lactose in processed cheese are browning and crystal growth. The processing times and temperatures in this study were not high enough to lead to significant discolouration. As mentioned in the review of the literature, crystal growth can usually be avoided by

ensuring that the lactose concentration is less than its saturation concentration at the appropriate temperature; for example, the saturation concentration is 16.9 g lactose/100 g water at 15°C and 11.0 g lactose/100 g water at 0°C. However, a lower lactose concentration is usually used in processed cheese because of the presence of precipitating nuclei and other competing water-soluble constituents, lower storage temperatures and the extended shelf life of the products (Lee et al., 1999).

4.1.1 Objectives

The objectives of this study were as follows.

(1) Develop a model system in which the protein and polysaccharide contents could be varied systematically while holding the other components constant.

(2) Characterise the evaporative losses in the model system.

(3) Measure the functional properties of the model system while varying the composition.

(4) Establish the limits or boundaries at which the product would be too soft or too viscous.

It was hoped that the study would lead to a better understanding of how proteins and polysaccharides interact in processed cheese.

4.2 Materials and methods

4.2.1 Model processed cheese formulation

The formulation chosen for the initial experimentation work is shown in Tables 14 and 15. This formulation was created by S. K. Lee, based on extensive research work on the

functional and rheological properties of processed cheeses (Foegeding, Lee, & Buwalda, 1996; Lee et al., 1999; Lee, Buwalda, Euston, Foegeding, & McKenna, 1995). It was constructed using software based on Microsoft Excel. Xanthan gum was chosen as a starting point for the addition of polysaccharide to processed cheese.

Table 14 The standard model cheese formulation used for the experimentation work

Ingredient	Quantity (g)	Quantity (%)
Soya oil	9.110	30.367
Rennet casein (90 MESH)	3.710	12.367
Lactose	1.045	3.483
Citric acid (CA)	0.215	0.717
Trisodium citrate ($2H_2O$) (TSC)	0.840	2.800
Salt (NaCl)	0.300	1.000
Added water	14.780	49.267
TOTAL	30.000	100.000

Table 15 Chemical analysis of the standard model processed cheese formulation calculated using software

Composition	Analysis
pH (target = midpoint range)	5.7
Moisture	50.99%
Fat	30.40%
Fat in dry matter (FDM)	62.03%
Salt (NaCl)	1.00%
Ash	1.89%
Total casein	9.99%
Total protein	10.00%
Lactose	3.48%
Calcium	0.346%

4.2.2 Choosing lactose as a filler

In this study, lactose was chosen as a floating ingredient to accommodate changes in the polysaccharide content. At concentrations below 5.0%, lactose could be used as a "filler" in model processed cheese formulations without any adverse effects on the functional, sensory or nutritional properties (Lee et al., 1999). Lactose contents below 5.0% appeared to have little effect on the fracture stress, fracture strain, meltability, colour change and microstructure of the model processed cheese. As the lactose concentration was lower than the saturation concentration (11.0 g lactose/100 g water at 0°C), crystal growth was not an issue in the chosen model processed cheese formulation. As shown

in Table 16, the polysaccharide content was varied from 0 to 2.5% wt/wt in the model formulation by replacing it with an equal amount of lactose.

Table 16 Formulation of model processed cheese at pH 5.7; polysaccharide was swapped with lactose and protein was swapped with moisture

Fat (%)	Polysaccharide (%)	Lactose (%)	Protein (%)	Moisture (%)	TSC + CA + Salt (%)
30.0	0–2.5	3.5–1.0	14.0–8.0	46.0–52.0	4.5

Polysaccharide + lactose = 3.5% Protein + water =60.0%

4.2.3 Varying the protein content

The protein content in the model processed cheese formulation was varied from 8.0 to 14.0% and the moisture content was adjusted to maintain 100% (as shown in Table 16). The other components in the model processed cheese were kept constant. Therefore, in this study, a high protein content meant a low moisture content. This model was chosen deliberately to achieve a strategic significant difference in terms of mechanical and textural properties of the processed cheese at each protein content. The moisture/protein ratio was varied across such a wide range to create texture differences to mimic different products, from spread processed cheese to hard processed cheese. The chemical compositions of a few processed cheese samples with different protein and moisture contents were analysed (Table 17).

Table 17 Average chemical composition of experimental model processed cheese samples analysed in duplicate.

Sample	Moisture (%)	Fat (%)	Protein (%)	Intact Casein (%)
1	53.6 ± 0.1	30.4 ± 0.2	8.0 ± 0.3	6.37±0.05
2	51.2 ± 0.2	30.4 ± 0.2	9.9 ± 0.3	7.96±0.05
3	48.8 ± 0.1	30.4 ± 0.2	11.9 ± 0.2	9.55± 0.1
4	46.5 ± 0.1	30.4 ± 0.2	14.0 ± 0.1	11.6±0.05

4.2.4 Processing in the RVA

Refer to Section 3.1.

4.2.5 Sample preparation for uniaxial compression

Refer to Section 3.2.

4.2.6 Dynamic oscillatory measurement

Refer to Section 3.3.

4.2.7 Selection of cheese model for lactose study

Gunasekaran and Mehmet (2003) have shown that the relation between the texture and the composition of several cheeses follows the sequence of a decreasing contribution to the texture parameter: protein > NaCl > water > pH > fat. Moreover, Foegeding et al. (1996) have suggested that the critical minimum fat content to alter the elastic rigidity (G') is 5.0%. Therefore, fat was chosen as a lactose replacer in the formulation (as shown in Table 18) to measure the effect of the variation in lactose content on the small

strain rheological properties of the model processed cheese. The lactose content in the formulation was varied from 3.5 to 1.0% and the fat content was varied from 30.0 to 32.5% to measure *G*' and *G*" at a constant protein content of 10% A frequency sweep at a strain of 1.0% (as explained in Section 2.6.3) was carried out at 5°C.

Table 18 Formulation of model processed cheese at pH 5.7; the fat content was swapped with lactose content

Fat (%)	Lactose (%)	Protein (%)	Moisture (%)	TSC + CA+ Salt (%)
30.0–32.5	3.5–1.0	10.0	50.0	4.5

Fat + lactose = 33.5%

4.2.8 Statistical analysis

The samples for small strain rheological measurement were prepared in triplicate. Statistical analysis of the *G*' and *G*" data was carried out using one-way analysis of variance (ANOVA) to study the effects of lactose content on *G*' and *G*" (general linear model procedure, Minitab Version 15.1). A *P* value of < 0.05 was declared to be significant. Means, standard deviations and *P* values are shown in Figure 15.

4.3 Results and discussion

4.3.1 Moisture losses during processing

In this study, the RVA processing profile was adapted (Section 3.1.2) based on the published literature (Kapoor et al., 2004; Kapoor & Metzger, 2005; Prow & Metzger, 2005). A temperature of 85°C, which is close to the commercial cook temperatures of the New Zealand dairy industry, was used as the cook temperature. Moisture loss

during RVA processing was characterised and the moisture losses were determined to adjust the formulation to keep the final processed cheese within the typical composition specifications.

The moisture loss study was carried out using the formulation shown in Table 14. The interconnected protein and moisture contents as well as the polysaccharide content were varied while keeping the remaining components in the composition unchanged (as shown in Table16). The moisture losses varied from 3.77 to 5.64% for the entire protein and polysaccharide range (as mentioned in Table 16) in the formulation. The moisture loss data were displayed using a contour plot, as shown in Figure 14. The moisture loss data for this study are provided in appendix E.

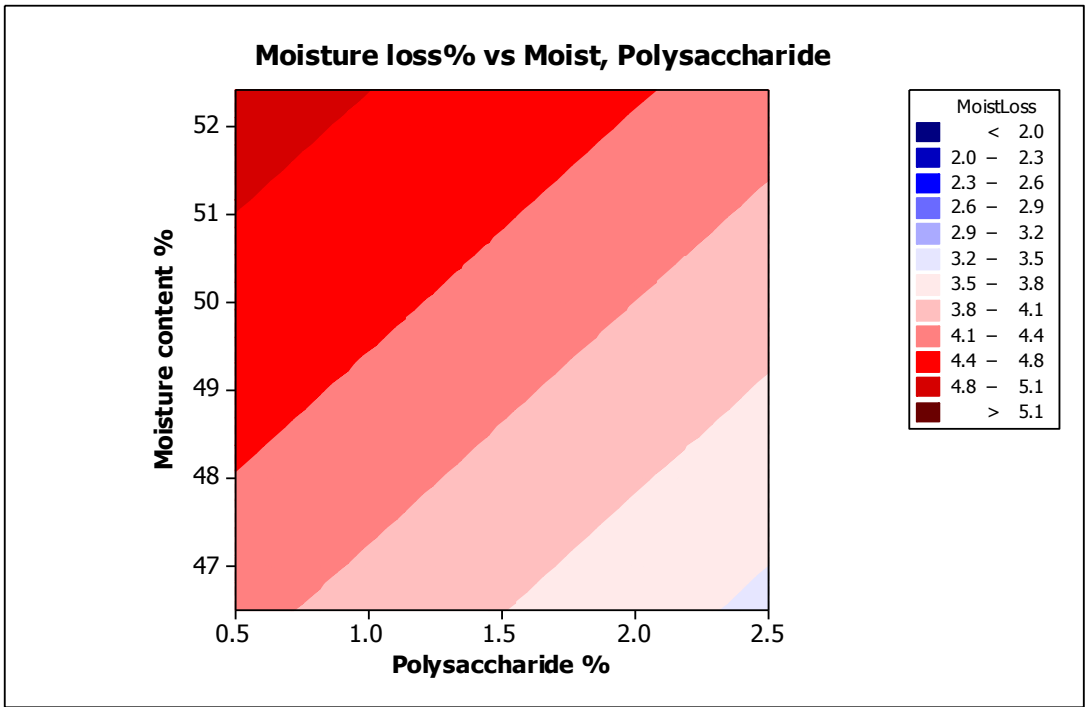


Figure 14 Contour plot of moisture losses for the model formulation with a polysaccharide content from 0.5 to 2.5% and a moisture content from 46.0% (14.0% protein) to 52.0% (8.0% protein).

As shown in the contour plot in Figure 14, the moisture loss increased with an increase in the moisture content and a corresponding decrease in the protein content. In addition, the moisture loss decreased with an increase in the polysaccharide content. The increased moisture loss with increased moisture content could have been because greater amounts of water were able to escape from the product. As the protein and polysaccharide contents increased, the moisture losses decreased. These decreases could have been because the polysaccharide and protein were able to bind the available moisture in the cheese mixture, which, in turn, allowed less moisture to escape during RVA processing.

4.3.2 Effect of lactose

Although it is thought that lactose has minimal effect on the rheological properties of a model processed cheese formulation, a study to check the ability of lactose to act as a filler in the current formulation was carried out.

The overall trend shown in the regression chart (Figures 15A and 15B) indicated that *G*' and *G*" decreased slightly with an increase in the lactose content in the model processed cheese formulation. However, the *P* values for *G*' (0.231) and *G*" (0.074) indicated that the means were not significantly different. Therefore, it can be inferred that an increase in the lactose content from 1.0 to 3.5% did not significantly alter the structure of the model processed cheese.

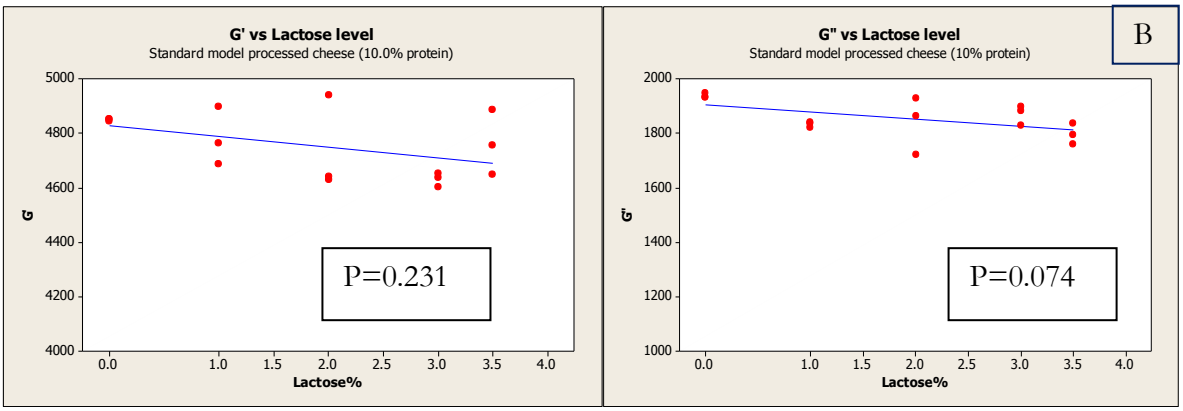


Figure 15 Scatter plots of (A) *G'* and (B) *G"* for processed cheeses containing lactose at 0.0, 1.0, 2.0, 3.0 and 3.5%. Values of *G'* and *G"* at a frequency of 0.1 Hz were selected for comparison.

The results agree with the findings of Lee et al. (1999). They observed that replacement with lactose up to 5.0% did not deplete the protein content to a level at which the protein network formation was affected; hence, the hardness did not change to a great extent. However, the hardness measured at 5°C showed a decreasing trend at lactose contents $\geq$ 5.0%.

4.3.3 Boundary conditions

RVA cooking is a fairly short process and can handle a small sample quantity. An experiment was carried out to determine the limit for the variation in the protein and polysaccharide contents in the model processed cheese formulation. The ability of the product to perform in terms of RVA processing, sample preparation and textural evaluations needed to be further investigated.

4.3.3.1 Processing in the RVA

As shown in Table 16, the model processed cheese formulations in which the protein content was varied from 8.0 to 14.0% were tested to determine the minimum and maximum amounts of protein that could give a stable homogeneous processed cheese at polysaccharide contents of 0–2.5%.

The visual observations, as shown in Table 19, and the RVA viscosity profiles of the model processed cheeses suggested that the flow behaviour was affected by the moisture content and the protein content of the samples. Increasing the moisture content and decreasing the protein content reduced the RVA viscosity, thus increasing the flow. At high polysaccharide and protein contents (2.5% polysaccharide and 14.0% protein), the cheese mix was very thick throughout the RVA processing. Moreover, one of the repeats showed phase separation at the end of the processing run. Three main factors could have caused this fat separation. (1) The high viscosity could have caused a spindle slip and, hence, the shear force of the RVA was not sufficient for the formation of a proper emulsion. (2) There could have been insufficient emulsifying salts for a higher protein/moisture ratio. A decrease in the added moisture and an increase in the protein content would have caused the creation of a much less open protein conformation, such that the emulsifying salts could not permeate easily between the casein molecules and emulsify them to a greater degree. (3) Competition for water between proteins and polysaccharides may have led to insufficient hydration of the proteins and a reduced ability to emulsify fat.

Table 19 Summary of observations from a visual grading of some of the RVA processed cheeses

Protein (%)	Water (%)	Polysaccharide (%)	Lactose (%)	Observation (during RVA processing)
8.0	52.0	0.0	3.48	Easy to process
8.0	52.0	1.0	2.48	Easy to process, no abnormalities
8.0	52.0	2.5	1.00	Easy to process, no abnormalities
10.0	50.0	0.0	3.48	Easy to process, no abnormalities
10.0	50.0	1.0	2.48	Easy to process, no abnormalities
10.0	50.0	2.5	1.00	Easy to process, slight swirl effect on the spindle of the RVA
12.0	48.0	0.0	3.48	Easy to process, no abnormalities
12.0	48.0	1.0	2.48	Easy to process, no abnormalities
12.0	48.0	2.5	1.00	High viscosity after 4 min of processing. Swirl effect on the spindle of the RVA
14.0	46.0	0.0	3.48	Easy to process, no abnormalities
14.0	46.0	1.0	2.48	Thick gel. Swirl effect on the spindle of the RVA
14.0	46.0	2.5	1.00	Very thick at the beginning of the process. Swirl effect on the spindle of the RVA

4.3.4 Sample preparation

4.3.4.1 Cylinder formation

At high protein and polysaccharide contents, two issues in this study were the transfer of the molten cheese from the RVA into the stainless steel cylinders (50 mm in height x 19 mm in diameter) and the formation of 2 mm thick slices. After RVA processing, as the processed cheese cooled, it thickened very quickly, especially for the samples containing polysaccharide. Such thickening made it difficult to transfer the cheese and pack it into a narrow-mouthed stainless steel cylinder. Moreover, because of the small size of each cheese sample, the whole sample was needed to prepare duplicates. To minimise any thickening while the cheese was being transferred, it was rapidly poured and scraped out of the RVA canister in one movement with the help of a Teflon spatula.

4.3.4.2 Air inclusion

Air bubbles were observed in the cheese cylinders if the molten processed cheese had not been poured out or transferred sufficiently rapidly. Air bubbles can affect the fracture initiation point in the cheese, which, in turn, can affect the double compression fracture stress and fracture strain results. Therefore, if a sample was observed to have significant bubbles or cracks, the results were omitted.

4.3.4.3 Slice preparation

To counter a similar problem with individually wrapped slices (IWS), a metal guide of 2 mm thickness and a heavy roller made from ceramic (Figure 16) were used to form an even slice from the hot RVA processed cheese, which thickened rapidly as it cooled.

During RVA processing, the cheese samples containing 2.5% polysaccharide and 14.0% protein were found to be too thick to pour/transfer from the RVA canister into a stainless steel cylinder.

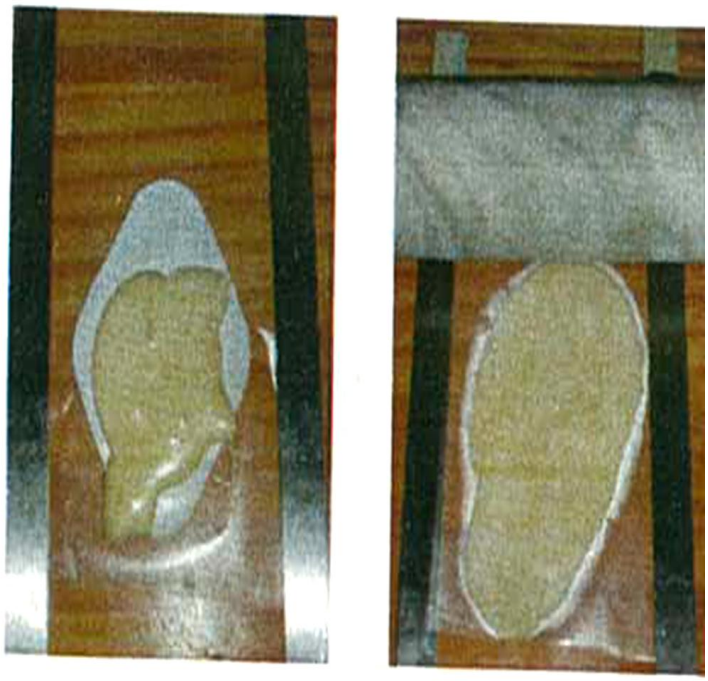


Figure 16 Slice formation: (left) molten processed cheese poured on to polypropylene film; (right) two metal strips flanking the molten processed cheese during rolling by a heavy roller.

4.3.5 Processed cheese texture

Figure 17 is based on visual observations after the processed cheese samples with protein contents from 8.0 to 14.0% and polysaccharide contents from 0.0 to 2.5% had been cooled to 5°C and stored for 24 h. The processed cheese with a protein content of 8.0% and no polysaccharide produced a gel that was too soft to remain solid. However, cracking was detected when the samples were compressed at 80% compression, indicated by a black dot with a red border in Figure 17. The processed cheese samples indicated by red dots had sufficient firmness to form a shape on cooling. Moreover, the

texture was smooth and homogeneous without any visible structural irregularities. A few samples with protein contents of less than 8.0% did not show any signs of a fracture point when compressed at 80% compression. Therefore, any samples with less than 8.0% protein were not used for this study.

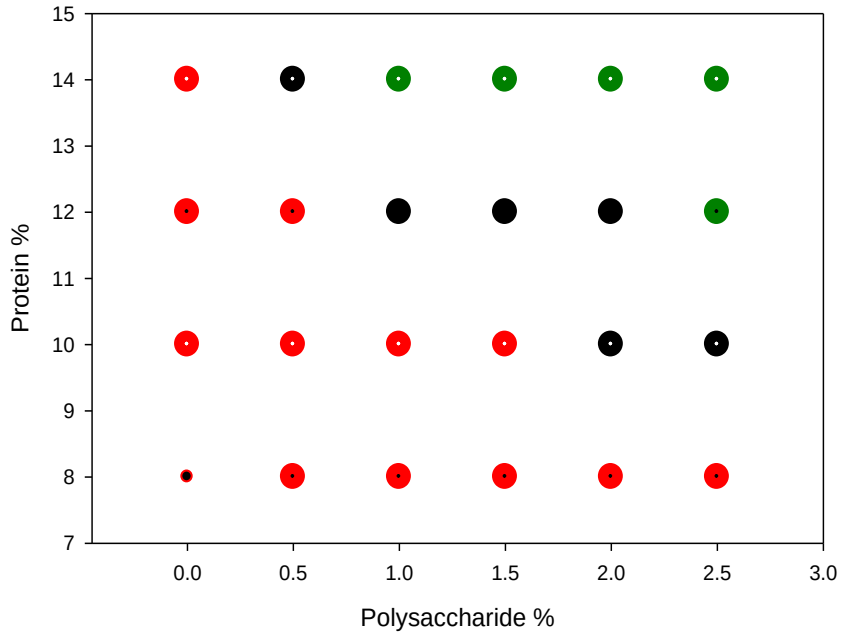


Figure 17 Textures of a range of processed cheeses at protein contents from 8.0 to 14.0% and polysaccharide contents from 0.0 to 2.5% are shown by different coloured dots: ●, very firm elastic gel; ●, firmer, elastic and homogeneous gel; ●, firm elastic and homogeneous gel; ◙, soft protein gel.

The processed cheese samples indicated by black dots were more firm, elastic and homogeneous and those indicated by green dots were very firm and elastic. At the extreme of the design, i.e. 14.0% protein and 2.5% polysaccharide, the processed cheese samples were extremely firm and very glossy.

4.3.6 Determination of linear viscoelastic region

It is well known that, at increased protein and polysaccharide contents, processed cheese behaves more like a viscoelastic solid. As this has an effect on the region of linear viscoelasticity, it was critical for this study to establish a linear viscoelastic region for a wide range of protein (8.0–14.0%) and polysaccharide (0.0–2.5%) contents. The dynamic rheological data obtained in this experiment included two components: the storage modulus component (G') and the loss modulus component (G'') (Figure 18). With the frequency (w) held constant at 1 Hz, values of G' and G'' were obtained at various shear strains, resulting in a strain sweep to determine the linear viscoelastic region.

The strain sweep behaviour of model processed cheeses with various protein and polysaccharide contents is presented in Figure 18. The dynamic mechanical properties of the processed cheeses at all protein and polysaccharide contents remained constant up to about 8.0% strain at a temperature of 5°C. Moreover, the strain sweep experiments suggested that there was no deviation from linear viscoelasticity at 0.1% strain for all processed cheese samples regardless of the protein and polysaccharide contents. Therefore, in future experimental work, 0.1% strain was often used to measure the rheological properties of the processed cheeses using a small amplitude oscillatory shear test.

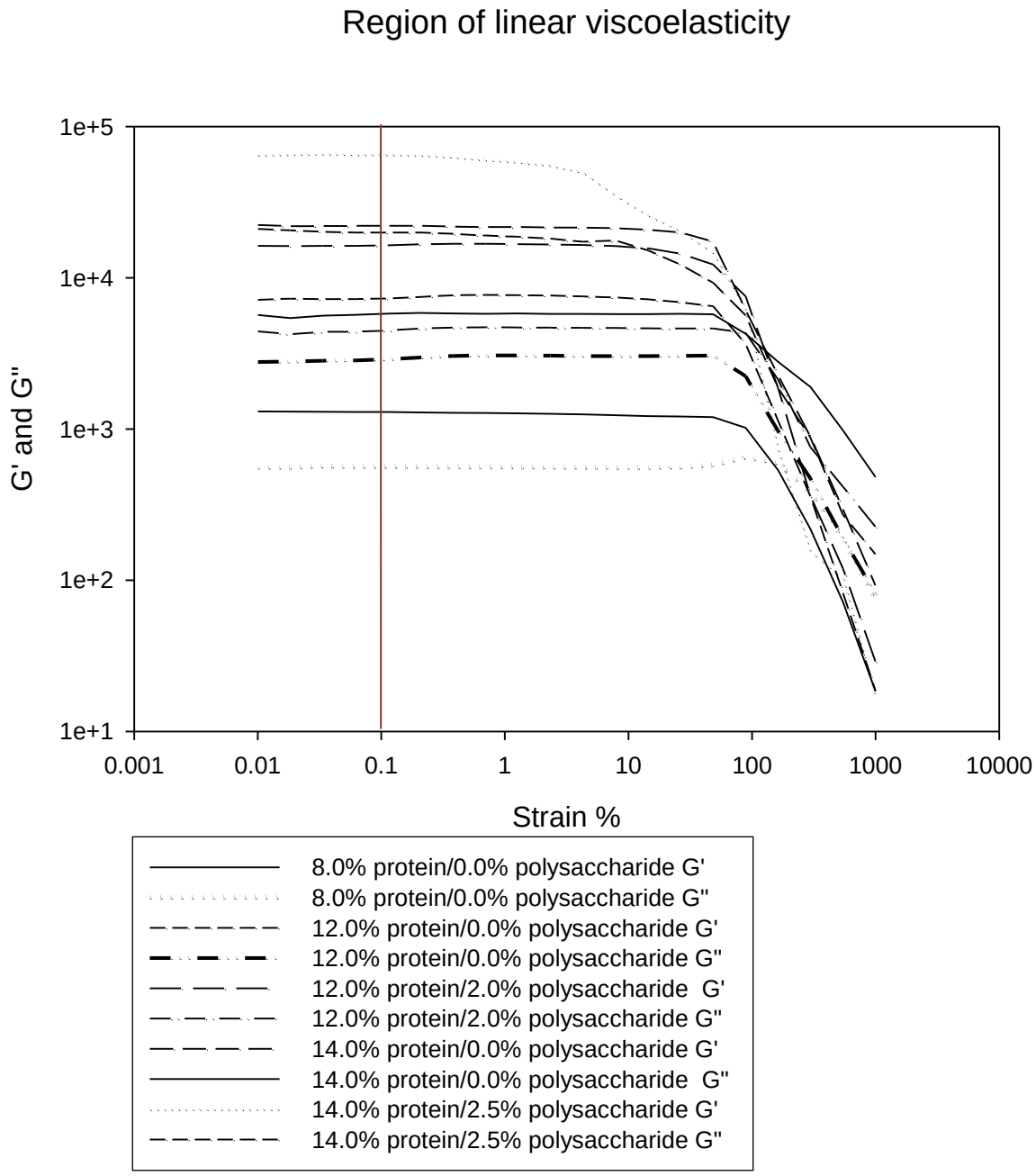


Figure 18 Strain sweeps carried out at 5°C for processed cheese formulations with protein contents from 8.0 to 14.0% and polysaccharide contents from 0.0 to 2.5%.

4.3.7 pH

The pH values of the final processed cheeses, regardless of whether they had higher protein or polysaccharide content, remained unchanged at 5.7 $\pm$ 0.05.

4.3.8 Complexity in compression testing

Compression fracture testing is used as a standard method for natural cheeses. However, compression is not often used for processed cheese because it has a different structure from natural cheese and therefore behaves differently. The yield point of processed cheese can be more difficult to detect, especially when tested at a higher temperature such as 13°C. Moreover, the homogeneous nature of the structure in processed cheese makes it more difficult to get a clear fracture under compression. Therefore, the test temperature chosen for all compression testing in this work was 5°C.

There are both positives and negatives when using compression as a texture measuring tool for model processed cheese. Compression fracture analysis is a simple and straightforward method that gives a lot of information about the product. Inclusion of any bubbles and crack formation when the molten processed cheese is poured into the stainless steel cylinders can lead to premature fracturing. However, samples with air were eliminated to reduce any unwanted variability.

4.4 Conclusions

Based on the knowledge gained from this work on numerous processed cheese formulations, it was concluded that the RVA could be used successfully to make processed cheeses with defined protein, moisture, polysaccharide and lactose contents.

Moisture was added back to compensate for the losses observed in the moisture loss study, thus standardising the composition of the processed cheeses against the targets. It was concluded that lactose contents from 1.0 to 3.5% did not change the cheese structure significantly. Therefore, lactose could be used as a filler in the model processed cheese formulation.

Minimum and maximum protein and polysaccharide contents were determined to ensure that the processed cheeses were homogeneous and suitable for textural analysis. As the cheese with 8.0% protein and no polysaccharide was too soft, it was decided that cheeses with protein contents below 8.0% could not be used for any high strain fracture analysis. In addition, 14.0% protein and 2.5% polysaccharide was a critical level at which the mixture was almost too "solid" and any cheese with above this protein content could not be processed into a homogeneous mass in the RVA. Moreover, at these protein and polysaccharide contents, the molten cheeses were very difficult to pour into the narrow-mouthed cylinders when preparing samples for double compression testing.

Chapter 5 The synergistic effect of xanthan gum and locust bean gum in model processed cheese

5.1 Introduction

Processed cheeses are usually produced by using specific amounts of a limited number of ingredients: water, fat, protein, emulsifying salts and stabilisers. A cheese-like structure is formed when these ingredients are mixed and heated to 80–85°C. The interactions resulting from the mixing of such ingredients are mainly responsible for the structural and textural properties of processed cheese (Fox et al., 2000). Polysaccharides are added to processed cheese to bind water and protein, resulting in altered textural properties of the final product.

The influence of non-starch polysaccharides on the functional properties of imitation cheese is well known (Kiziloz, Cumhur, & Kilic, 2009). The properties of one polysaccharide can be modified by interactions with other polysaccharides (Jana, Patel, Suneeta, & Prajapati, 2010). Synergistic interactions between polysaccharides are of commercial interest because they offer the prospect of generating novel functionality. They can also produce given rheological or textural characteristics using reduced levels of polysaccharides and thereby can possibly reduce cost.

The synergistic interaction between xanthan gum (xanthan) and galactomannans, such as locust bean gum (LBG), is well known and widely exploited by the food industry. Xanthan has been found to form stronger, elastic, thermoreversible gels when mixed with LBG (Zhan et al., 1993). Some studies suggest that the intrinsic viscosities and gel

strengths obtained by a xanthan and LBG blend are higher than those obtained by the two polysaccharides individually (Higiro, Herald, & Alavi, 2006; Wang, Wang, & Sun, 2002). The interactions between xanthan and LBG generally give a positive effect, such as enhancement of the viscosity or gelation, especially when combined in a particular ratio (Cheetham & Mashimba, 1988; Sandolo et al., 2010). A 1:1 blend of xanthan and LBG is known to give the optimum synergistic effect (Higiro et al., 2006; Lundin & Hermansson, 1995). In a recent study (Jana et al., 2010), a 1:1 xanthan+LBG blend was used in a Mozzarella cheese analogue to obtain improved rheological and sensory properties.

Despite numerous studies of such synergistic interactions in aqueous solutions (Chandrasekaran & Radha, 1997; Sandolo et al., 2010; Shatwell, Sutherland, Ross-Murphy, & Dea, 1991; Tako, Asato, & Nakamura, 1984; Wang et al., 2002), the exact nature of the interactions between xanthan and LBG when they are used in blends in complex and multi-ionic emulsions (i.e. processed cheese) is still controversial. Polysaccharides such as xanthan and xanthan+LBG are susceptible to interaction within the complex ionic environment of processed cheese at high temperatures (about 85°C). These interactions at higher temperatures may lead to changes in the end-use rheological properties of the processed cheese.

Although such polysaccharides are widely used in cheese spreads and soft cheeses, there is little published literature on their effects on processed cheese. This present work aimed to bring some insight into the effects of xanthan and a 1:1 xanthan+LBG blend on the textural changes in model processed cheese. The model processed cheese

containing xanthan (see Chapter 4) was investigated further; the effects of a blend of xanthan+LBG added at different concentrations on the following functional properties were determined.

(1) Rheology related to end-use textural properties: modulus of deformability, fracture strain and adhesion area.

(2) Viscoelasticity as a function of temperature and structural changes: elastic modulus (G'), loss modulus (G'') and tan δ.

(3) Changes in microstructure of the processed cheese samples, as characterised by confocal laser scanning microscopy (CLSM).

5.2 Materials and methods

5.2.1 Manufacture of model processed cheese

Manufacture of the model processed cheese was as described in Section 3.1.

This standard formulation (10.0% protein) was used to determine particle size and for CLSM in the current study.

5.2.2 Experimental design

In the model processed cheeses selected for this study, protein was replaced with water to achieve different protein contents. In order to keep the total solids content constant at each protein concentration (8.0, 10.0, 12.0 and 14.0% wt/wt), lactose was used to replace the different polysaccharide concentrations (from 0 to 2.5% wt/wt). The selected model had a constant volume fraction (30.0% vol/wt) of soya oil in all samples.

Five polysaccharide concentrations (0.5, 1.0, 1.5, 2.0 and 2.5% wt/wt) and four protein concentrations (8.0, 10.0, 12.0 and 14.0% wt/wt) were chosen to study the effects of xanthan and a 1:1 blend of xanthan and LBG on the changes in modulus of deformability and fracture strain (ε_f) of the model processed cheese. The standard formulation (refer to Table 14) at 10.0% (wt/wt) protein and 30.0% (wt/wt) fat was used for the CLSM study. The minimum and maximum concentrations of protein (8.0 and 14.0% wt/wt) and polysaccharide (0.0 and 2.5% wt/wt) were chosen based on preliminary experiments (refer to Chapter 4) on the model processed cheese formulation. Samples containing less than 8.0% (wt/wt) protein were too soft and could not retain their shape for the compression test. An upper limit of 14.0% (wt/wt) protein ensured the right consistency without experiencing difficulties in pouring the hot molten cheese into stainless steel cylinders. Cheese samples with polysaccharide concentrations as high as 2.5% (wt/wt) exhibited good emulsion formation under the current Rapid Visco Analyser (RVA)-based manufacturing method, possibly because of the thermoreversible properties of the xanthan and the xanthan+LBG blend used in this experiment.

5.2.3 Uniaxial compression

Refer to Section 3.2.

5.2.4 Dynamic oscillatory measurement

Refer to Section 3.3.

A temperature sweep from 5 to 85°C (2°C/min) was carried out at a constant frequency of 0.1 Hz and a strain of 0.1%.

5.2.5 Sample sections and CLSM

Refer to Section 3.4.

5.2.6 Statistical analysis

The samples were prepared in triplicate. Statistical analysis of the modulus of deformability and fracture strain data was carried out using three-way analysis of variance (ANOVA) to study the effects of polysaccharide type, polysaccharide concentration, protein concentration and their interactions on modulus of deformability and fracture strain (general linear model procedure, Minitab Version 15.1). Surface plots were obtained using a central composite type response surface design, Minitab Version 15.1.

5.3 Results and discussion

5.3.1 Modulus of deformability

Although not consistent, the modulus of deformability (resistance of the cheese to deformation) tended to increase with an increase in the polysaccharide content (refer to Figures 19A and 19B). The following hypothesis is based on the principles of phase separation (Chan et al., 2007) and the fact that the modulus increases significantly with a decrease in the moisture content of the cheese (Visser, 1991). More polysaccharide (assuming phase separation between the polysaccharide and the protein matrix, shown by CLSM and discussed later) means that more water leaves the continuous protein phase. Alternatively, absorption of water by polysaccharide in a phase other than the protein phase leads to effective concentrations of both protein and polysaccharide that

are higher than the original nominal concentrations in the mixture (Chan et al., 2007). Thus the protein continuous phase effectively has a higher concentration, which gives a higher modulus.

The magnitude of the increase in stiffness was not the same for both polysaccharides. The processed cheese containing the xanthan+LBG blend showed marginally greater stiffness at all protein and polysaccharide contents than the processed cheese containing xanthan. For the processed cheese samples containing the xanthan+LBG blend, the increase in stiffness was almost linear with the increase in protein content from 8.0 to 14.0%. However, for the processed cheese samples containing xanthan, there was no linear increase in stiffness as xanthan level increases at higher protein contents.

The cheese samples containing the xanthan+LBG blend showed a two-way increase in the stiffness of the cheese structure when the protein and polysaccharide contents were increased (Figures 19A and 19B). This indicated that, at all protein contents, the xanthan+LBG blend provided more strength to the structure of the model processed cheese than xanthan alone.

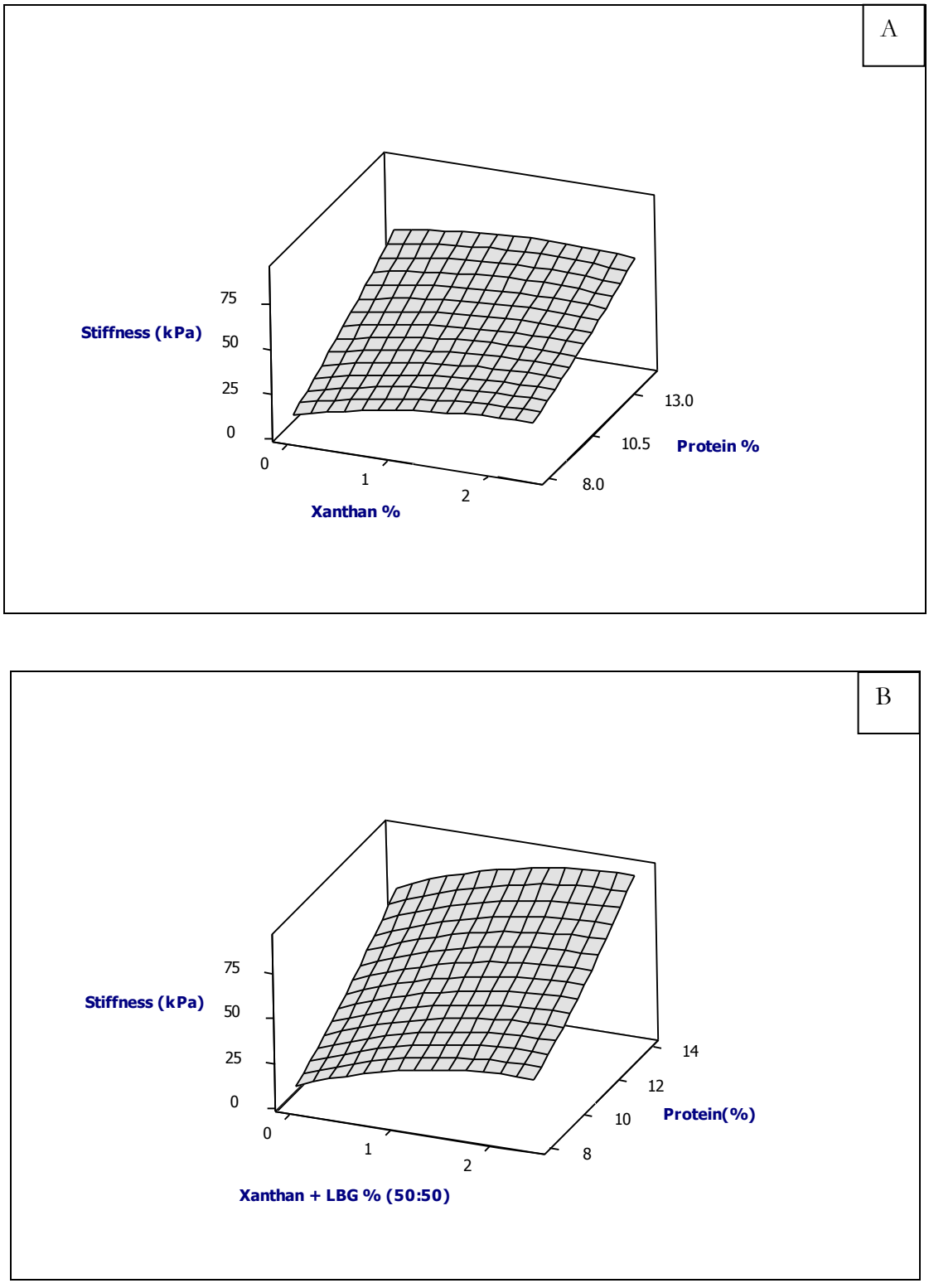


Figure 19 Stiffness at different protein and polysaccharide contents in processed cheeses containing (A) xanthan and (B) xanthan+LBG (1:1) .

5.3.2 Adhesion area

In terms of adhesion area (i.e. the force to pull the top and bottom plates apart after compressing the cheese between the plates), there was no significant difference between the cheese containing xanthan and the cheese containing the xanthan+LBG blend, when the added polysaccharide content was below 0.5% (Figures 20A and 20B).

Cheese with no added polysaccharide had a very similar reduction in adhesion with an increase in the protein content from 8.0 to 14.0%. As explained earlier, because of the manufacturing procedure, a cheese formulation with lower protein content had a higher moisture content. Processed cheese with higher moisture content had a lower modulus, as discussed in Section 5.3.1.

This approximate negative correlation between adhesion area and modulus is broadly consistent with the Dahlquist criterion, which states that stickiness does "not" occur in hard materials (i.e. as an approximation only, there is no (or less) adhesion at shear storage modulus G' values > 100 kPa (Dahlquist, 1969)). However, this is a guideline, not an exact equation in this case.

Equation 9

$G = 0.5*E / (1+ \nu)$ (Mezger, 2011)

where E = Young's modulus, G = shear modulus and ν = Poisson's ratio.

If strain rate effects are ignored and it is assumed that $G' \sim G$ and that Poisson's ratio is about 0.5 for soft viscoelastic materials (Mezger, 2011), then, to a first approximation, $G' \sim E/3$. Thus, the maximum G' from the data on this current processed cheese is about

75/3 = 25 kPa, implying that all these processed cheeses are likely to be sticky, with $G' <$ 100 kPa.

The adhesion of a pressure-sensitive material such as cheese is determined not only by viscoelasticity, as discussed above, but also by the surface energy of the adhesive and the adherents (Heddleson, Hamann, & Lineback, 1993). However, to a first approximation, this is again omitted. For example, Childs, Daubert, Stefanski & Foegeding (2007) found that surface energy did not correlate with the adhesion of cheese to steel.

A dramatic decrease in adhesion was observed for cheese samples containing xanthan+LBG when the polysaccharide content was increased from 0.5 to 2.5%. The decrease in adhesion with an increase in the xanthan+LBG content may have been due to the ability of the polysaccharide to increase the storage modulus (and the Dahlquist theorem broadly applies). The modulus increases possibly because the polysaccharide interacts with the cheese matrix and provides more mechanical interlocking. The use of xanthan alone as a stabiliser in Mozzarella cheese analogues often leads to a stickier product; this problem could be overcome by using xanthan in a mixture with LBG (Jana, Suneeta, & Solanky, 2008; Kiziloz et al., 2009). That the processed cheese samples containing xanthan were stickier than those containing xanthan+LBG is again partly reflected by the lower modulus of the processed cheese samples containing xanthan. This decrease in stickiness as the xanthan+LBG content increases from about 0.5 to 2.5% is desirable for sliced cheeses.

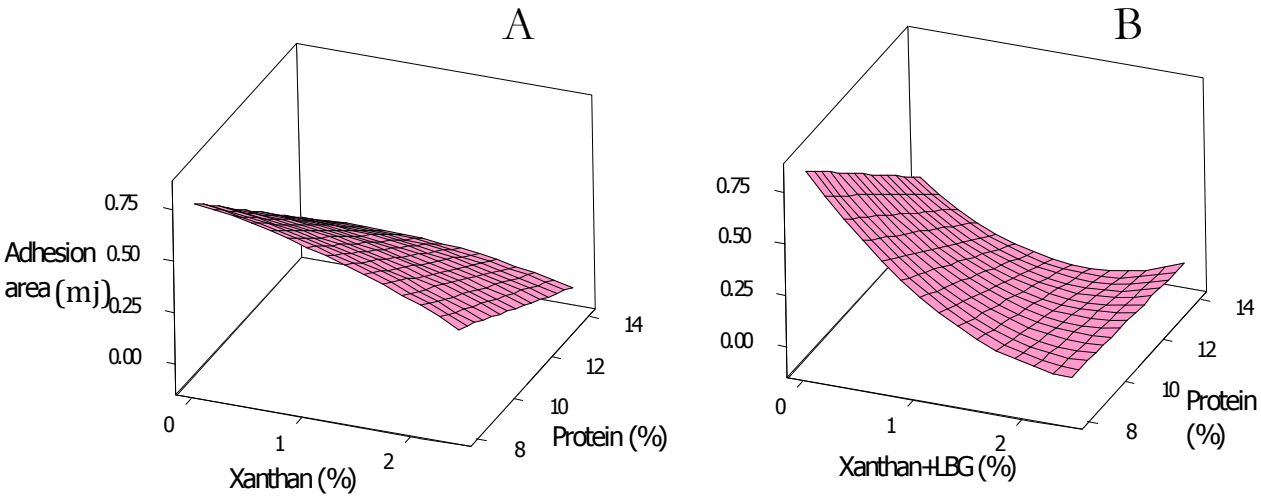


Figure 20 Stickiness at different protein and polysaccharide contents of processed cheeses containing (A) xanthan and (B) xanthan+LBG (1:1).

5.3.3 Fracture strain

The fracture strain decreased with an increase in the polysaccharide content, as shown in Figure 21. The discrete polysaccharide structures (as shown in Figures 22A and 22B) induce more inhomogeneity in the cheese matrix and give more pathways for cracking. This causes cheese samples to fail more readily under deformation, as shown by the lower fracture strain. The intensity of decrease in fracture strain was higher in xanthan added model cheese compared with xanthan+LBG added model cheese. The difference in fracture strain was not signification at and above 1.5% polysaccharide addition.

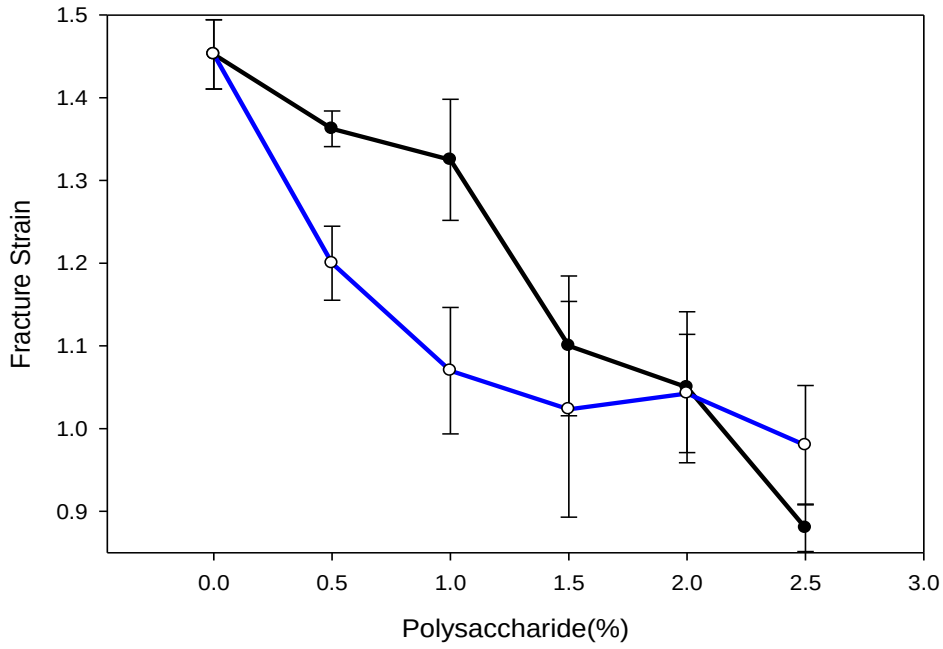


Figure 21 Effect of polysaccharide content on the fracture strain of model processed cheese containing 10.0% protein (this protein content is normally used for sliced cheese), 30% fat and 1.0% xanthan (–o–) or 1.0% xanthan+LBG (1:1) (–●–).

5.3.4 Microstructure

The texture of a cheese is a reflection of its microstructure (Jack & Paterson, 1992). The microstructure of the model processed cheeses is shown in the confocal micrographs in Figures 22A and 22B, which clearly indicate the changes in the components that occurred as a result of the presence of polysaccharide in the samples. These processed cheese samples had the same composition apart from the type of polysaccharide used. The effect on the cheese structure of 1.0% xanthan addition was clearly different from that of 1.0% xanthan+LBG blend addition.

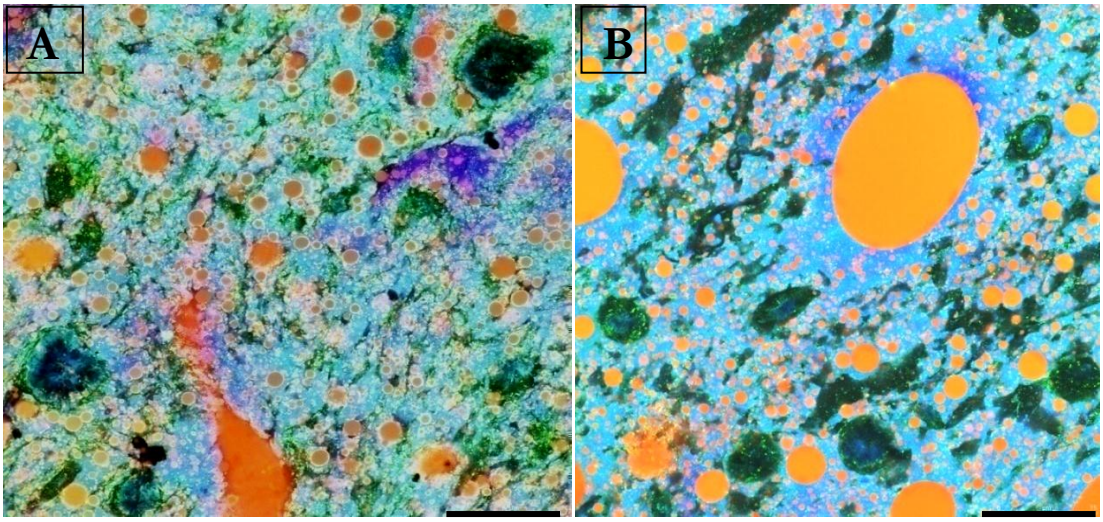


Figure 22 CLSM images of model processed cheeses containing (A) 1.0% (wt/wt) xanthan and (B) 1.0% (wt/wt) xanthan+LBG (1:1). Protein is blue, fat is orange and polysaccharide is dark green. The scale bars represent 75 µm. Polysaccharide structures are visible as discrete entities in the protein matrix.

5.3.5 Viscoelasticity as a function of temperature and structural changes

Model processed cheese samples with added xanthan and added xanthan+LBG have essentially the same dry matter contents and the same pHs. Such standardised model processed cheeses mean that their rheological properties at different hydrocolloid concentrations can be compared. Behavioural changes in the cheese matrix were evident when the viscoelastic properties were measured over a slow temperature rise from 5 to 85°C.

The viscoelastic flow behaviour, shown in Figure 23 as tan δ, indicated that the cheeses containing polysaccharide did not flow to the same extent as the control cheese at elevated temperature. This could have been because a stronger gel structure was retained at higher temperature by the cheeses containing xanthan and xanthan+LBG. The tan δ values increased dramatically between temperatures of 50 and 60°C for all three processed cheese samples containing xanthan+LBG.

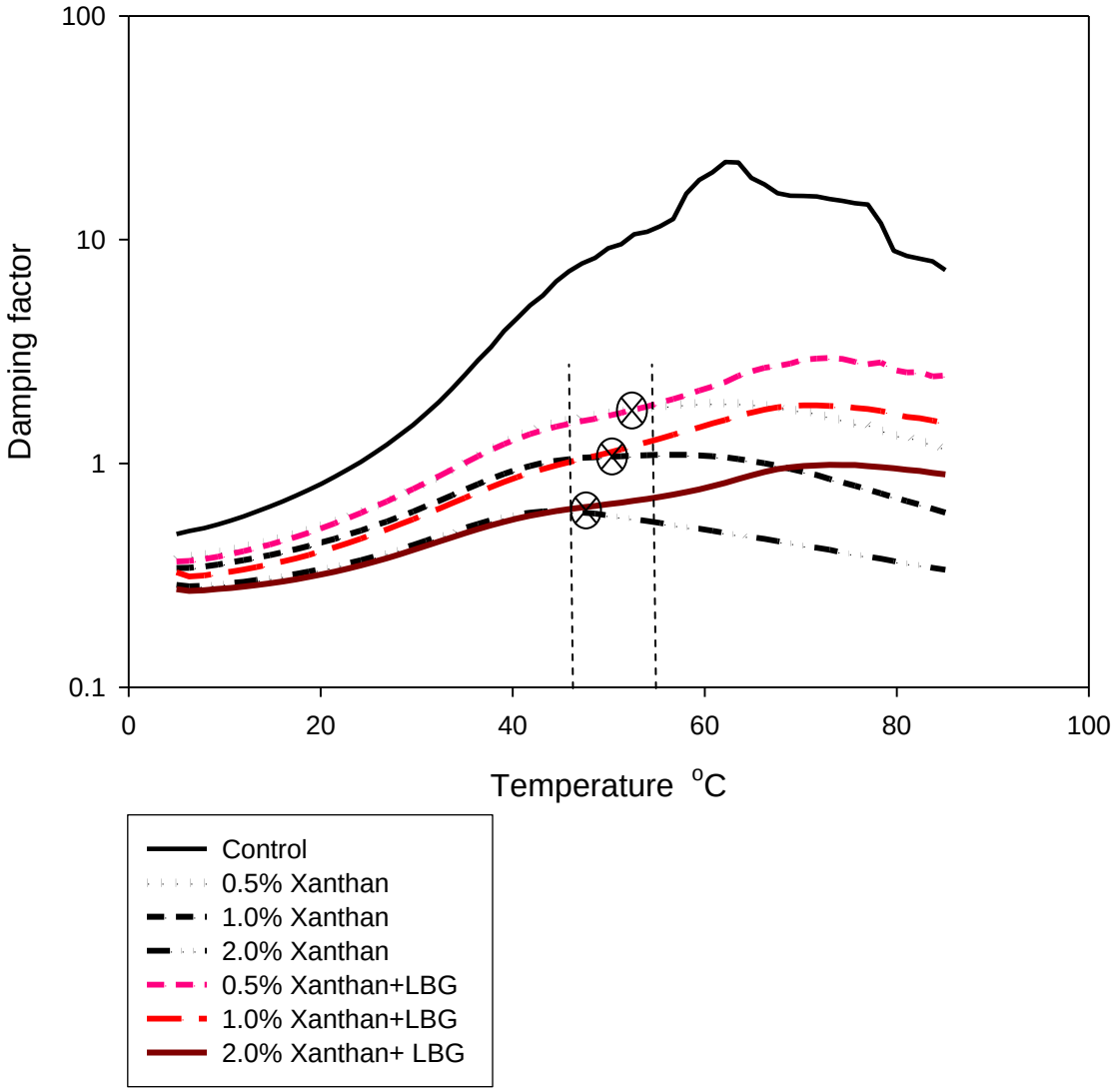


Figure 23 Temperature sweeps at a frequency of 0.1 Hz and a strain of 0.1%, showing changes in damping factor (tan δ) values for model processed cheeses containing xanthan and a 1:1 xanthan+LBG blend at the same protein content of 10.0% (wt/wt) and different polysaccharide contents of 0.0, 1.0 and 2.0% (wt/wt).

Figure 24 shows the results of dynamic oscillatory rheometry temperature sweeps for the model processed cheeses with and without polysaccharide. An increase in the content of both xanthan and xanthan+LBG increased the gel hardness and the gel strength. The increase in the elastic modulus (G') could have been the result of intensive interactions between the polysaccharide and the cheese, which led to the formation of a

more extensive cheese network. It was evident from the results that the *G*' values of the processed cheeses containing xanthan+LBG were greater than those of the processed cheese containing xanthan up to a temperature of about 46–56°C (As shown in Figure 23 by.×symbols). However, a significant decrease in the *G*' values was observed when the temperature was increased from about 60 to 85°C. The cheeses containing the xanthan+LBG blend showed a greater decrease in *G*' values than the cheeses containing xanthan.

The increased flow (tan δ) at higher temperature in the processed cheese samples containing LBG could be attributed to the reduced elastic modulus (*G*') and the corresponding increase in the loss modulus (*G*") of the cheese, as shown in Figure 24. This behaviour indicated that the melting property of the cheese containing xanthan was enhanced when LBG was added to the mixture. On a commercial scale, there may be repercussions for polysaccharide-containing cheeses because the equipment may not be able to handle products with high viscosity at high polysaccharide contents (such as 2.5% xanthan). However, xanthan+LBG may be a desirable solution for such viscosity problems in large scale product manufacture

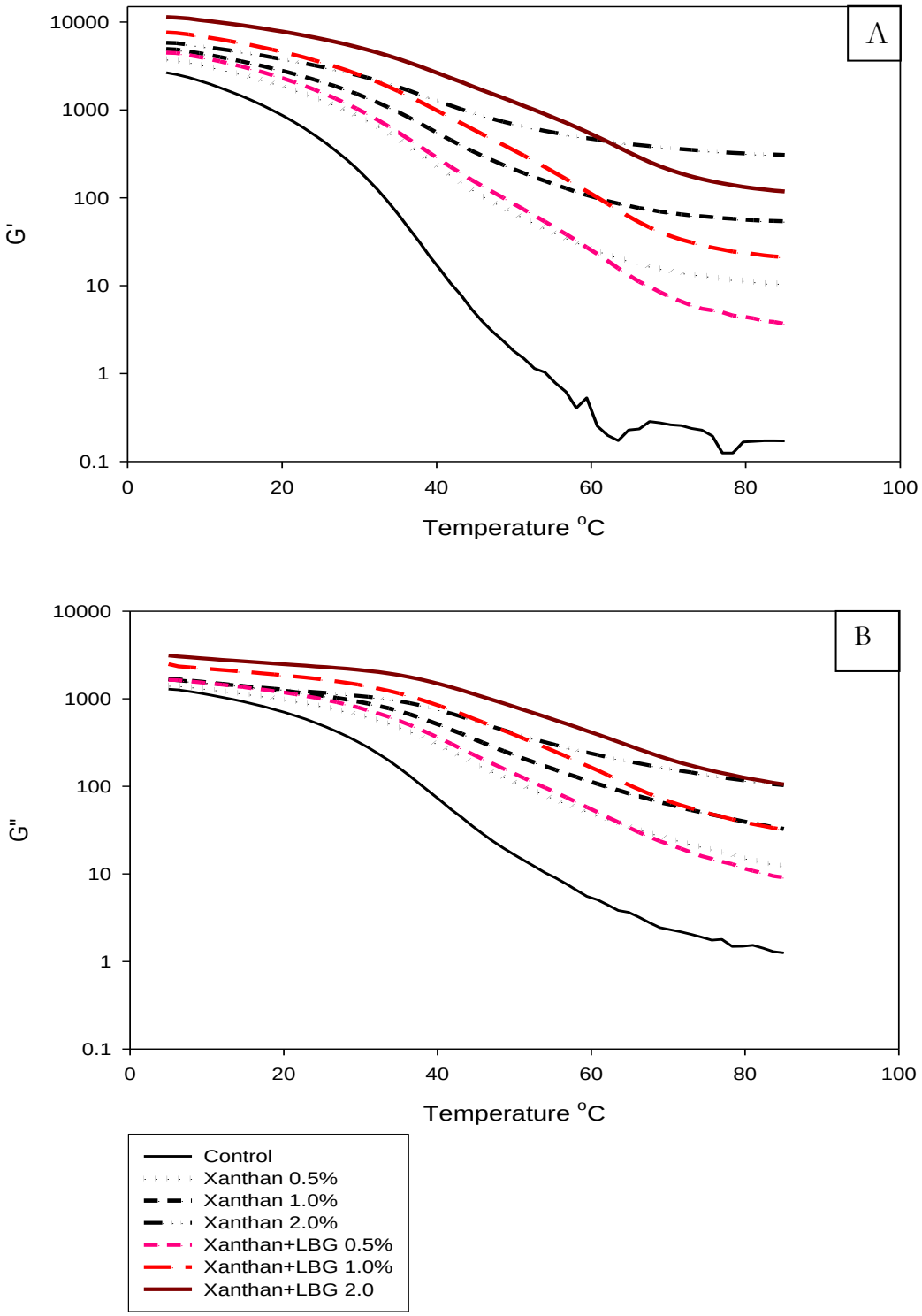


Figure 24 Temperature sweeps at a frequency of 0.1 Hz and a strain of 0.1%, showing changes in (A) *G'* and (B) *G"* values for model processed cheeses containing xanthan and a 1:1 xanthan+LBG blend at the same protein content of 10.0% (wt/wt) and different polysaccharide contents of 0.0, 1.0 and 2.0% (wt/wt).

5.4 Conclusions

Table 20 **Textural and microstructural properties of the processed cheeses and interpretations of the various tests used to measure the textural properties**

Texture of processed cheese	**Compressive rheology and fracture properties**			**Dynamic small strain stress rheometry**		
	Modulus of deform-ability	Fracture strain	Adhesion area	*G'* high temperature 65–90°C	*G"* high temperature 65–90°C	tan δ
Xanthan	↑	↓	↓	↑	↓	↓
Xanthan+LBG	↑↑	↓↓	↓↓	↓	↑	↑

Note: ↑ and ↓ indicate whether the rheological properties would increase or decrease respectively, and the number of ↑ or ↓ is a rough indicator of the magnitude of the increase or decrease.

The results confirmed that the 1:1 blend of xanthan+LBG imparted greater stiffness to the model processed cheese compared with xanthan. Either xanthan or a blend of xanthan and LBG may be incorporated into the formulation at amounts up to 2.5%. However, an increase in the xanthan+LBG content gave greater stiffness at all protein contents from 8.0 to 14.0%, which was not evident for model processed cheeses containing xanthan. Larger amounts of xanthan+LBG (1:1) reduced the stickiness, which is a desirable end-use property for sliced cheeses. Increased amounts of xanthan and xanthan+LBG reduced the fracture strain (resistance to crumbling). However, at the highest addition rate of 2.5%, lower fracture strain is not a desirable property of cheese slices. Unique thermoreversible behaviour was observed at higher temperatures

(60–85°C) for the cheeses containing the xanthan+LBG blend. This thermoreversible property at higher temperatures provides processed cheese with a unique quality for some food applications, such as cheese slices for burgers, for which melting is considered to be a desired characteristic.

Based on the findings, use of a 1:1 blend of xanthan+LBG, rather than xanthan, is recommended in a processed cheese formulation. The processed cheese made with the xanthan+LBG blend was superior to that made with xanthan, in terms of greater stiffness, lower stickiness and greater flowability at higher temperatures.

Chapter 6 Effect of the microbial polysaccharide gellan gum on the functionality of a model processed cheese

6.1 Introduction

Pocessed cheese is an oil-in-water emulsion in which dairy proteins play an important role in building the structure of the product. Rennet casein normally contributes to the protein component of analogue cheese. The functional properties of imitation cheese such as stability, texture and melting are influenced by the ingredients (e.g. protein, polysaccharides, water, fat, salts and surfactants) and the processing conditions employed during manufacture. Research on the use of polysaccharides in cheese has been carried out over the last decade. It has been reported that the addition of polysaccharides affects the extent of hydration of various components in imitation cheese, especially protein (Mounsey & O'Riordan, 2001; Mounsey & O'Riordan, 2008). Water, which is a key ingredient, plays an important role in dissolving the calcium chelating salts and hydrating the proteins and other components (Lee et al., 2004). Varying the concentration and type of polysaccharide has been shown to alter the mobility of water and thus the functionality of the cheese. For example, cheese samples with more mobile water (i.e. free water) had soft texture and were easy to melt, whereas cheese samples with less mobile water (i.e. a high proportion of bound water) were firmer and non-melting (Noronha, Duggan, Ziegler, O'Riordan, & O'Sullivan, 2008).

Non-starch polysaccharides, such as κ-carrageenan, locust bean gum and inulin, have been used in processed cheese (Gustaw & Mleko, 2007; Hennelly, Dunne, O'Sullivan, &

O'Riordan, 2006; Kiziloz et al., 2009). Their addition increased the hardness and cohesiveness and reduced the melting of the cheese (Kiziloz et al., 2009). The use of microbial polysaccharides in the processed cheese system has not been thoroughly studied but is important because microbial polysaccharides are safe for human consumption and can be produced from dairy media. Among the microbial polysaccharides, gellan gum is one of a few bacterial exopolysaccharides that have US Food and Drug Administration approval for use as direct food additives (Roller & Dea, 1992). The published literature shows that gellan gum is not just a gelling agent; it is also a texturising, stabilising, viscosifying and flavour-releasing polymer. Gellan gum is available in two forms (high and low acyl content). Although high acyl gellan gum (Gellan-H) gels are normally softer than low acyl gellan gum (Gellan-L) gels, Gellan-H has an intrinsically more stable structure and a greater capacity to hold water (Huang, Tang, Swanson, & Rasco, 2003). Moreover, the properties of the gellan gum gel are influenced by the presence of monovalent cations (Na^{+}), divalent cations (Ca^{2+}) and the chelating agents (Moritaka, Fukuba, Kumeno, Nakahama, & Nishinari, 1991; Tang, Tung, & Zeng, 1997) that are an essential part of the processed cheese formulation.

In the present study, Gellan-H and Gellan-L were added to a model processed cheese and changes in the mechanical properties and the microstructure of the model processed cheese samples were characterised by rheological techniques and confocal laser scanning microscopy (CLSM). The addition of gellan gum to a model processed cheese is a novel concept and has rarely been reported in the literature.

6.2 Materials and methods

6.2.1 Manufacture of model processed cheese

Refer to Section 3.1.

6.2.2 Experimental design

In the model processed cheeses selected for study, protein was replaced with moisture to achieve different protein contents. In order to keep the total solids content constant at each protein content (8.0, 10.0, 12.0 and 14.0% wt/wt), lactose was used to replace the different polysaccharide contents (from 0 to 2.0% wt/wt). Lactose is considered to be a filler at low concentrations (< 5.0% wt/wt), as it is known to have no effect on the deformability and meltability of processed cheese at these low concentrations. The selected model had a constant volume fraction (30.0% vol/wt) of soya oil in all samples.

Four polysaccharide contents (0.5, 1.0, 1.5 and 2.0% wt/wt) and four protein contents (8.0, 10.0, 12.0 and 14.0% wt/wt)(refer to Figure 16) were chosen to study the effect of Gellan-H and Gellan-L on the changes in fracture stress (σ_f) and fracture strain (ε_f) of the model processed cheeses. The standard formulation at 10.0% (wt/wt) protein and 30.0% (wt/wt) (refer to Figure 14) fat was used for the CLSM study. The minimum and maximum protein (8.0 and 14.0% wt/wt) and polysaccharide (0.0 and 2.0% wt/wt) contents were chosen based on preliminary experiments on the model processed cheese formulation. Samples containing less than 8.0% (wt/wt) protein were too soft and could not retain their shape for the compression test. An upper limit of 14.0% (wt/wt) protein ensured the right consistency without experiencing difficulties in

pouring the hot molten cheese into stainless steel cylinders. Cheese samples with polysaccharide contents above 2.0% (wt/wt) exhibited poor emulsion formation, showing fat separation under the current Rapid Visco Analyser (RVA)-based manufacturing method.

6.2.3 Uniaxial compression

Refer to Section 3.2.

6.2.4 Dynamic oscillatory measurement

Refer to Section 3.3.

A temperature sweep from 5 to 85°C (2°C/min) was carried out at a constant frequency of 0.1 Hz and a strain of 0.1%.

6.2.5 Determination of average droplet size

Refer to Section 3.5.

6.2.6 Sample sections and CLSM

Refer to Section 3.4.

6.2.7 Statistical analysis

The samples were prepared in triplicate. Statistical analysis of the σ_f data was carried out using regression to study the effects of polysaccharide type and polysaccharide concentration (general linear model procedure, Minitab Version 15.1). The ε_f data were

analysed and surface plots were obtained using a central composite type response surface design, Minitab Version 15.1.

6.3 Results and discussion

6.3.1 Emulsion formation

Emulsification time is defined as the time required for the formation of an emulsion as indicated by an abrupt increase in viscosity of the RVA graph during processed cheese manufacture (Kapoor & Metzger, 2005). The increase in the RVA viscosity can also be attributed to other interactions such as protein association at higher temperature during processing of the cheese (Lee et al., 2003). During RVA processing, it was observed that an increase in the Gellan-H concentration increased the time required to form the emulsion (Figure 25A). Gellan-H swells and absorbs water rapidly (Chandrasekaran, Radha, & Thailambal, 1992) during the initial cold dispersion (hydration for 40 min) of the ingredients. Consequently, the competition for water between casein and gellan gum has significant effects on the microstructure and hence the physical properties of processed cheese. As the emulsification of processed cheese is dependent on the interaction of trisodium citrate (TSC) with casein micelles (Gupta et al., 1984; Savello et al., 1989; Shimp, 1985), the resultant increase in the emulsification time suggests insufficient availability of water to hydrate the protein and/or the melting salt system. Therefore, increasing the amount of Gellan-H reduced the amount of TSC available to interact with the casein micelles and consequently reduced the conversion of rennet casein to more soluble paracaseinates (Lee et al., 2004). Figure 25A shows that the total time for emulsification of the samples containing Gellan-H (i.e. the time

between the peak viscosity and the end of processing – 10 min) decreased with increasing concentration. However, the increase in RVA viscosity for Gellan-L (Figure 25B) was different from that for Gellan-H (Figure 25A). The time to achieve the peak viscosity did not change markedly; however, the viscosity increased noticeably with an increase in the Gellan-L concentration. The lower water-holding capacity of Gellan-L compared with Gellan-H (Huang et al., 2003) could result in a greater amount of moisture being available for casein hydration. Therefore, the increased efficiency of emulsification indicated by the greater increase in peak viscosity (Figure 25B) in the model processed cheese could have been due to an increased extent of casein hydration.

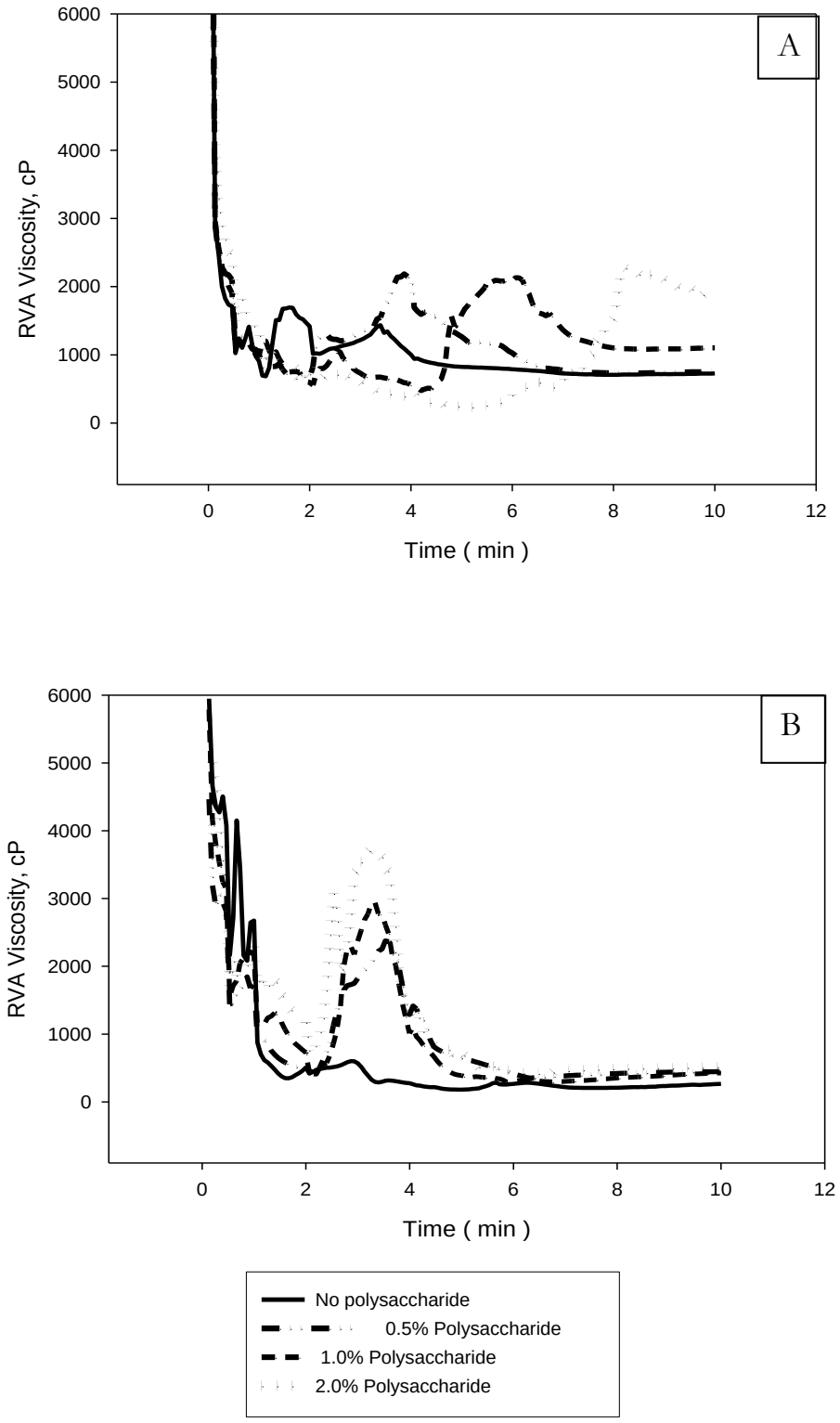


Figure 25 RVA viscosity profile obtained during the manufacture of model processed cheeses containing 10.0% (wt/wt) protein, 30.00% (wt/wt) fat and 50.00% moisture: (A) Gellan-H; (B) Gellan-L.

It is evident from Figures 26A and 26B that the fat globule particle size in the standard cheese formulation (10% (wt/wt) protein, 30.0% (wt/wt) fat) increased with an increase in the concentration of Gellan-H but decreased with an increase in the concentration of Gellan-L. The changes in the particle size of the cheese samples containing Gellan-L were in line with the published literature, which indicates that increasing the viscosity of the continuous phase generates higher shear stress during processing, which is therefore better able to reduce the fat particle size (Lee et al., 2004). The lower availability of water for protein hydration and the reduced action of TSC in the processed cheese samples containing Gellan-H would have resulted in poorly hydrated casein, which consequently would have decreased the ability of protein to diffuse to the fat interface, resulting in a poor emulsion and a greater fat globule particle size in the cheese matrix (Figure 26A). Another possible reason for not achieving efficient fat globule breakdown could have been retardation of the mixing efficiency at extremely high viscosity during the RVA processing at high polysaccharide (Gellan-H) concentrations. The observed behaviour was consistent with the work of Mounsey and O'Riordan (2008), who reported retardation in the emulsification properties with an increase in pregelatinised starch.

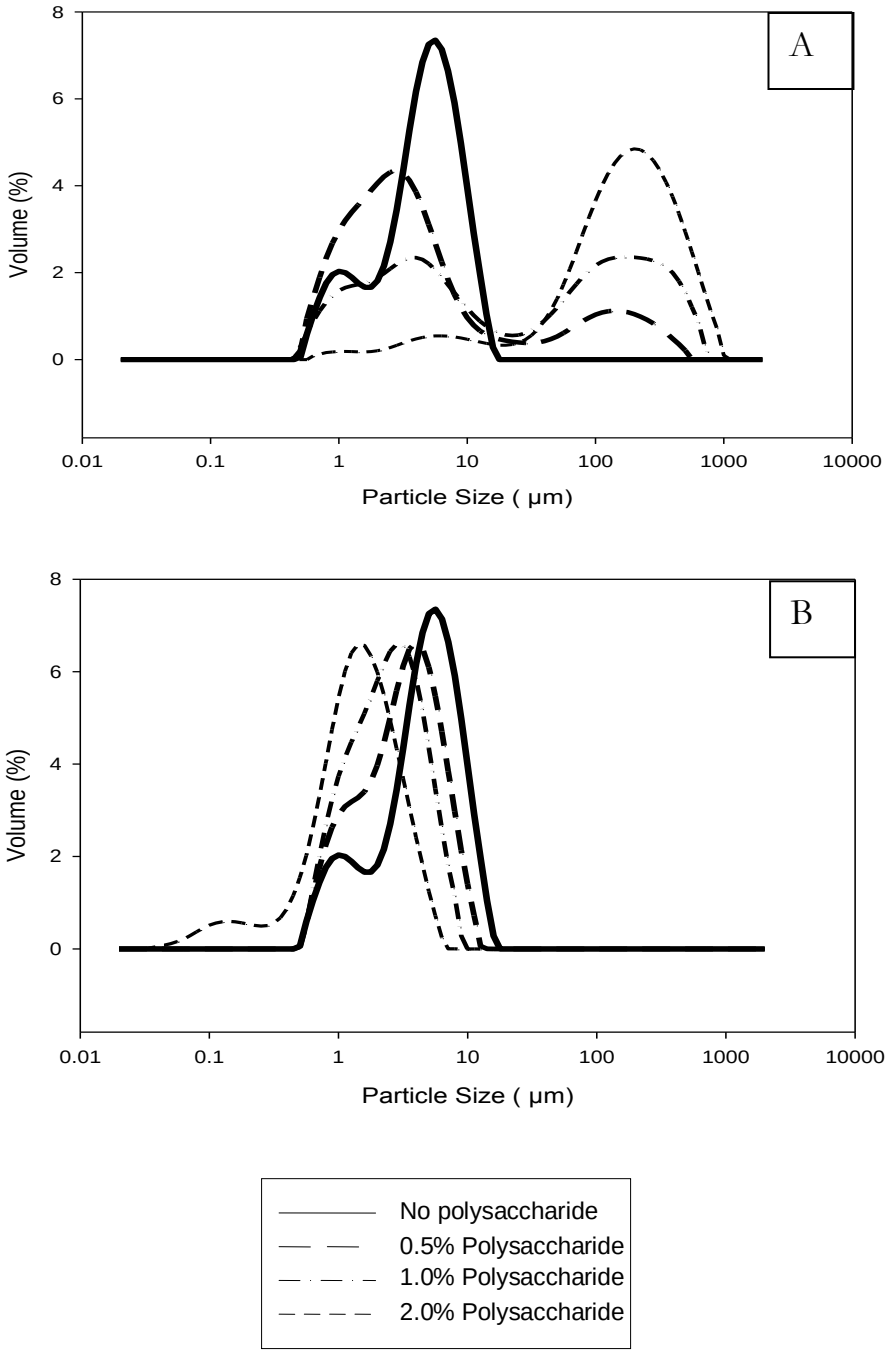


Figure 26 Fat globule particle size in the cheese matrix for various concentrations of polysaccharides in model processed cheeses containing 10.0% (wt/wt) protein, 30.00% (wt/wt) fat and 50.00% moisture: (A) Gellan-H; (B) Gellan-L.

It was also noticed while establishing the limits for the protein content (in Chapter 4) that, at 8.0% (wt/wt) protein, a sample containing greater than 2.0% (wt/wt) Gellan-H resulted in poor incorporation of the oil and a separated layer of oil was observed at the end of processing. A relatively lower concentration of protein available for emulsification of the fat could also have been a reason why fat separation was more evident at low (8.0% wt/wt) protein contents.

6.3.2 Microstructure

CLSM images of the processed cheese samples are shown in Figures 27A, 27B and 27C. The sample shown in Figure 27A contained no polysaccharide whereas the samples shown in Figures 27B and 27C contained 1.0% (wt/wt) Gellan-H and Gellan-L respectively. The protein matrices of all samples appeared to be continuous because no apparent change in the colour intensity of the background was observed. In the presence of chelating agents, the calcium–phosphate bridges that hold the protein units together are broken down, allowing the protein units to dissociate. This dissociation gives a continuous (dissolved) structure of the proteins in the processed cheese system (Mounsey & O'Riordan, 2008; Savello et al., 1989; Shimp, 1985; Tolstoguzov, 1997).

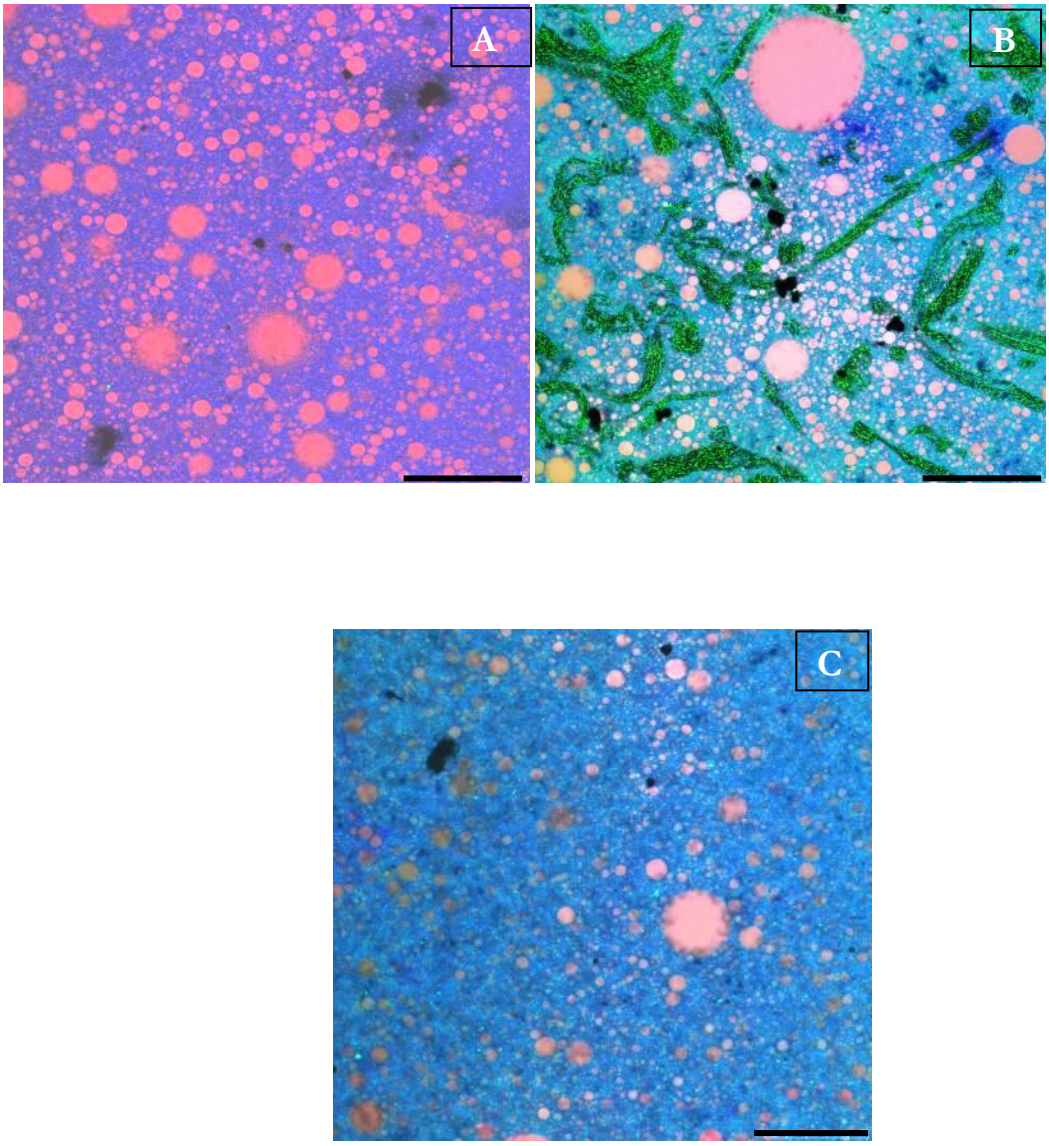


Figure 27 CLSM images of model processed cheeses containing 10.0% (wt/wt) protein, 30.00% (wt/wt) fat and 50.00% moisture: (A) no polysaccharide; (B) 1.0% (wt/wt) Gellan-H; (C) 1.0% (wt/wt) Gellan-L. Protein is blue, fat is pink and polysaccharide is green. The scale bars represent 75 µm. Polysaccharide structures are visible as discrete entities in the protein matrix.

The micrographs show distinct differences in the protein matrices of the processed cheese samples containing Gellan-H and Gellan-L. Gellan-H appeared as distinct clusters (green) among the homogeneous protein matrix. The bright green particles visible in

the micrograph were gellan gum, which appeared to be only partially dissolved because of the low availability of water in the matrix. Although the temperature used to manufacture the model processed cheese (85°C) was sufficiently high for Gellan-H to be soluble, the relatively short hydration time (40 min) and processing time (10 min) also implied limited time to complete the solubilisation of Gellan-H. The micrograph in Figure 27B shows that the Gellan-H gel clusters were dispersed within a continuous protein matrix. The pre-gel solution (during the hydration step) of Gellan-H, when processed under shear, resulted in the trapping of individual swollen granules in a dispersed phase within the continuous protein matrix. A closer look at these filament-shaped swollen clusters of Gellan-H revealed that the water attracted by the polysaccharide was observed against a dark background rather than a blue background (protein matrix), indicating a discontinuous serum phase with clear boundaries that was isolated from the continuous protein matrix. The CLSM image (Figure 27B) shows that polysaccharides do not become adsorbed on to the fat globule surface, but remain in the emulsion and modify the viscosity and water binding because of their extended, hydrated structure in the protein matrix.

The CLSM image of the sample containing Gellan-L (Figure 27C) did not show similar polysaccharide clusters in the continuous protein network. The smooth and continuous protein matrix indicated that the Gellan-L was well solubilised and dispersed in the processed cheese matrix.

6.3.3 Fracture stress (σ_f)

Fracture stress indicates the textural property of firmness (Watkinson et al., 2001). The data for the mean values in Figures 28A and 28B showed an increase in σ_f with an increase in the protein content. The high σ_f at high protein content (i.e. at low moisture content) meant that the processed cheese had a higher resistance to deformation at fracture. As the protein content increased, the moisture content in the protein matrix, and hence the ability to dissipate the energy at large strains, decreased (Lee, Imoto, & Rha, 1978). The stress at fracture decreases with increasing moisture content (Prentice, 1992) and decreasing protein content (Visser, 1991).

The processed cheese samples containing Gellan-H and Gellan-L showed similar trends of an increase in fracture stress with an increase in the polysaccharide content. The slopes for fracture stress versus Gellan-H concentration were similar for protein contents of 8–12%. The σ_f values for the cheese samples containing Gellan-H were more dependent on polysaccharide content, because the slopes of the fracture stress versus polysaccharide curves were steeper for Gellan-H (ranging from 2.2 to 3.6 kPa/%) than for Gellan-L (ranging from 0.6 to 1.7 kPa/%). The fracture stress values for Gellan-H showed a marginal increase over those for Gellan-L when the means were plotted at various protein contents from 8.0 to 14.0% (wt/wt) (Figure 28). The Gellan-L slopes were similar at all protein contents above 8%.

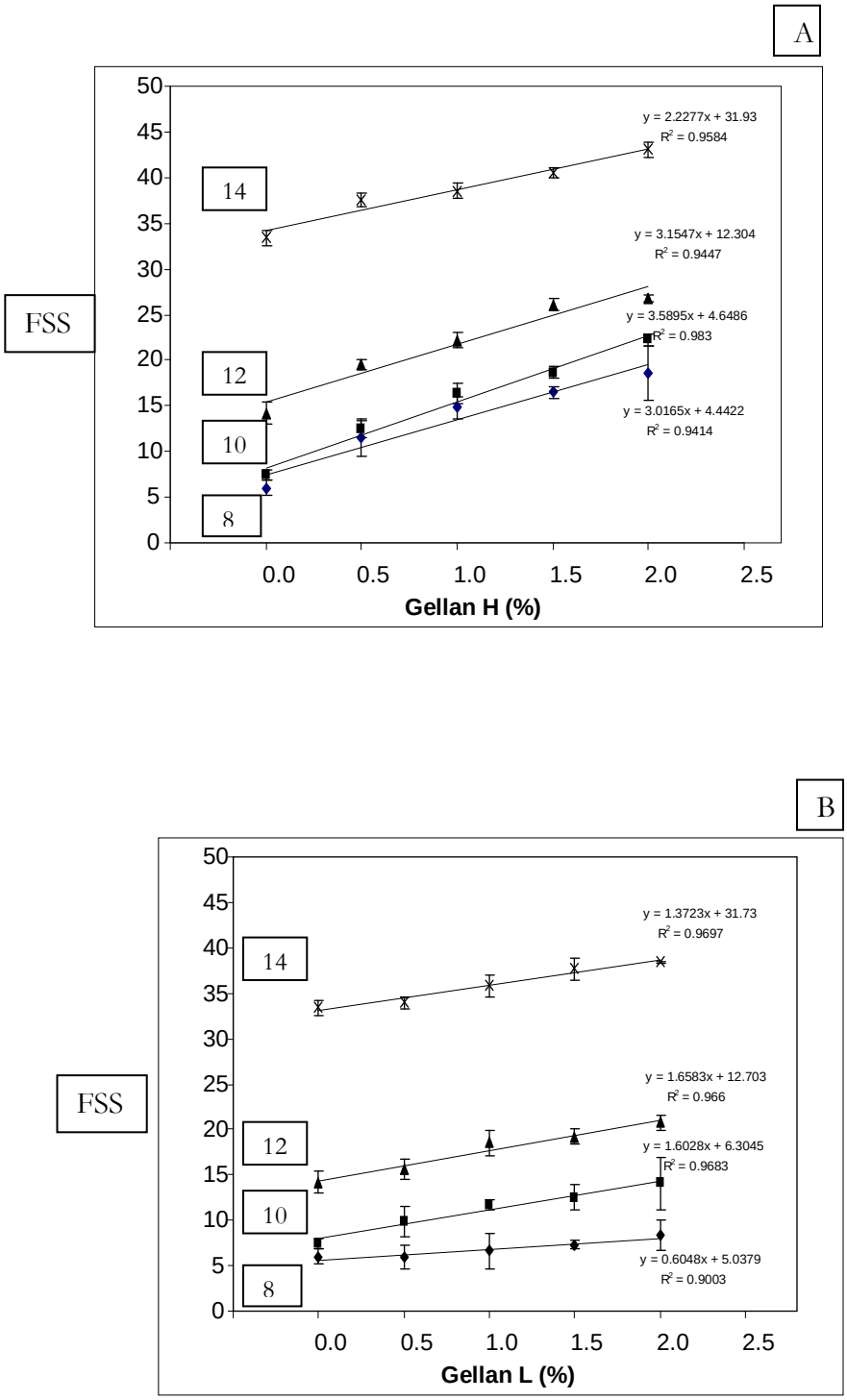


Figure 28 Effect of polysaccharide content at various protein contents on the fracture stress (FSS) of RVA model processed cheese: (A) Gellan-H; (B) Gellan-L. ♦ = 8.0% (wt/wt) protein; ■ = 10.0% (wt/wt) protein; ▲ = 12.0% (wt/wt) protein; x = 14.0% (wt/wt) protein.

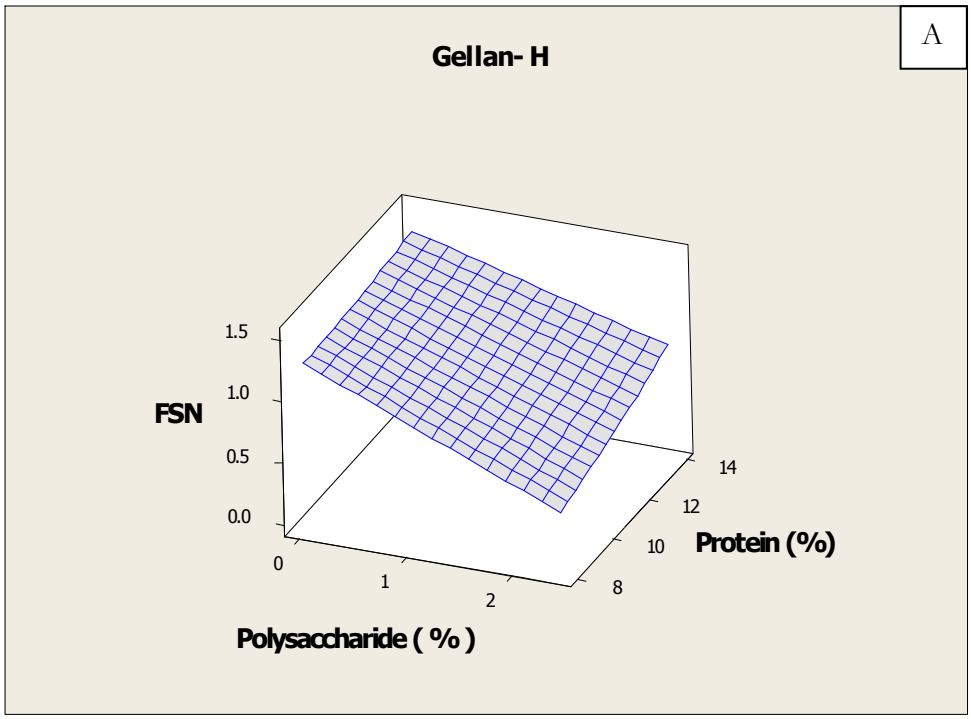
The following is an extension of an hypothesis from earlier work (Chan et al., 2007) to explain why gellan gum gives higher fracture stress than that in the control processed cheese. Chan et al. (2007) stated that the higher viscosity induced in mixtures of casein and waxy maize starch, compared with casein, (assuming the usual phase separation between protein and starch) is probably the result of effective protein dehydration caused by the polysaccharide taking some of the water. Phase separation means that the effective concentrations of both protein and starch within their own phases are considerably higher than their original nominal concentrations in the mixture. We assume that this hypothesis applies more generally to phase-separated protein and non-starch polysaccharides. We also assume that this applies not only to viscosity but also to another stress-related property, i.e. fracture stress.

The following hypothesis is proposed to explain why Gellan-H induced higher σ_f than Gellan-L in processed cheese. Gellan-H swells and absorbs water faster (Chandrasekaran et al., 1992) during the hydration step, possibly leaving less moisture available for hydration of the protein. The protein phase therefore remains more concentrated in the processed cheese, giving more structure reinforcement. The increase in σ_f with increasing Gellan-H content can be supported by the phenomenon of filler–matrix interactions in composites such as processed cheese. The swollen Gellan-H in this study was in a dispersed phase within a continuous protein matrix (evident from the micrograph in Figure 27B). A previous study (Manksi, Kretzers, van Brenk, van der Goot, & Boom, 2007) concluded that an increased volume fraction of a rigid dispersed phase causes an increase in σ_f. This increase in σ_f as a result of an increase in the Gellan-

H (filler phase) content (and volume fraction) in the processed cheese may have been because of the strong interaction between the matrix and the filler phase. The increase in volume fraction was inferred from the CLSM images by the increase in the number of Gellan-H particles and their particle size (Figure 27B). The fracture stress of a matrix filler is particularly sensitive to the filler–matrix interaction (Manksi et al., 2007).

6.3.4 Fracture strain (ε_f)

Fracture strain indicates the textural property of longness or the resistance of the sample to crumbling (Watkinson et al., 1997). The ε_f results obtained indicated that the incorporation of both Gellan-H and Gellan-L contributed to very different mechanical properties in the model processed cheese, as shown in Figures 29A and 29B. The surface plots showed entirely different trends for ε_f compared with σ_f. The ε_f values decreased slightly with increasing Gellan-L content from 0.0 to 2.0% (wt/wt) at all protein contents (Figure 29B). However, the ε_f values decreased markedly (Figure 29A) when the Gellan-H content was increased at lower protein contents. The reduction in ε_f was also clearly evident at higher protein contents but the magnitude of the decrease was less than at the lower protein contents.



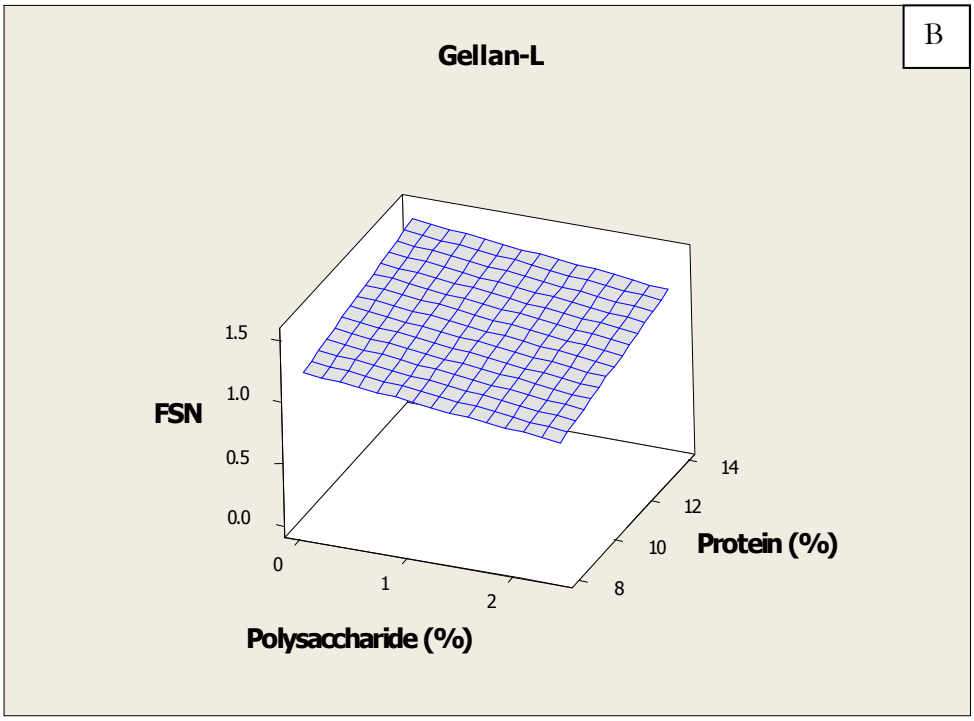


Figure 29 Effect of polysaccharides on the fracture strain (FSN) of model processed cheeses: (A) Gellan-H; (B) Gellan-L.

The cause of the large decrease in the εf of the model processed cheese at high Gellan-H content can be explained by combining the microstructural and rheological results. A low εf can be correlated with the crumbly (cracking) behaviour of the product (Luyten, van Vliet, & Walstra, 1987). The steep reduction in εf caused by Gellan-H indicates greater initialisation of cracking (structure failure) induced by deformation. Such brittle, crumbly processed cheese sometimes results from strain-weakening behaviour, where the structure yields prior to fracture (Bowland & Foegeding, 1999). This phenomenon in the current work could have been due to fracture points at the protein–polysaccharide interface.

The slope ratio (Bowland & Foegeding, 1999) is defined as the ratio of the fracture stress to fracture strain divided by the ratio of stress at 0.3 × fracture strain to strain at 0.3 × fracture strain (Equation 11).

Equation 10

$$G_f = \frac{\sigma_f}{\varepsilon_f}$$

Equation 11

$$\text{Slope Ratio} = \frac{G_f}{G_{30\%\varepsilon_f}}$$

This ratio is less than 1 for strain weakening and the stress versus strain curve is concave down in shape. The ratio is greater than 1 for strain hardening and the stress versus strain curve is mostly concave up. See Figure 30 for illustrative concave-up and concave-down stress versus strain curves.

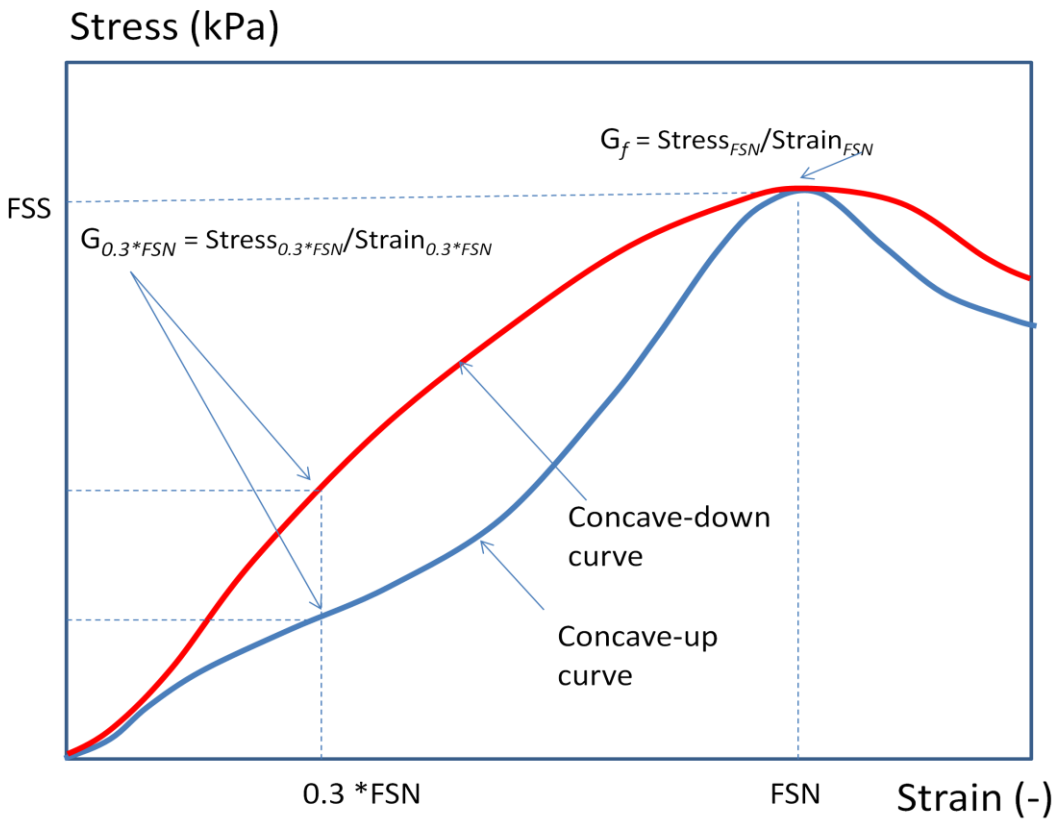


Figure 30 Illustrative concave-up and concave-down stress versus strain curves.FSN=Fracture strain, FSS=Fracture stress.

For the current work, this slope ratio for the cheese containing Gellan-H at 8.0 and 12.0% (wt/wt) protein (Figure 31) was typically less than 1 (strain weakening), having a range of about 0.7–1.1 (Figure 31). The exception was for the cheese containing 8.0% (wt/wt) protein and 0.2% (wt/wt) Gellan-H, which had a slope ratio of just over 1 (mostly concave up). There was no very clear fracture point at which to measure the slope ratio at 8.0% (wt/wt) protein and no polysaccharide, but the stress versus strain curve was mostly concave up. A mostly concave-up stress versus strain curve has a region of less stress increase with strain or greater flow during deformation (Luyten, van Vliet, & Walstra, 1991). This greater apparent flow or liquid-like behaviour is partly

explained in the current work by the higher moisture content (with lower protein content) and little (or no) polysaccharide. Luyten et al. (1991) found two types of compressive stress versus strain curves for Gouda cheese: concave down and mostly concave up. A concave-up type curve was exhibited by young cheese (higher moisture content) that was low in acidity.

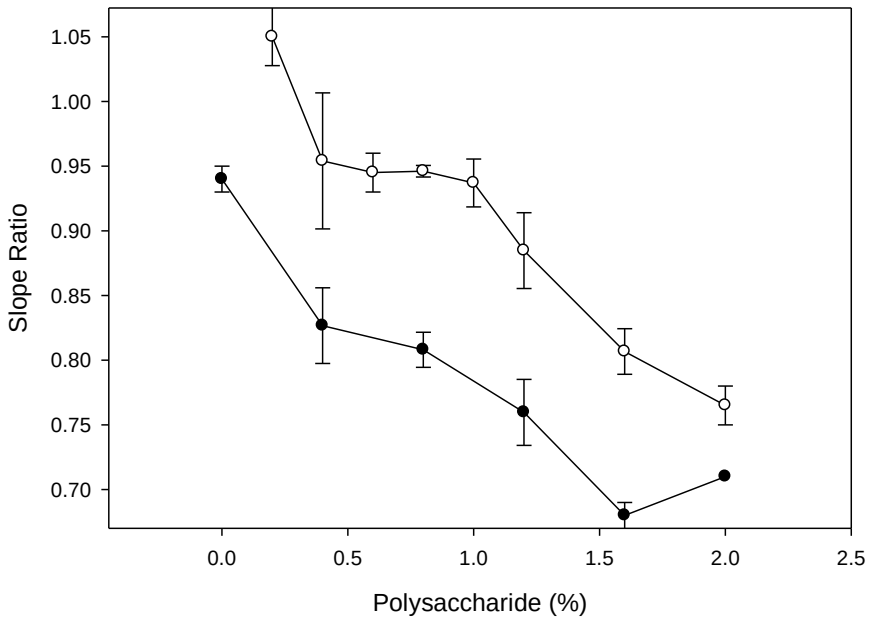


Figure 31 The relationship between slope ratio and concentration of Gellan-H in model processed cheese: ○ = 8.0% (wt/wt) protein; ● = 12.0% (wt/wt) protein. Error bars show the standard error of the mean.

From Figures 32A, 32B and 32C, it is evident that the size and the length of the discontinuous Gellan-H structures increased with an increase in the moisture content in the cheese matrix (i.e. with a decrease in the protein content).

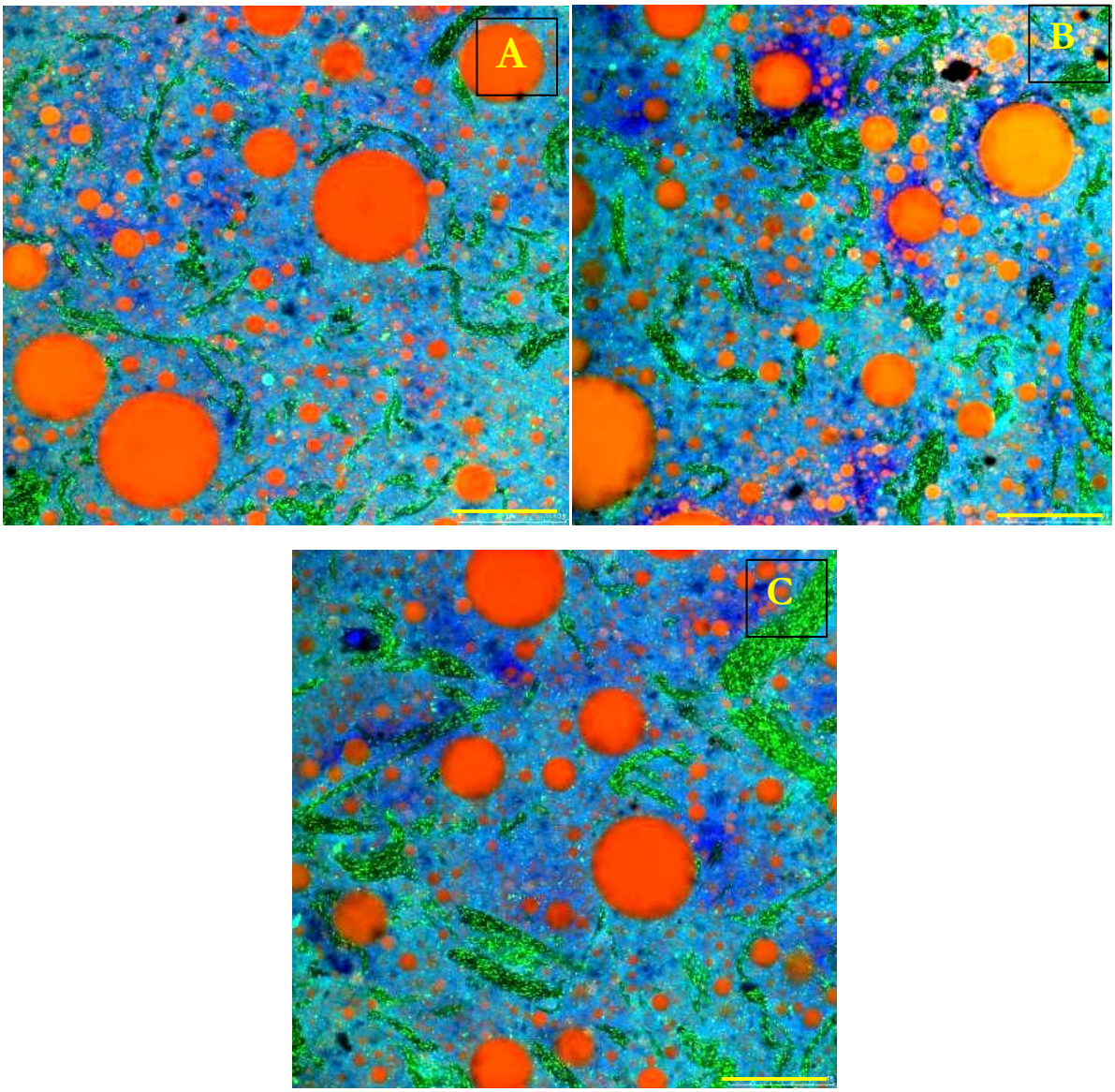


Figure 32 CLSM images of model processed cheeses containing 1.0% (wt/wt) Gellan-H: (A) 14.0% (wt/wt) protein; (B) 12.0% (wt/wt) protein; (C) 8.0% (wt/wt) protein. Protein is blue, fat is red and polysaccharide is dark green. The scale bars represent 75 µm.

The increase in the length scale of the discrete Gellan-H structures at lower protein contents might give more pathways for cracking and lower ε_f. The CLSM micrograph in Figure 32A shows that the Gellan-H structures became more numerous, thin and well distributed in the cheese matrix with an increase in the protein content. This reduced length of inhomogeneity in the structure therefore reduced the susceptibility of the

cheese sample to fail under deformation and hence reduced the magnitude of the reduction in ε_f.

The almost constant ε_f values for Gellan-L (Figure 29B) at all protein contents were consistent with the observed microstructure in the CLSM images (Figure 27C). With increasing Gellan-L content, the continuous protein phase remained nearly unchanged and the microstructure was homogeneous.

6.3.5 Loss tangent (tan δ)

Meltability is another important parameter that determines the functionality of processed cheese because these cheeses are normally manufactured at high temperature and are used as an ingredient in cooked food. The loss tangent (tan $\delta = G''/G'$), as determined by dynamic rheology (Steffe, 1996), has been reported to be a useful predictor of the meltability of imitation cheese (Mounsey & O'Riordan, 2001). The change in tan δ with increasing temperature reflects the changing viscoelastic behaviour of processed cheese when heated. The temperature at the crossover point of G' and G'' (tan $\delta = 1$) is normally defined as the "melting" temperature of the cheese (Sutheerawattananonda & Bastian, 1998). Above this temperature, the viscous behaviour of the cheese dominates over its elastic behaviour ($G'' > G'$).

The increase in tan δ values with increasing temperature from 5 to 85°C (Figures 33A and 33B) showed that the cheese matrix began to soften at 40–45°C for cheese containing Gellan-H, at 35–40°C for cheese containing Gellan-L and at 25–30°C for cheese with no added polysaccharide.

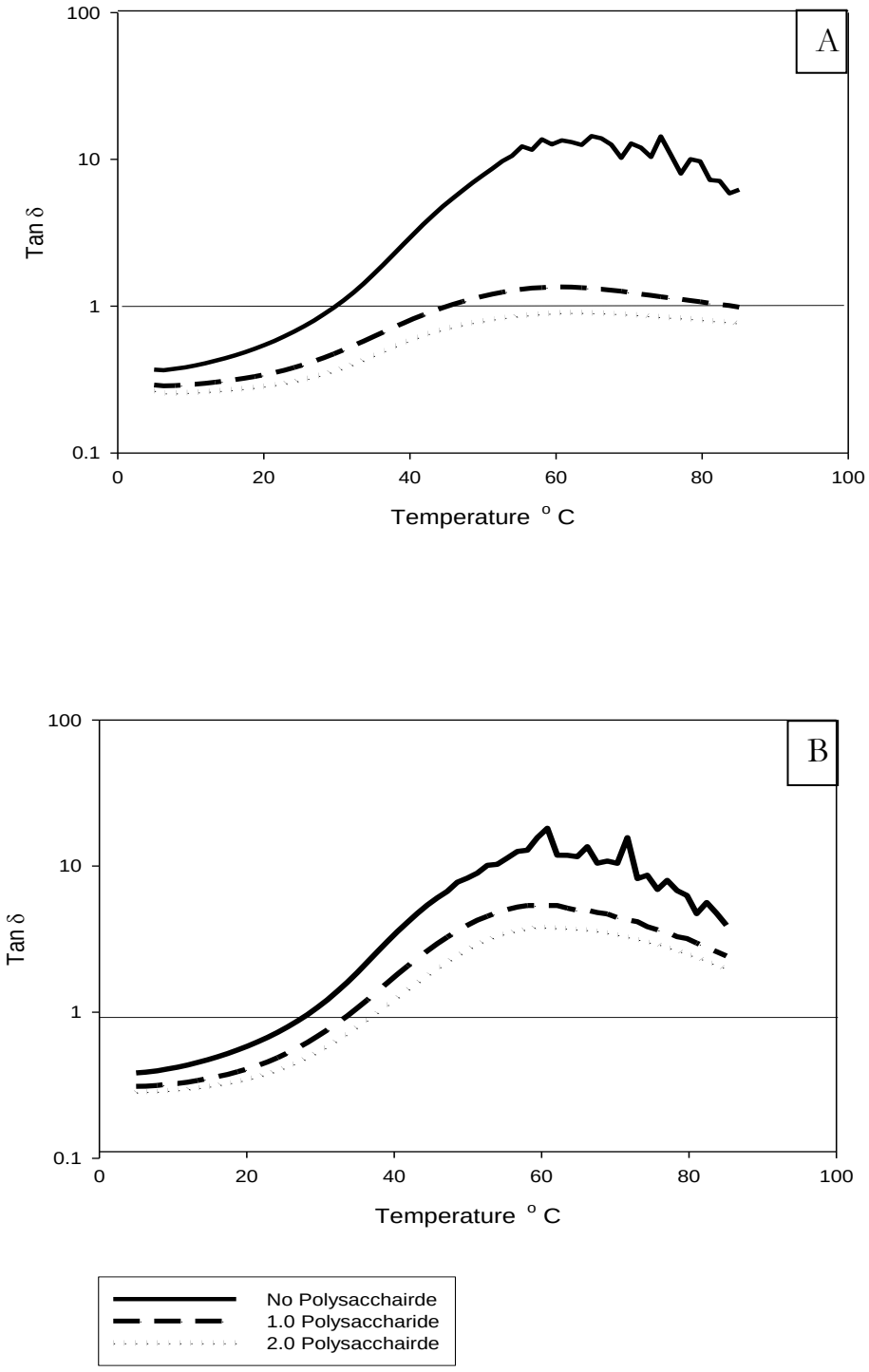


Figure 33 Temperature sweeps at a frequency of 0.1 Hz and a strain of 0.1%, showing changes in tan δ values for model processed cheeses containing (A) Gellan-H and (B) Gellan-L at the same protein content of 10.0% (wt/wt) and different polysaccharide contents of 0.0, 1.0 and 2.0% (wt/wt).

The melting temperature (tan δ = 1) increased with an increase in the concentration of both polysaccharides compared with the samples with no added polysaccharide. The differences in melting temperature for Gellan-H compared with Gellan-L were quite remarkable. Processed cheese containing 2.0% (wt/wt) Gellan-H did not melt even at high temperature (tan δ < 1). The immobilisation of water by the swollen Gellan-H may have resulted in greater dehydration of the protein matrix, leading to increased protein–protein hydrophobic interactions and ultimately poor melting properties (low tan δ values). Mounsey and O'Riordan (2001) reported similar behaviour in imitation cheese in the presence of swollen starch granules.

A maximum in tan δ at around 60°C was found for all cheeses (Figures 33A and 33B). This maximum has previously been described to be the result of the dissociation of protein followed by re-association to form a stronger network (Lee et al., 2003). The tan δ behaviours of Gellan-H and Gellan-L with increasing temperature at two different protein contents in processed cheese (8.0 and 12.0% wt/wt) are presented in Figures 34A and 34B. At low protein content (8.0% wt/wt), all processed cheeses, with or without polysaccharide, showed a plateau in tan δ at above 50°C, perhaps indicating a low level of protein re-association (Figure 34A). A maximum, rather than a plateau, in tan δ was detected at 12% (wt/wt) protein (Figure 34B). At both high protein content and low protein content, the addition of Gellan-H to the processed cheese reduced tan δ more than did the addition of Gellan-L. This effect of reduced tan δ was even more pronounced at low protein content because the addition of 1.0% (wt/wt) Gellan-H resulted in a large reduction in cheese meltability, as defined by tan δ < 1 (Figure 34A).

As mentioned earlier, such behaviour may indicate the dominating effect of the embedded polysaccharide (Gellan-H) gel over the protein phase in the cheese matrix.

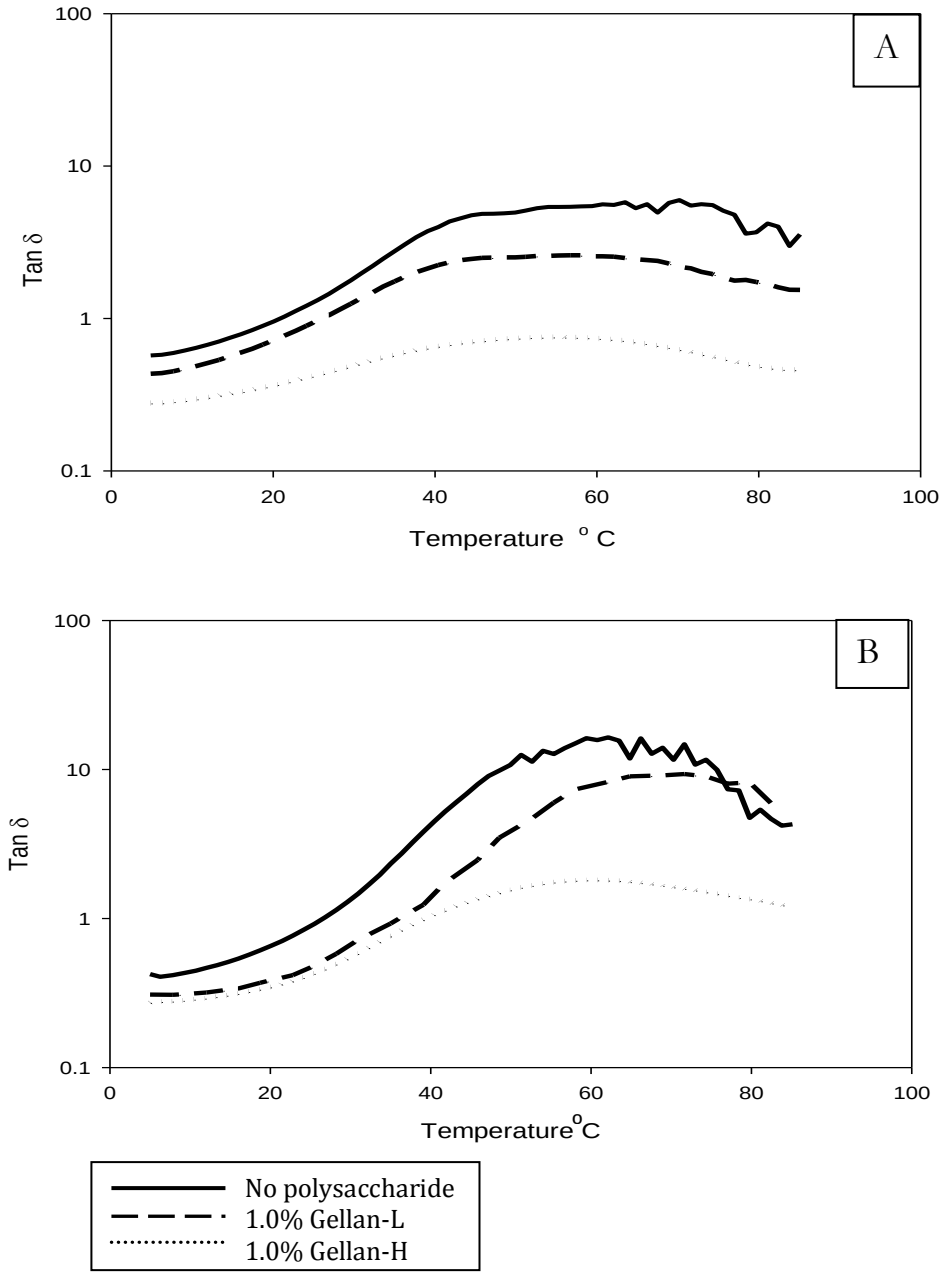


Figure 34 Temperature sweeps at a frequency of 0.1 Hz and a strain of 0.1%, showing changes in tan δ values for model processed cheeses at two different protein contents of (A) 8.0% (wt/wt) and (B) 12.0 (wt/wt), at polysaccharide contents of 0.0 and 1.0% (wt/wt) each for Gellan-H and Gellan-L.

The type of serum phase present and the hydration status of the protein matrix are important in controlling the melting of imitation cheese (Zhou & Mulvaney, 1998). In general, for the current work, cheese with high protein content (12.0% wt/wt) resulted in higher tan δ values, and thus greater flow of the product at higher temperature, than cheese with lower protein content (e.g. 8.0% wt/wt) irrespective of whether or not polysaccharide was added (Figures 34A and 34B). There is no obvious rationale for the higher tan δ values at higher (50–70°C) temperatures of the high protein, low moisture cheese compared with the lower protein, higher moisture cheese, both having no polysaccharide.

These results indicate the important role of casein – as a temperature-dependent biopolymer (Udayarajan, Lucey, & Horne, 2005) – in weakening the resistance to flow even at low moisture contents for the current model cheese system. Casein gels were found to be more dependent on temperature in a mixed polymer system (casein and starch) (Chan et al., 2007); this study on the effect of temperature on the viscosity of a mixed casein–waxy maize starch system revealed that the mixed polymer system with a higher amount of casein (17.0%) and a lower amount of starch (5.0%) showed higher extensional viscosity at higher temperature than a mixture with a low amount of casein (5.0%) and a high amount of starch (25.0%).

The different effects of Gellan-H and Gellan-L on processed cheese may also be related to the different behaviours of the polysaccharides with temperature and in the presence of salts. In the absence of protein and fat and using only TSC, citric acid and NaCl, Gellan-H showed very strong gelling properties compared with Gellan-L (results not shown) at

the same concentration and over the whole temperature range (5–85°C). It is well known that Gellan-H forms thermoreversible gels that normally set at higher temperature (above 65°C) compared with the heat-stable gels formed by Gellan-L, which set at low temperature (30–50°C) (Sworn, 2000a). The presence of TSC, citric acid and NaCl changed the gelling behaviour with temperature of both polysaccharides and completely inhibited the thermoreversibility of Gellan-H. In this case, the effect of monovalent cations (Na^+), divalent cations (Ca^{2+}) and chelating agents on the sol–gel transition of gellan gum, caused by aggregation of double helices, may have determined the behaviour of the polysaccharide in the final processed cheese.

6.4 Conclusions

The textural and melting properties of model processed cheese containing the microbial polysaccharide gellan gum were dependent on the type and concentration of polysaccharide present. Gellan-H increased the "emulsification time" during the cheese-making process probably because of dehydration of protein whereas Gellan-L had no effect on this parameter. The less hydrated protein network formed by Gellan-H compared with Gellan-L showed less effective fat globule particle size breakdown in the model processed cheese. The confocal images revealed that the swollen Gellan-H particles embedded in the protein matrix were separate entities. This may have been the cause of the reduction in fracture strain at low protein content, because the matrix appeared to be disrupted by the polysaccharide phase. The fracture stress increased with increasing Gellan-H content, indicating that this polysaccharide still reinforced the structure of the model processed cheese. However, poor flow behaviour at higher

melting temperatures indicated a lack of gel thermoreversibility of the embedded Gellan-H, together with a certain degree of protein dehydration. Gellan-L had less strengthening effect on the textural and melting properties of the model processed cheese because it had less interaction with the protein matrix.

Chapter 7 Influence of polysaccharide on elongational viscosity, water mobility and microstructure of model processed cheese

7.1 Introduction

Imitation cheese is a multi-component oil-in-water emulsion, with fat and protein as its major components. All the performance characteristics of cheese analogues, such as hardness, stretchability and flow behaviour, are influenced by the precise nature of the protein in the aqueous phase (Ennis, O'Sullivan, & Mulvihill, 1998). Rennet casein is often used as a protein source in processed cheese and its influence on the final product depends on the level of hydration during manufacture. The use of chelating salts in imitation cheese disrupts the calcium-mediated cross-bridges between proteins and thus allows the protein to hydrate (Ennis & Mulvihill, 1999). Moreover, water plays an important role in the hydration of protein by dissolving the calcium chelating salts and dispersing the components (Lee et al., 2004). Therefore, the correct combination of chelating salts with water is important in the manufacture of processed cheese. Together, they have the effect of dispersing and hydrating casein, and promoting emulsification (Ennis & Mulvihill, 1999). However, the presence of polysaccharide in the processed cheese system creates competition for water, mainly between casein and polysaccharide. Depending on the ability of the polysaccharide to interact with water in the cheese system, the protein–water interactions, and therefore the extent and the nature of the hydration of the protein, differ because of the different availabilities of water for interaction. The functionality of a processed cheese containing polysaccharide

is governed by the type and the amount of polysaccharide present in the cheese matrix (Noronha et al., 2008).

Fundamental rheological measurements, such as lubricated squeezing flow, are preferred for evaluating the flow characteristics of soft foods, e.g. their spreadability under constant force (Sun & Gunasekaran, 2009). The term "spreadability" can be defined as the ease with which soft foods will flow or spread under a small force. Biaxial elongational viscosity is a good measure of the relative spreadability of soft foods (Sun & Gunasekaran, 2009) and the method is well established for evaluating the flow characteristics of cheese (Wang et al., 1998).

The flow characteristics of soft cheeses depend on the amount (and mobility) of water present in the continuous cheese matrix. The presence of polysaccharides that are phase separated in imitation cheese effectively changes the amount of water (and its mobility) in the continuous protein phase (Chan et al., 2007) and, thus, the functionality of the cheese. Therefore, measurement of the physical distribution of the water and fat in the processed cheese structure (and whether the protein and polysaccharide are phase separated) is of interest for cheese scientists because of the influence of this distribution on the functional properties of the cheese.

Water molecules in biological materials can be classified into several fractions according to their mobility and the extent of their association with or "binding" to macromolecules within the material (Kuo, Gunasekaran, Johnson, & Chen, 2001). To gain an understanding of the functionality of a multi-component food system, it is necessary to understand the role and the contribution of the individual components in

the system. Nuclear magnetic resonance (NMR) has been used by many researchers to measure the mobility of water and its relation to the functionality of many types of cheese (Gianferri, Maioli, Delfini, & Brosio, 2007; McMahon, Fife, & Oberg, 1999). The application of NMR to determine the actual amount and the mobility of water molecules that neighbour various polysaccharide and protein domains could provide useful information on the role of water in changes in functionality. The presence of polysaccharide in imitation cheese has been hypothesised to affect casein hydration. A recent study on imitation cheeses containing starch (Noronha et al., 2008) revealed that the spin–lattice and spin–spin relaxation times can be used to distinguish between the tightly bound water and the moderately bound water in multi-component imitation cheeses. However, there is as yet no direct evidence for the hypothesis of casein hardening in cheese containing polysaccharide. Moreover, there is no published literature on the association of water with the individual components in processed cheese containing a microbial polysaccharide such as gellan gum.

This chapter shows that a liquid-state NMR and cross-polarisation/magic-angle spinning (CP/MAS) solid-state NMR study of processed cheese containing gellan gum provides information about the changes in the state of water and fat that are induced by the presence of polysaccharide in the cheese matrix, and thus insight into changes in the spreadability of the cheese.

7.2 Materials and methods

7.2.1 Manufacture of model processed cheese

Refer to Section 3.1.

7.2.2 Sample preparation for squeezing flow measurement

The molten cheese was carefully poured from the Rapid Visco Analyser (RVA) canister into two stainless steel cylinders (7 mm in height x 30 mm in diameter) to the brim to give accurate height and shape to the sample. The inner surface of the cylinders was lined with polypropylene film. The bottom and upper ends of the cylinders were lined with polypropylene film and closed using aluminium foil. The cheese samples were then placed in an air-tight container and were kept at 5 ± 1°C for 24 h prior to physical testing. They were removed from the cylinders and placed into the UW melt meter cup for measurement.

7.2.3 Constant force test

The flows of model processed cheeses containing high acyl gellan gum (Gellan-H) and low acyl gellan gum (Gellan-L) were studied at a constant temperature of 25°C under a constant force at 0.55 N. This force was found to give a convenient final height for the cheeses used.

The constant force test was carried out as described in Section 3.6.

7.2.4 Determination of fat globule size

Refer to Section 3.5.

7.2.5 Proton transverse (T_2) relaxation measurements

Refer to Section 3.7.

7.2.6 ^{13}C MAS solid-state NMR

Carbon (^{13}C) direct polarisation (DP) and cross-polarisation (CP) MAS NMR spectra of the cheese samples were acquired. For the CP/MAS spectra, the proton magnetisation was cross-polarised to adjacent carbons (^{1}H–^{13}C cross-polarisation). CP/MAS spectra represent only the solid-like components of the sample; any liquid-like component will not cross-polarise efficiently enough to be observed. However, DP/MAS spectra represent all the carbon species, both solid like and liquid like, present in the sample (Morgan, Furneaux, & Larsen, 1995).

^{13}C CP/MAS spectra were acquired with a ^{1}H 90^{o} pulse of 5.5 ms, a cross-polarisation contact time of 1000 ms, an acquisition time of 30 ms, a relaxation time of 2 s and 3K scans. ^{13}C DP/MAS spectra were acquired with a 90^{o} pulse of 5.5 ms, an acquisition time of 30 ms, a relaxation time of 2 s and 5K scans. The same receiver gain was used for both methods.

7.2.7 Transmission electron microscopy (TEM)

7.2.7.1 Fixation

Samples were cut into ~ 1 mm^3 cubes and put into bijoux bottles containing 6.25% glutaraldehyde in 0.2 M imidazole buffer.They were stored at 5°C for 48 h before being fixed. The glutaraldehyde solution was rinsed twice with 0.2 M imidazole buffer over 2 h. The buffer was then removed and the embedded liquid samples were placed into 1% osmium tetroxide in 0.2 M sodium cacodylate for 24 h. The samples were then rinsed twice with distilled water.

7.2.7.2 Dehydration

The dehydration process was carried out at 5°C in 25% acetone (15 min), then in 50, 70 and 90% acetone (for 30 min each) and finally in 100% acetone (three changes over 90 min).

7.2.7.3 Embedding

The acetone was then replaced with 1:1 100% acetone:Procure 812 embedding resin, and put on rollers for 12 h. This was then replaced with 100% Procure 812 for 12–24 h before a cube of sample was placed into a BEEM embedding capsule and cured at 60°C for 48 h.

7.2.7.4 Sectioning

The embedded samples were sectioned to a thickness of 90 nm using a Leica Ultracut R microtome. These sections were mounted on 3 mm copper/rhodium grids and stained

using uranyl acetate and lead citrate before examination in a Philips transmission electron microscope (Philips 201C, Eindhoven, The Netherlands) at an accelerating voltage of 60 kV.

7.3 Results and discussion

Confocal micrographs showed that Gellan-H and protein were phase separated (Figure 27B) and that Gellan-L and protein were not so clearly phase separated (Figure 27C). In an earlier study, (Chan et al., 2007) stated that the higher viscosity induced in casein–waxy maize starch mixtures (assuming the usual phase separation between protein and starch) is probably the result of effective protein dehydration caused by the polysaccharide taking some of the water. Phase separation means that the effective concentrations of both protein and starch within their own phases are considerably higher than their original nominal concentrations in the mixture. We assume that this hypothesis applies more generally to phase-separated protein and non-starch polysaccharides.

We hypothesise that water mobility (as measured by NMR) is an indirect measure of the effective concentrations of protein and polysaccharide. Thus, in general, the addition of polysaccharide together with its phase separation from protein should give lower water mobility and higher viscosity than in polysaccharide-free cheese.

7.3.1 Spin–spin relaxation (T_2)

7.3.1.1 Water

Spin–spin relaxation (T_2) measurements were obtained using the multi-component model (Budiman, Stroshine, & Campanella, 2000; Noronha et al., 2008) to analyse the model processed cheese. The major components of the multi-component system model processed cheese are casein, fat, water and polysaccharide. The fast relaxing component can be attributed to water molecules entrapped in the protein matrix; they form an integral part of the protein structure and can interact with it by diffusion from the bulk to the biopolymer interface. The slowly relaxing component can be attributed to water molecules present in open channels in the protein matrix (Gianferri, D'Aiuto, Curini, Delfini, & Brosio, 2007).

Figure 35 shows the T_2 profiles of the Gellan-H gel in water and of the processed cheese containing Gellan-H at two different protein contents, 8.0 and 14.0%. The peak T_2,tb (i.e. the lower T_2 value) corresponds to protons in a less mobile fraction of water within the cheese, i.e. water that is tightly bound. The second peak T_2,mb (i.e. the higher T_2 value) corresponds to the moderately bound water within the cheese matrix (Noronha et al., 2008). The T_2 profiles in this work showed decreases in T_2,tb and T_2,mb when the protein content was increased from 8.0 to 14.0%. This indicated that protein played a major role in binding the water more tightly in the cheese matrix. Figure 35 shows just one peak for the Gellan-H gel in water, whereas the presence of protein in the cheese formulation split the T_2 relaxation time into two different peaks. This means that, in addition to bound water, water may be physically entrapped by assemblies of protein

molecules in the processed cheese which is still not completely hydrated. The diffusive behaviour of a large fraction of entrapped water has been reported to be very much like that of bulk liquid water, even in closely packed protein crystals (containing between 25 and 80 wt% water) (Dickinson & McClements, 1995).

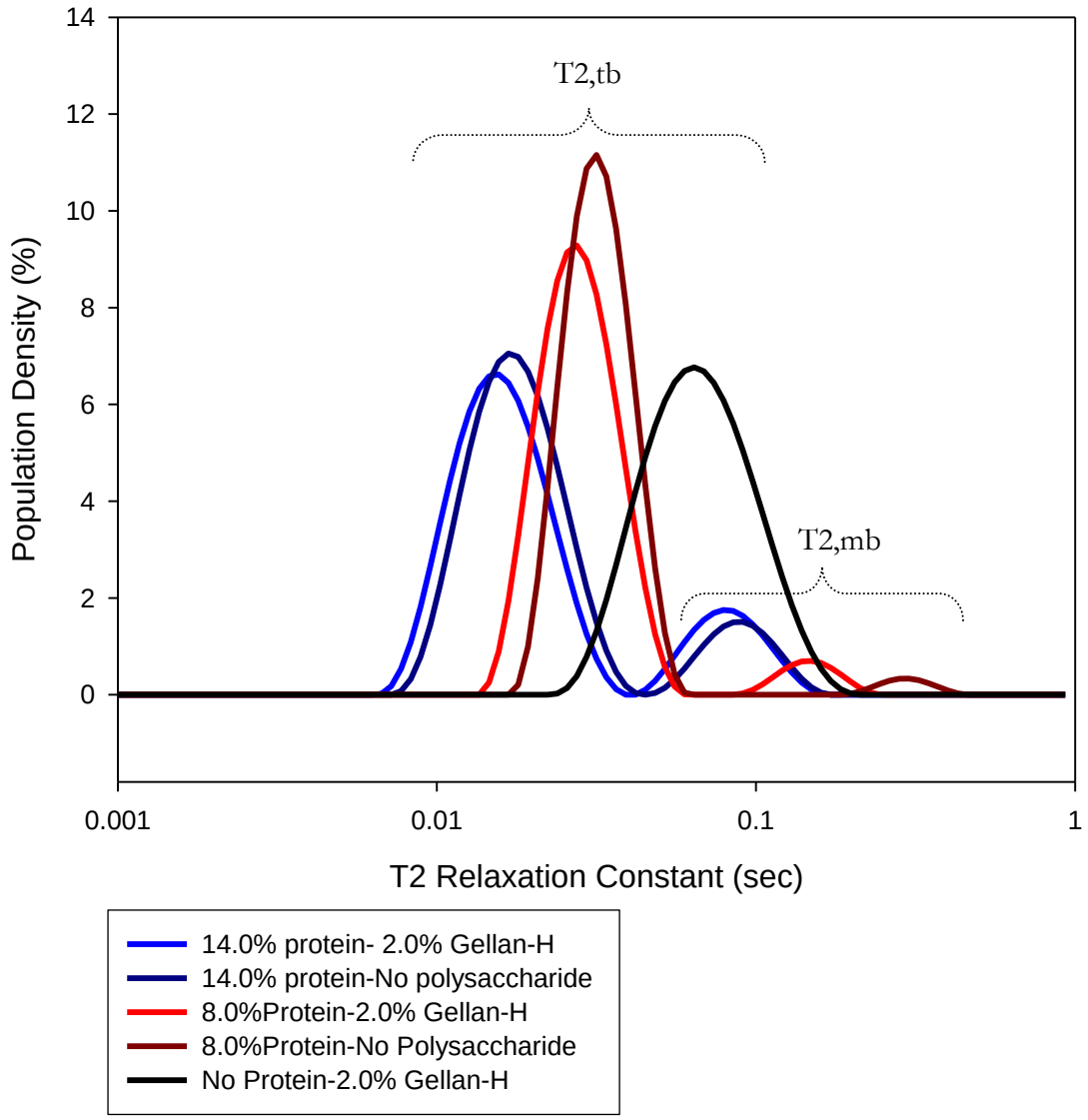
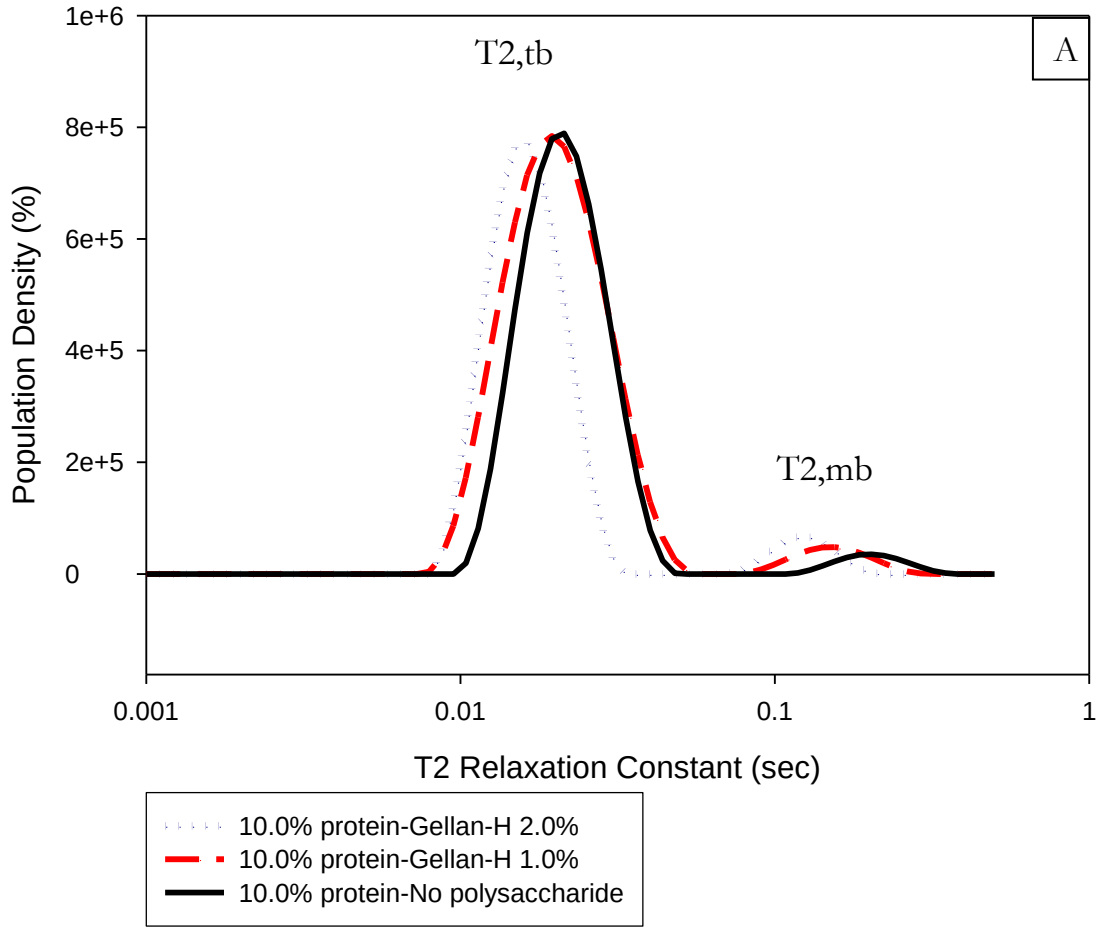


Figure 35 Spin–spin relaxation (T_2) of the water component of model processed cheeses.

Noticeable differences in the T_2 values were observed (Figures 36A and 36B) when standard processed cheese formulations (10.0% protein and 30.0% fat) containing Gellan-H and Gellan-L were compared. As the Gellan-H content increased, the T_2,tb peak decreased from 0.2 to 0.12 s, and the T_2,mb peak became narrower and the peak intensity increased. This can be attributed to the presence of Gellan-H reducing the water mobility in the processed cheese. Also the narrowing of the T_2,mb peak suggests that there was an increase in the uniformity of the chemical and physical states of the water molecules in the processed cheese.



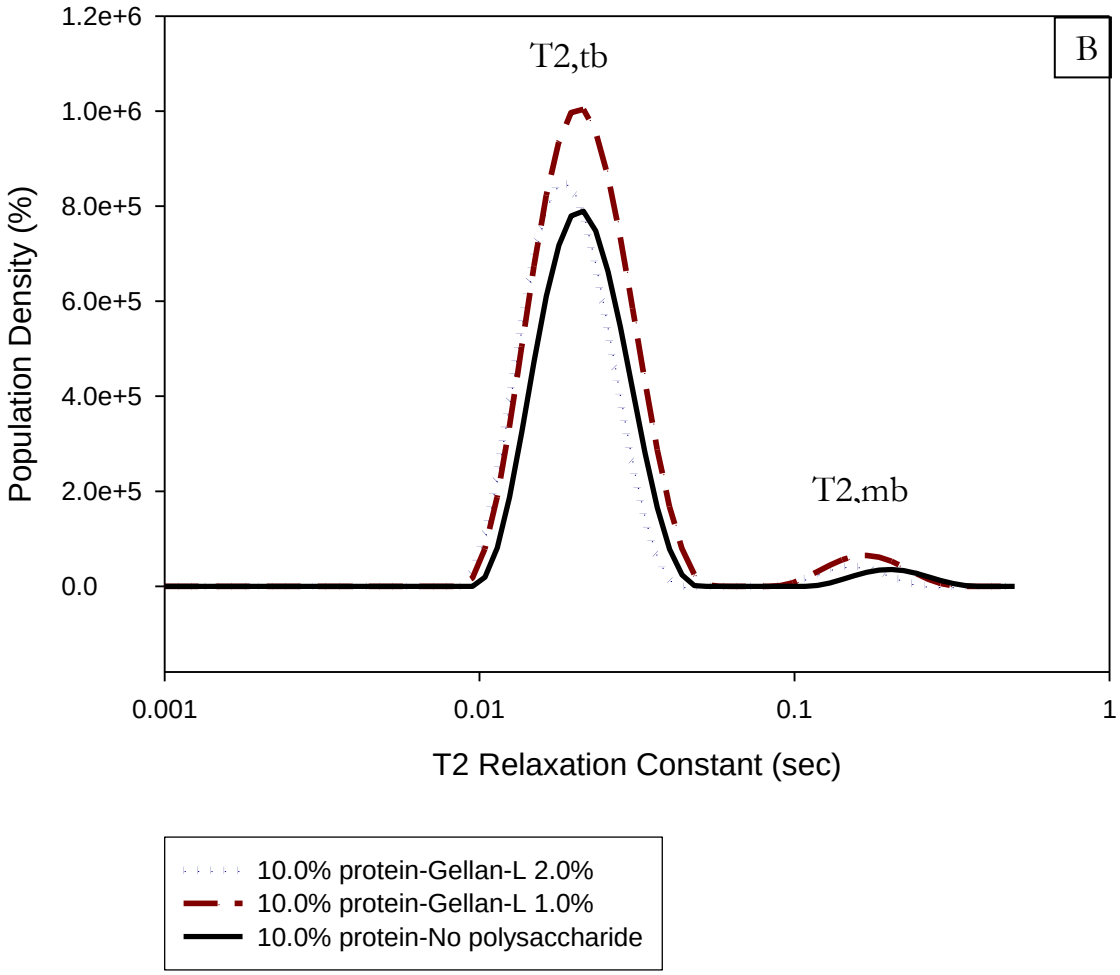


Figure 36 Spin–spin relaxation (T_2) of the water component of model processed cheeses containing 10.0% (wt/wt) protein, 30.00 % (wt/wt) fat and 50.00% moisture: (A) Gellan-H; (B) Gellan-L.

Increasing the Gellan-L content from 0.0 to 2.0% gave a slight reduction in the water mobility (T_2,tb) and the peak width tended to narrow. This behaviour was not observed for Gellan-H. Therefore, it can be inferred that the inclusion of Gellan-L induced more uniformity in the samples. The small but noticeable changes in the less mobile fraction (T_2,tb), revealed that this fraction might be associated with the solids portion in the

processed cheeses. At 1.0% Gellan-H and Gellan-L, changes in T_2,tb were not noticeable. However, at 2.0% Gellan-H, a small reduction was observed. The reduction in T_2,tb values were even smaller for Gellan-L at 2.0% than for Gellan-H at 2.0%.

Thus our hypothesis that the addition of polysaccharide together with its phase separation from protein should give lower water mobility in processed cheese is supported by these results for Gellan-H and, to a lesser extent, for Gellan-L.

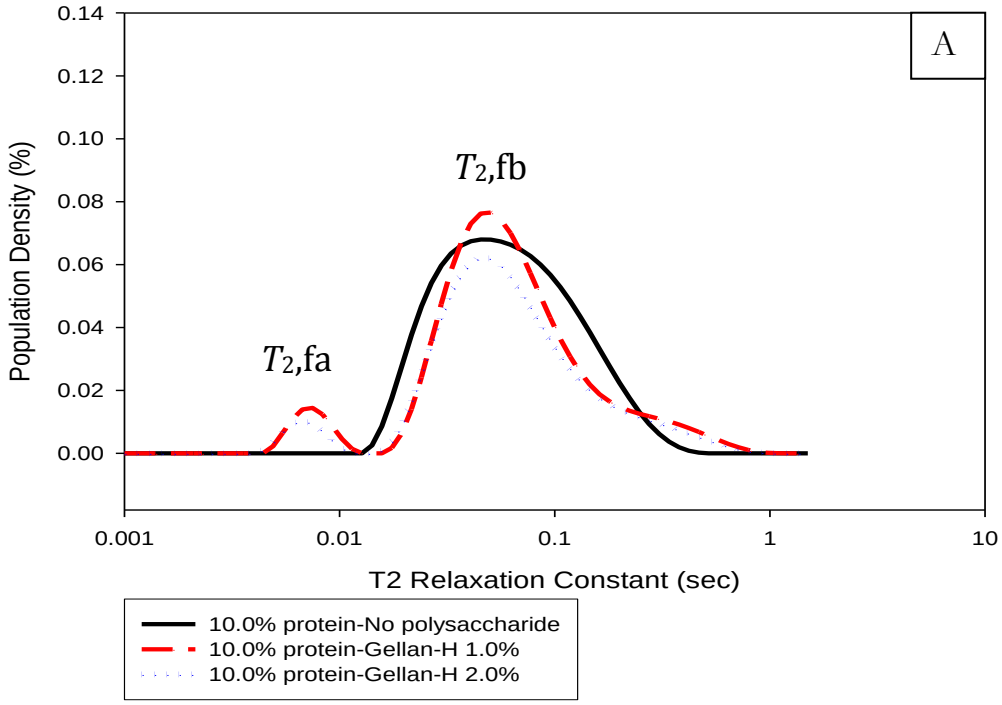
7.3.1.2 Fat

The competition for water by protein and polysaccharide in processed cheese increases the viscosity of the continuous phase (assuming that protein is continuous and loses water to polysaccharide). An increased viscosity of the continuous phase generates higher shear stress during processing; the higher shear stress is better able to reduce the size of the fat globules (Lee et al., 2004). A small fat globule size and an increase in homogeneity enhance the incorporation of fat into the protein matrix (Jost, Baechler, & Masson, 1986). A definite trend of the distribution of fat in the cheese was evident when the component (T_2,f) representing protons from the fat phase was relaxed.

The inclusion of polysaccharide split the single peak (polysaccharide-free cheese) into two peaks: T_2,fa and T_2,fb (Figures 37A and 37B). The fat with lower mobility (T_2,fa) can be described as being more emulsified and having finely distributed small globules, whereas the fat with higher mobility (T_2,fb) can be described as being fat in larger globules in the cheese matrix. Figure 37A shows that the addition of Gellan-H generated less emulsified fat in the processed cheese than the addition of Gellan-L. According to

the interpretation discussed in Chapter 6, the larger fat globules in the matrix containing Gellan-H could have been due to less water being available for protein hydration. This then reduced the action of trisodium citrate (TSC) in the cheese samples containing Gellan-H, resulting in poorly hydrated casein. Insufficient hydration of the protein may result in an impaired ability to stabilise the oil-in-water emulsion formed during cheese analogue manufacture (Ennis et al., 1998), leading to larger fat globules in the cheese matrix.

Another possible reason for not achieving efficient fat globule breakdown could have been poor mixing efficiency during RVA processing at the extremely high viscosities generated at high Gellan-H contents. The more intense and narrow T_2,fa peak observed in the cheese containing Gellan-L (Figure 37B) indicated finely distributed small fat globules in the structure and, therefore, that the fat globules were more efficiently broken down during RVA processing. The greater amount of moisture left by Gellan-L to hydrate the protein in the cheese matrix could have been why there was more efficient interaction of protein with fat. Extensive hydration of the protein molecules permitted their greater interaction with the oil droplet surface, giving better stabilisation of the dispersed oil phase than was obtained at low levels of protein hydration (Ennis & Mulvihill, 1999).



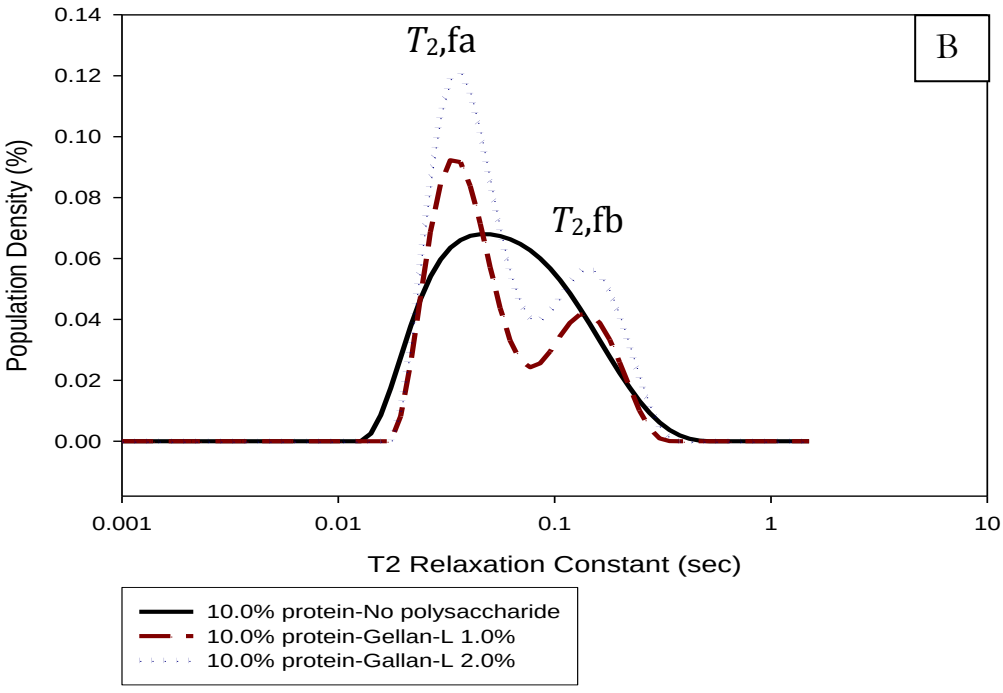


Figure 37 Spin–spin relaxation (T_2) of the fat component of model processed cheeses containing 10.0% (wt/wt) protein, 30.00 % (wt/wt) fat and 50.00% moisture: (A) Gellan-H; (B) Gellan-L.

The results for NMR relaxation of the fat phase (Figures 37A and 37B) correlated with the Malvern particle size distribution in the same processed cheese sample. The increase in the number of finely broken down fat globules in the cheese with the addition of Gellan-L, as measured by the Malvern method (Figure 38B), was similar to the increase in less mobile fat with the addition of Gellan-L, as measured by NMR relaxation (Figure 37B). As discussed earlier, the reduced amount of mobile water left in the cheese matrix as a result of the competition for water between Gellan-L and protein in the cheese matrix could have resulted in an increase in viscosity and, in turn, increased the efficiency of fat globule breakdown. Confocal microscopy (Pereira, Bennett, Hemar, & Campanella, 2001) and particle size distribution (Lee et al., 2004) results for model processed cheeses showed a similar decrease in fat particle size with decreasing available moisture content in the cheese matrix. The observed decrease in fat proton relaxation time and intensity for the cheese containing Gellan-H could have been due to strong emulsification of the partial volume of fat through Gellan-H, which generated a small volume of highly immobile fat but left the rest of the fat in a mobile state. As discussed earlier, the generation of mobile fat could have been the effect of a spindle slip during RVA processing. The state of the fat correlated positively with the fat particle size in the cheese matrix, when evaluated by Malvern particle size distribution.

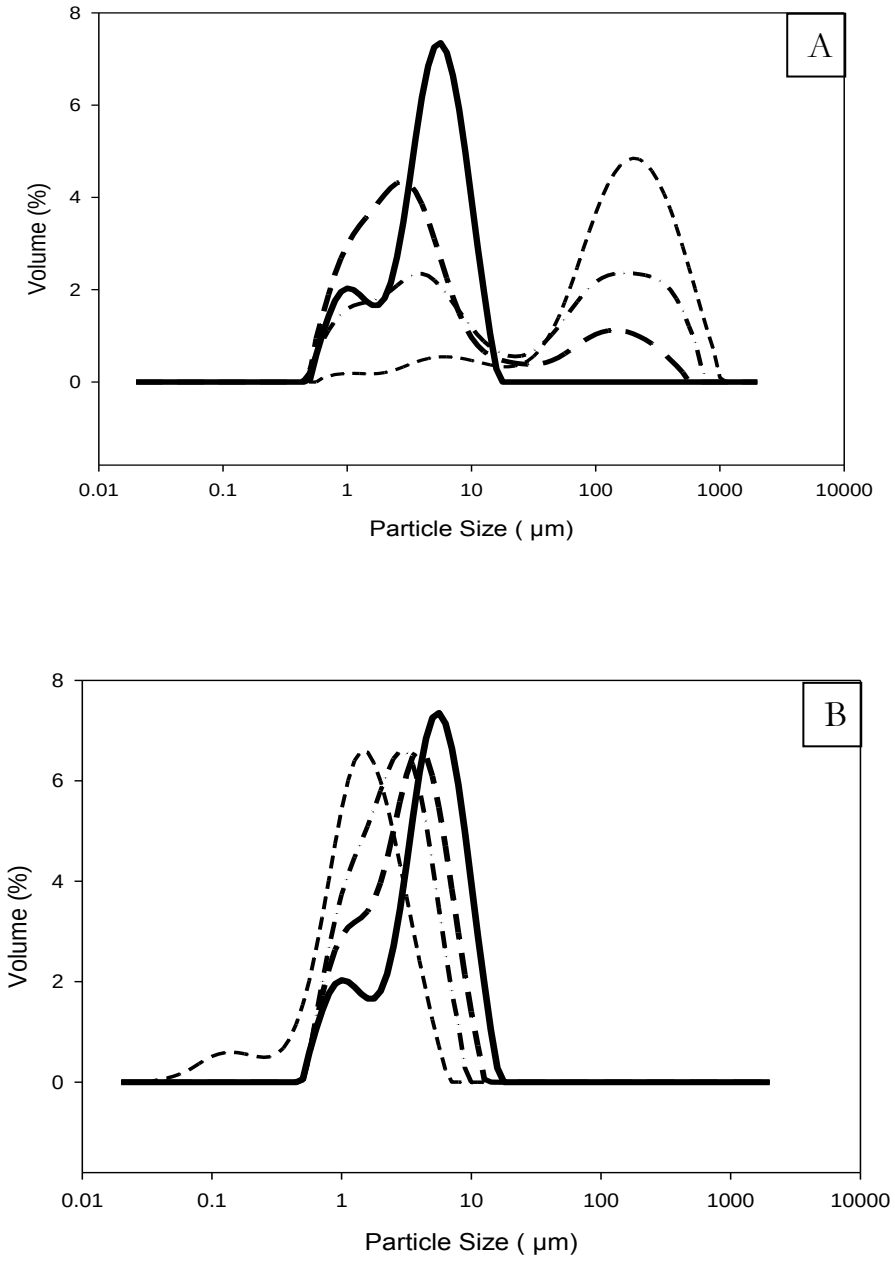


Figure 38 Fat globule particle size in the cheese matrix for various polysaccharide contents in model processed cheeses containing 10.0% (wt/wt) protein, 30.00% (wt/wt) fat and 50.00% moisture: (A) Gellan-H; (B) Gellan-L.

7.3.1.3 Protein

TSC disrupts the calcium-mediated cross-bridges between the proteins in cheese analogues and model processed cheese (Ennis & Mulvihill, 1999; Lee et al., 2004). The level of casein hydration obtained depends on the extent of this disruption (Caric & Kaláb, 1993). The presence of polysaccharide in processed cheese may reduce the availability of water for chelating salts to reach the cross-bridges of rennet casein and, therefore, the efficiency of water to interact with casein. In this study, the addition of Gellan-H at 2.0% wt/wt at two different protein contents, 8.0 and 14.0%, significantly reduced the intensities of the T_2,tb peaks (Figure 35). However, the changes were more pronounced at low protein contents (formulations with higher moisture contents), which could have been due to the increased water binding capability of Gellan-H in the cheese with more bulk moisture. The intensity of the shift in the T_2,mb values indicated that the presence of Gellan-H shifted moderately mobile water to less mobile water. The changes were greater at 8.0% protein than at 14.0% protein. Moreover, the broader peak width for cheese with a higher protein content indicated a relatively less uniform sample in terms of the distribution of water within the cheese matrix. In general, the peak width relates to the uniformity of the sample, with broader peaks corresponding to less uniform samples (Noronha et al., 2008).

As expected, the influence of water mobility was reflected in the spreadability when the squeezing flow at constant force (0.55 N) and temperature (25°C) of the model processed cheeses was measured. As shown in Figure 39, the processed cheese containing 12.0% protein and 2.0% Gellan-H showed a slower decrease in height than

the processed cheese containing 8.0% protein and 2.0% Gellan-H. This indicated that a higher protein content imparted more solid-like character to the cheese formulation at high polysaccharide contents.

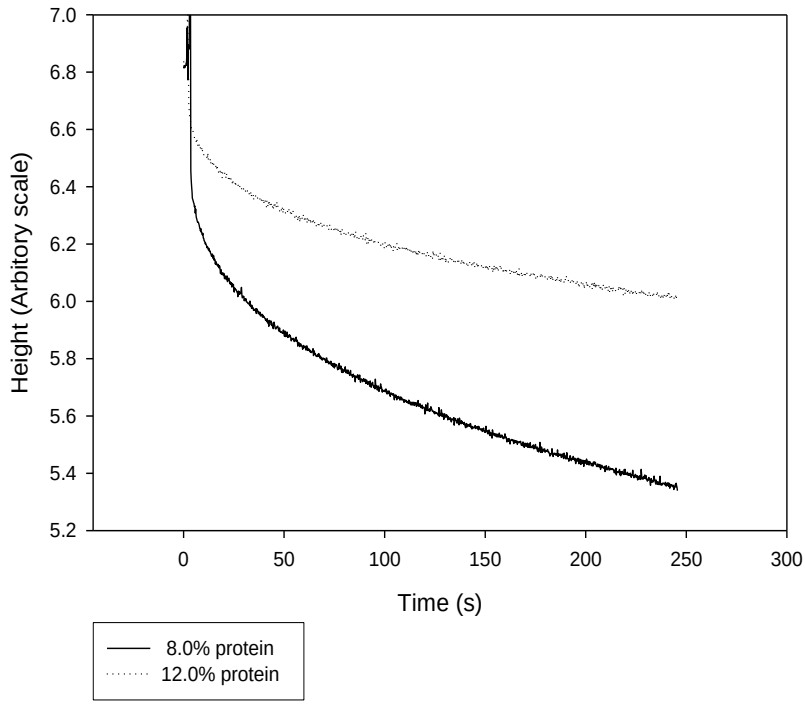
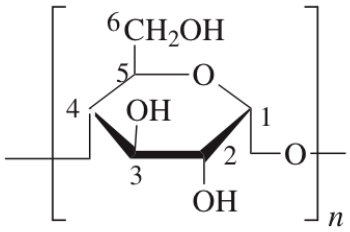


Figure 39 Height under constant force (0.55 N) at 25°C of control model processed cheeses containing 2.0% Gellan-H and two different protein contents: ●, 8.0% protein; ○, 12.0% protein.

The T_2 relaxation component supported the observation of a more solid-like character when the less mobile water in the cheese matrix was increased. However, it is difficult to define the increase in the solid-like character of the cheese that results from increased protein dehydration caused by polysaccharide using the T_2,tb and T_2,mb components. The solid-state ^{13}C CP/MAS NMR technique has the potential to physicochemically characterise an intact food sample (Pizzoferrato et al., 2000). Higher

intensity ^{13}C CP/MAS NMR has also been applied to interpret the presence of more rigid, crystalline and glassy components of starch (Morgan et al., 1995; Morgan, Gerrard, Every, Ross, & Gilpin, 1997).

Figure 40 shows the ^{13}C CP/MAS NMR spectra of processed cheese samples containing 2.0% Gellan-H and two different protein contents, 8.0 and 12.0%. For ^{13}C CP/MAS NMR, the peaks between 50 and 110 ppm can be assigned to the C1 to C6 carbons of the glucan units of carbohydrates (Gidley & Bociek, 1988).



The R(CO)X peak (~ 175 ppm) can be assigned to the main chain peptide carbonyl carbons of proteins and the C–H_n aliphatic carbon peaks (20–40 ppm) can be assigned to protein and lipid side chains (Baik, Dickinson, & Chinachoti, 2003). The C=C peak (135 ppm) and the CH_n peaks (15–40 ppm) can be assigned to lipids and fats. In Figure 40, the signal resonance at about 150 ppm was attributed to the C1 carbons of protein moieties, and the bands due to the carbons of the amino acids of the proteins were clearly distinguishable from those due to the carbons of the polysaccharides. Changes in the peak intensities of ^{13}C CP/MAS NMR spectra have been related to changes in molecular mobility, with higher peak intensities being due to decreases in segmental mobility, resulting in more efficient cross-polarisation.

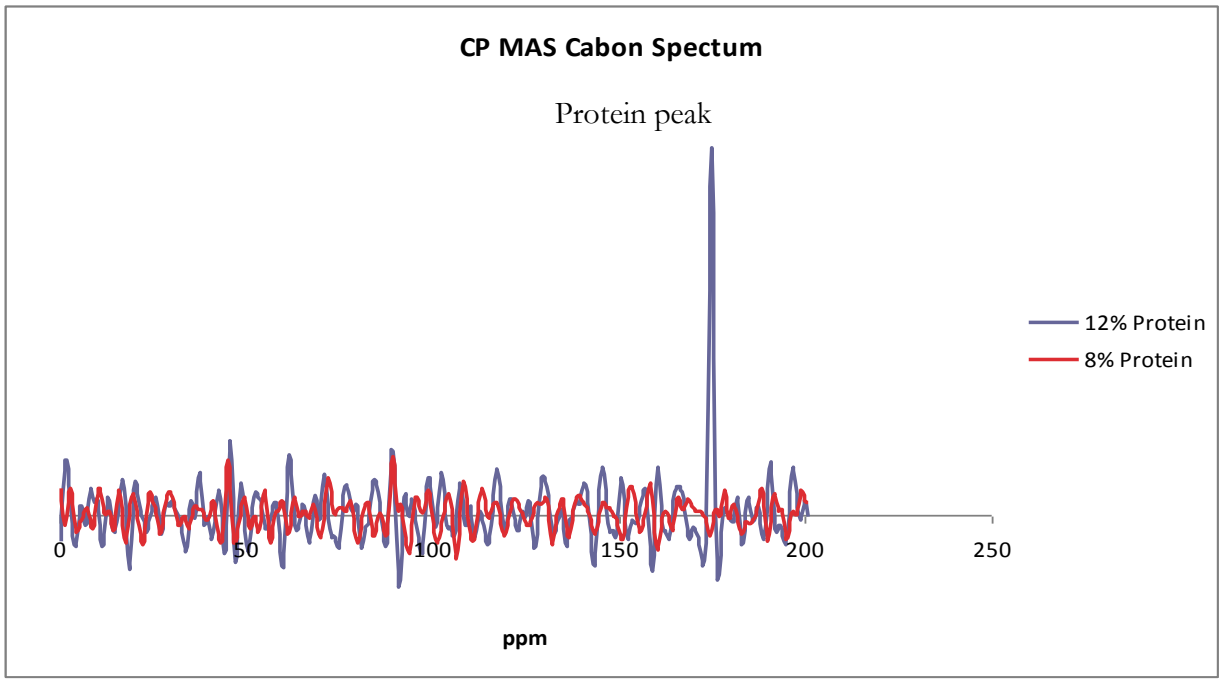


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Figure 40 ^{13}C CP/MAS NMR spectra under constant force (0.55 N) at 25°C of model processed cheeses containing 2.0% Gellan-H and two different protein contents: —, 8.0% protein; —, 12.0% protein.

The model processed cheese containing 8.0% protein and 2.0% Gellan-H exhibited a significant loss in the signal intensity in the ^{13}C CP/MAS NMR spectrum. This indicated that the major components of this cheese had more mobile and liquid-like behaviour. The clear and sharp protein peak observed in the ^{13}C CP/MAS NMR spectrum for the model processed cheese containing 12.0% protein and 2.0% Gellan-H indicated a hard protein component, which was interpreted as being dehydrated protein in the cheese. The exchange of water from the hydration layer around the exterior of the protein to the polysaccharide phase in the cheese matrix possibly altered the association of water molecules with the protein and made more bulk water available for Gellan-H. The dehydration of the protein in the presence of polysaccharide may have been caused by

migration of bulk water from hydrated casein particles to the polysaccharide phase. A very small amount of water is located in the interior of folded protein molecules. This water forms hydrogen bonds with the polar groups and helps to maintain the tertiary structure of the protein. The exterior of protein molecules is enveloped in a water hydration layer; this water is predominantly bound to the proteins and exchanges rapidly with the bulk water (Dickinson & McClements, 1995).

The spectra in Figure 40 showed that there was no marked difference in the carbohydrate components between 50 and 110 ppm in the two samples. This indicated that the embedded discontinuous polysaccharide in the continuous protein phase behaved like a soft gel in the cheese matrix.

The protein component in a cheese analogue stabilises the oil-in-water emulsion by reducing the tension at the aqueous phase–oil interface (Ennis & Mulvihill, 1999). As expected, increasing the protein content from 8.0 to 14.0%, and simultaneously reducing the moisture content, in the processed cheese formulation resulted in marked changes in both fat components (as shown in Figure 41).

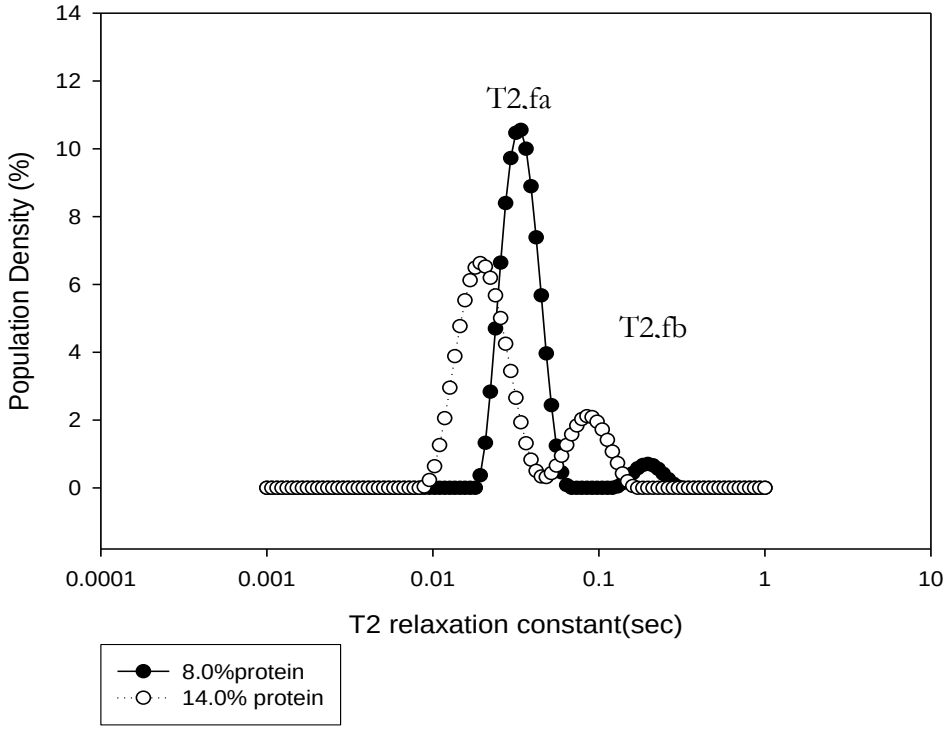

Figure 41 Spin–spin relaxation (T_2) of the fat component for control model processed cheeses with different protein contents: ●, 8.0% protein; ○, 14.0% protein.

The decrease in the intensity of the T_2,fa peak and the increase in the intensity of the T_2,fb peak indicated an increase in less mobile fat with an increase in the protein content. This could have been an effect of the viscosity increase in the serum phase during manufacture of the processed cheese. The higher viscosity of the continuous phase because of the higher volume fraction of protein means that it is easier to break the fat particles into smaller sizes in model processed cheese spreads (Lee et al., 2004).

7.3.2 Constant force measurement

The water mobility, as measured by NMR relaxometry, supported the rheological measurements from lubricated squeezing flow of the model processed cheese. As noted

in Section 7.1, the viscosity from squeezing flow is a transient biaxial elongational viscosity, which is better described as a biaxial stress growth coefficient (BSGC). The objective of this study was to understand the effect of Gellan-H and Gellan-L on the BSGC and water binding in the model processed cheese. A UW melt meter, operated in the lubricated squeezing flow configuration, was used at constant force. This device provides controlled heating, a constant sample temperature and rheologically well defined test and data analysis. Figure 42 shows the decreases in sample height at 0.55 N during tests on standard model processed cheese formulations (12.00% wt/wt protein, and 30.00% wt/wt fat) containing Gellan-H and Gellan-L. Each curve represents the mean of three replicates.

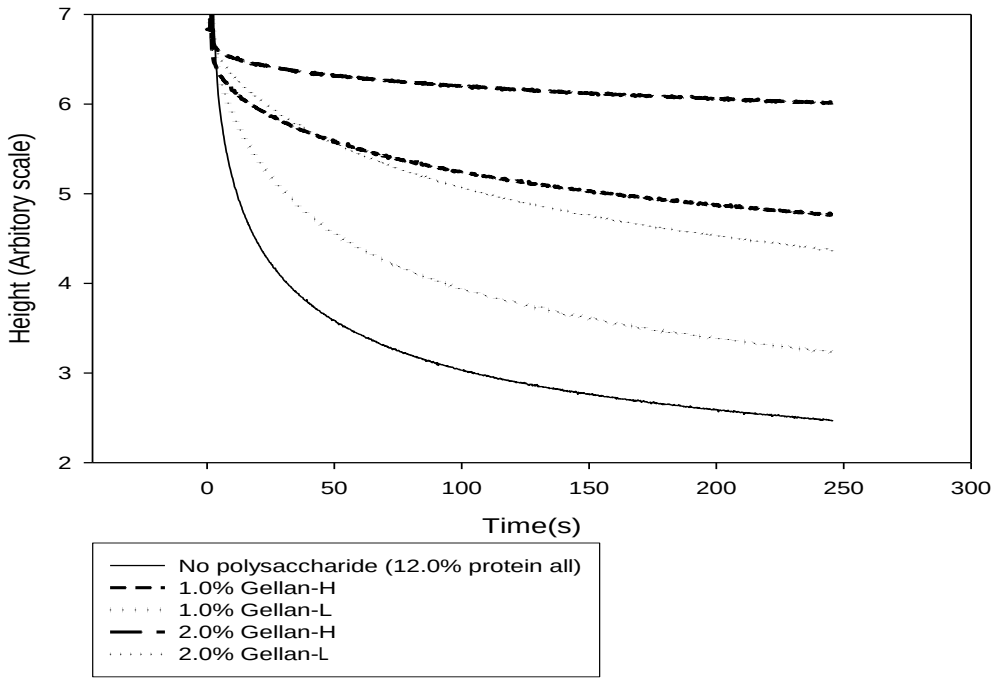

Figure 42 Height under constant force (0.55 N) at 25°C of control model processed cheeses (30% fat and 12.0% protein) containing Gellan-H and Gellan-L.

The curves show that there was a decrease in height of the processed cheeses containing Gellan-L and Gellan-H with an increase in the polysaccharide content, with the decrease being more rapid and greater in the cheeses containing Gellan-L than in those containing Gellan-H. This indicated that the processed cheeses containing Gellan-L exhibited a lower BSGC (more liquid-like behaviour), whereas the processed cheeses containing Gellan-H exhibited a higher BSGC (more solid-like behaviour).

Figure 43 shows the mean BSGC values for model processed cheeses with different polysaccharide contents. The values indicate that the BSGC for Gellan-H was higher in general than the BSGC for Gellan-L.

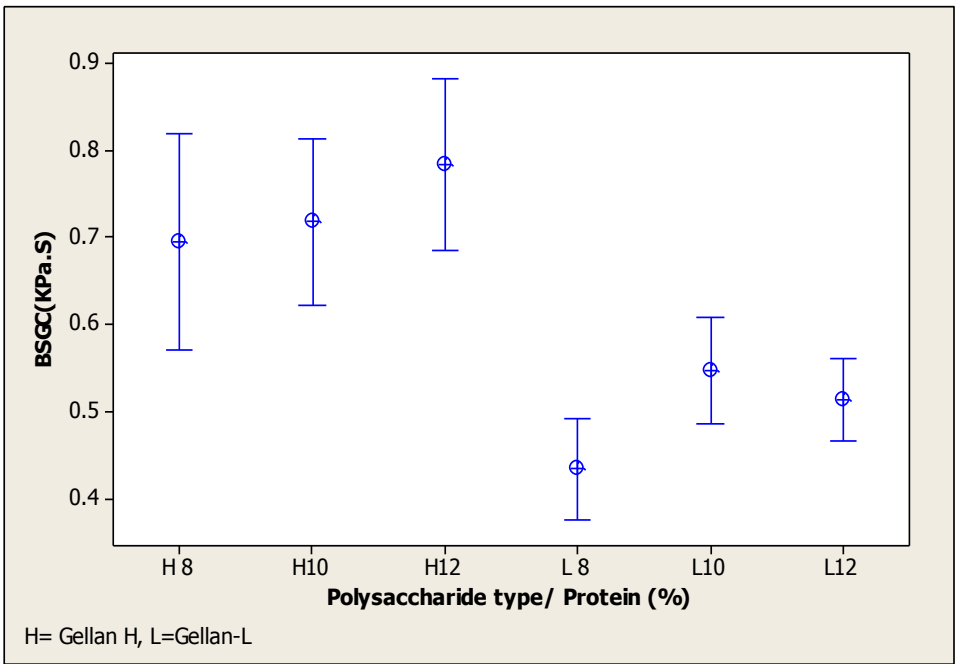

Figure 43 Overall BSGC means under constant force (0.55 N) at 25°C for model processed cheeses containing Gellan-H and Gellan-L and three different protein contents, 8.0, 10.0 and 12.0%.

It is clearly evident from Figure 44 that the decrease in the rate of deformation with an increase in the protein content was greater for Gellan-L than for Gellan-H. The maximum strain rate, which is the maximum rate of deformation of the sample and is always found at the start of the test, was measured. Figure 44 shows that there was a relatively large decrease in the strain rate when Gellan-H was added to the processed cheese. The small incremental decreases with an increase in the protein content clearly showed that the rate of reduction at each protein content was not significant for the processed cheeses containing Gellan-H. However, the difference in strain rate between a low protein content (8.0%) and a high protein (12.0%) was marginally significant for the processed cheeses containing Gellan-H. In contrast, the processed cheeses containing Gellan-L exhibited a very high strain rate at low protein content and each increase in protein content significantly reduced the strain rate.

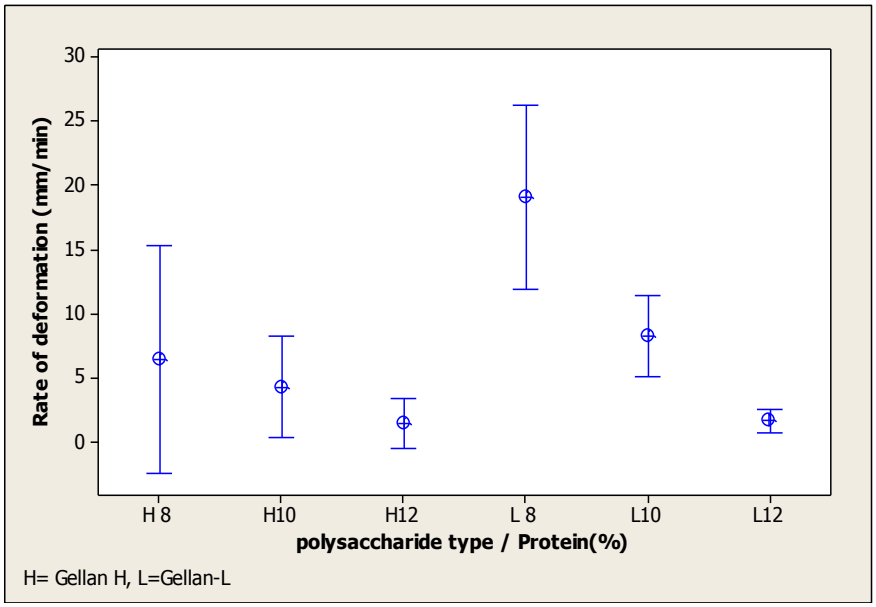

Figure 44 Overall mean rates of deformation under constant force (0.55 N) at 25°C for model processed cheeses containing Gellan-H and Gellan-L and three different protein contents, 8.0, 10.0 and 12.0%.

The increases in the BSGC (and the associated decreases in the strain rate) indicate a shift from relatively mobile water in processed cheese with no polysaccharide to less mobile water in processed cheese containing Gellan-H. It is known that both the amount of water bound to casein and the presence of free water influence the rigidity of the casein network, which ultimately influences the rheological properties of cheese analogues (Prentice et al., 1993). However, in this study, the mean BSGC values indicated that the polysaccharide had more influence than the protein. The addition of Gellan-H resulted in a greater increase in BSGC than an increase in the protein content.

7.3.3 Transmission electron microscopy

Our hypothesis that the addition of polysaccharide together with its phase separation from protein should give a higher cheese viscosity is supported by the TEM results for Gellan H (Figure 45C) and, to a lesser extent, also by those for Gellan L (Figure 45B). Moreover, it is evident from Figure 45C that the unhydrated Gellan-H particles in the gel structure could have been a result of poor dispersibility of the polysaccharide. Hydration takes place quickly, forming lumps that are wet on the outside but dry in the centre. This problem also occurs in the manufacture of pasteurised processed cheese spreads, resulting in a defect called fish eyes (Zehren & Nusbaum, 1992b).

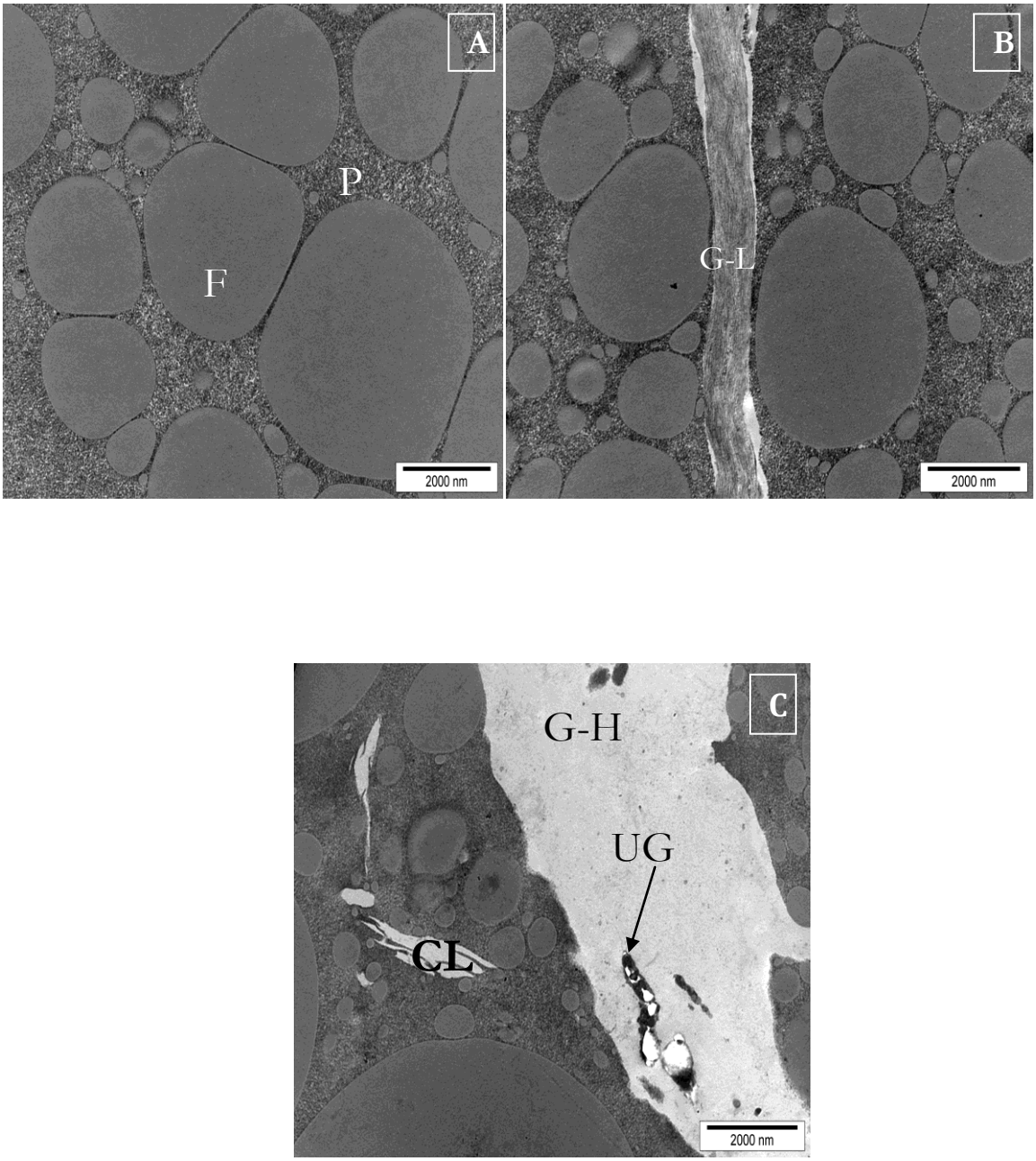

Figure 45 TEM images of model processed cheeses (10.0% protein) containing: (A) no polysaccharide; (B) 1.0% (wt/wt) Gellan-L; (C) 1.0% (wt/wt) Gellan-H. F = fat; P = protein; G-L = Gellan-L polysaccharide filaments in the protein matrix; G-H = Gellan-H polysaccharide filaments in the protein matrix; UG = un-hydrated polysaccharide; CL =Calcium lactate crystals.

7.4 Conclusions

The transmission electron micrographs showed that Gellan-H was phase separated from the protein whereas Gellan-L was not so clearly phase separated from the protein. Phase separation increases the effective concentrations of both protein and polysaccharide compared with a mixture of the two biopolymers. We hypothesise that water mobility (as measured by NMR) is an indirect measure of the effective concentrations of protein and polysaccharide. Thus, the addition of polysaccharide together with its phase separation from protein should give a lower water mobility measurement and a higher viscosity (BSGC) measurement (because of the increased concentration) than for the polysaccharide-free cheese.

The NMR data revealed that both polysaccharides significantly reduced the water mobility in the cheese but that the reduction was greater for Gellan-H.

The rheology data showed that the addition of polysaccharide increased the viscosity (BSGC) and reduced the deformation rate (strain rate) for processed cheese containing both Gellan-H and Gellan-L.

The reduction in the T_2,tb water value (time) as the polysaccharide concentration was increased was evidence that there was reduced mobility of the water in the cheese. Therefore, the relative spreadability of processed cheese containing Gellan-H reduced (the BSGC increased) because of the reduced availability of water in the protein phase.

Thus, our hypothesis that the addition of polysaccharides together with their phase separation from the protein gives a higher cheese viscosity and a lower water mobility is supported by these results for Gellan-H and, to a lesser extent, for Gellan-L.

^{13}C CP/MAS NMR confirmed that there was an increase in the rigidity of the protein in the processed cheeses containing 12% protein and polysaccharide, which contributed to the reduction in spreadability of the cheese.

The T_2 relaxation of the fat component also showed the effect of Gellan-H and Gellan-L on the increase in viscosity of the serum phase, which in turn affected the breakdown of the fat globules and the generation of strongly emulsified fat and less emulsified fat during RVA processing.

Chapter 8 Development of a dairy-based exopolysaccharide bioingredient

8.1 Introduction

Water-soluble and dispersible food biopolymers are widely used in many food formulations. These polymers, mostly derived from plants or seaweeds, are long chain, high molecular mass biopolymers that have thickening or gelling properties. However, they may not always possess the desired rheological properties or be available at the required quality (De Vuyst & Degeest, 1999). Most of the plant carbohydrates used are chemically modified to improve their structure and rheological properties (Roller & Dea, 1992). Therefore, their addition is restricted and is allowed in only some food products in the European Union (De Vuyst & Degeest, 1999). Moreover, there are sometimes difficulties in applying the "all-dairy" label to dairy foods because of the added plant polysaccharides.

An alternative potential source of biothickeners could be exopolysaccharides (EPSs) produced by lactic acid bacteria (LAB) (Ayala-Hernández, Hassan, Goff, & Corredig, 2009; De Vuyst, De Vin, Vaningelgem, & Degeest, 2001; Khurana, 2007). These polysaccharides either are associated with the cell surface in the form of capsules or are secreted into the extracellular environment in the form of slime (Hassan, 2008). LAB EPS is known to act as a texturiser, by increasing the viscosity of the final product through the binding of water of hydration and by interacting with other milk constituents, such as protein and micelles, to strengthen the rigidity of the casein

network (Ayala-Hernández, 2010). Consequently, EPS can decrease the syneresis and improve the stability in dairy products (Khurana & Kanawjia, 2007).

The role of EPS-producing *Streptococcus thermophilus* cultures in the manufacture of cheese is well established. They are known for improving the structure of cheese by improving its textural characteristics. The ability of EPS to bind water and increase the moisture in the non-fat substance (MNFS) without modifying the cheese manufacturing protocol is also well known (Dabour, Kheadr, Benhamou, Fliss, & LaPointe, 2006). EPSs increase moisture retention by trapping water within the three-dimensional cheese network (Awad et al., 2005; Broadbent et al., 2003; Khurana & Kanawjia, 2007). Additionally, EPSs have been observed to function as nuclei for the formation of large pores in the cheese matrix (Hassan et al., 2005). EPSs have been reported to increase the efficiency of the manufacture of half-fat Cheddar cheese by improving the rennet coagulation properties, reducing the cheese-making time and increasing the moisture content (Dabour et al., 2006; Rynne et al., 2007). EPSs can also increase the viscosity of the aqueous phase in cheese and modify its viscoelastic properties (Hassan et al., 2005); Ruas-Madiedo, Tuinier, Kanning, & Zoon, 2002).

However, the production of EPSs using LAB has some disadvantages that make these EPSs uncompetitive with other polymers of plant or microbial origin. Unlike many other bacterial species, LAB generally produce a limited quantity of polymer (typically < 200 mg/L) (Ricciardi & Clementi, 2000). However, some strains such as *Lactobacillus sake* 0-1 and *Lactobacillus rhamnosus* RW-9595M can produce larger quantities of EPS, of around 1.4 g/L (van den Berg et al., 1995) and 2.7 g/L (Macedo, Lacroix, Gardner, &

Champagne, 2002); higher yields could potentially justify their production and use as a food ingredient. Ideally, such a biothickener should contain a sufficient level of highly functional EPS and a concomitant lower viable cell count, so as to avoid interference with the action of natural dairy starter systems. The amount and the composition of the EPS produced by thermophilic LAB are markedly influenced by the culture and the fermentation conditions and are growth associated (De Vuyst, Vanderveken, Van de Ven, & Degeest, 1998; Gancel & Novel, 1994; Zisu & Shah, 2003). The production of EPS is also dependent on temperature, the pH of the medium and the carbon, nitrogen, mineral and vitamin contents of the medium (Ayala-Hernández, Hassan, Goff, de Orduña, & Corredig, 2008; Zisu & Shah, 2003). With respect to amino acid requirements, *S. thermophilus* requires primarily glutamic acid, histidine and methionine, as well as cystine, valine, leucine, isoleucine, tryptophan, arginine and tyrosine, for growth(Neviani, Giraffa, Brizzi, & Carminati, 1995) . It also utilises peptides and proteins in the growth medium through an enzyme system (Zourari, Accolas, & Desmazeaud, 1992).

In this study, an attempt was made, using dairy protein sources, to produce an ingredient with a high level of functional EPS for inclusion in cheese; the aim was to increase the texturising ability by enhancing bacterial growth (and hence EPS production) and potentially through EPS–protein interactions.

8.2 Materials and methods

8.2.1 Bacterial strains and media

An EPS-producing *S. thermophilus* strain ST10 was obtained from the culture bank of the Microbial Fermentation Unit, Fonterra Co-operative Group Limited, Palmerston North, New Zealand. Whey protein hydrolysate (WPH), milk protein hydrolysate (MPH), whey protein concentrate (WPC) and whey protein isolate (WPI) samples were procured from New Zealand Milk Products (NZMP), Fonterra Co-operative Group Limited, New Zealand. Preliminary studies showed that a concentration of 2.0% wt/vol of each of these samples was adequate for improved growth.

8.2.2 Fermentation

8.2.2.1 Flask fermentation

Low-heat skim milk powder (LHSMP) was obtained from NZMP, and was dissolved in Milli-Q water to make 10% wt/vol reconstituted skim milk (RSM). Different media, containing RSM (10% wt/vol) and enriched milk medium (10.0% RSM; 0.5% wt/vol yeast extract (YE) and/or 1.0% wt/vol casamino acids (DIFCO-USA)), were tested in Erlenmeyer flasks on a 50-mL scale for their capacity to support good acidification and growth of the *S. thermophilus* strain. The RSM contained 64.1% lactose and 33.4% protein.

8.2.2.2 Batch fermentation

The fermentations were performed in a computer-controlled laboratory fermenter (Bio-controller, Applikon Biotechnology, Schiedam, The Netherlands). The fermenter vessel was steam sterilised at 121°C for 20 min. The RSM (10.0% wt/vol) was then transferred into the sterile batch fermenter. Additional nitrogen sources were sterilised separately and aseptically pumped into the milk medium.

Citric acid (50% wt/vol) and 3 M NaOH were sterilised separately at 121°C for 30 min. The initial pH of the medium was adjusted with the citric acid and the medium was then maintained at the required pH (5.0, 6.0 and 7.0) with automatic addition of 3 M NaOH as required to neutralise the lactic acid produced by the microorganisms. To maintain the homogeneity of the medium, the fermenters were operated at 150 rev/min with a stirrer composed of two impellers each having three flat blades. The fermenter was inoculated with 1.0% (vol/vol) of a 24-h-grown culture of the *S. thermophilus*. All batch fermentations were performed at 37°C.

A sample was withdrawn aseptically every 6 h for quantification of the EPS and enumeration of the bacterial count. Another sample was stored at –30°C and subsequently analysed for EPS and viscosity within 60 h of sampling.

8.2.3 EPS quantification

A modified phenol–sulphuric acid method (Goh, Haisman, Archer, & Singh, 2005) was used for EPS quantification. A frozen sample (50 mL) was thawed to 4°C and swirl mixed, and the pH was adjusted to 7 using NaOH. A 100 µL aliquot of filter-sterilised Flavourzyme (10% wt/wt) was added to 10 mL of sample in a polypropylene Nalgene™ centrifuge tube. It was then incubated at 50°C in a shaker for 4 h and mixed for approximately 15 s. Distilled water (2.9 mL) and chilled absolute ethanol (7 mL) were pipetted into the centrifuge tube, followed by 100 µL of culture medium. The sample was left overnight at 4°C and then centrifuged at 27,000 *g* and 4°C for 40 min using a Sorvall ss34 rotor. The supernatant was decanted carefully and the tube was inverted on a piece of paper towel for approximately 10 min. A 3 mL aliquot of distilled water was pipetted into the centrifuge tube to resuspend the pellet, 7 mL of chilled 99.7% ethanol was added and the EPS concentration was then quantified using the phenol–sulphuric acid method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).

8.2.4 Freeze drying process

After transfer from the batch fermenter to sterile flasks, samples were frozen at –30°C using an ethanol bath. The sample flasks were then connected to the ports of an FTS Dura-Dry II™ freeze dryer for freeze drying. The freeze dryer was operated at a condenser temperature of –85°C and a vacuum of –75 mm Hg for 72 h.

The freeze-dried powder was stored at room temperature (20–25°C) in an anaerobic chamber. An anaerobic atmosphere (5.0 ± 0.1% and hydrogen in nitrogen 10.2 ± 0.2%)

was created using gas-generating kits. Silica gel was placed in the chamber to absorb moisture.

8.2.5 Bacterial enumeration

The spread plate technique was used to determine viable bacterial cell counts. Serial dilutions were prepared in sterile 0.1% peptone solution and the bacteria were enumerated using M17 agar (agar made with M17 broth supplied by Difco™, Fort Richard Laboratories, Auckland, New Zealand). The plates were incubated for 72 h at 37°C under anaerobic conditions. Plating was performed in triplicate. The average of the three values thus obtained was expressed in units of colony-forming units (cfu) per millilitre.

The freeze-dried samples used for bacterial enumeration were rehydrated in Milli-Q water at 30°C.

8.2.6 Viscosity measurement

The viscosity of the fermented medium (at 9.12% total solids) was measured at 5°C using a controlled stress Paar-Physica 2000 rheometer (Physica Messtechnik GmbH, Stuttgart, Germany) with a cone and plate geometry with the following specifications: 40 mm diameter stainless steel cone, 2° angle, fixed gap 50 µm. The samples were equilibrated for 10 min and then a shear rate ramp from 0.1 to 200 s^{-1} was applied. Freeze-dried powder was reconstituted to the same concentration as the fermented medium by adding sterile distilled water at 20°C.

8.3 Results and discussion

8.3.1 Influence of medium composition on growth and EPS production

The *S. thermophilus* strain was examined for its capacity for EPS production in different complex nitrogen sources. Most *S. thermophilus* strains do not grow well in the absence of peptide or amino acid sources because they lack an efficient proteinase that is capable of proteolytically degrading the caseins (Zourari et al., 1992).

As an initial experiment, RSM was supplemented with various dairy proteins in order to identify any influence that these ingredients might have on the growth of *S. thermophilus* (no pH control). Supplementation at 2.0% solids concentration was shown in preliminary experiments (at 1.0, 2.0 and 3.0% wt/vol solids) to achieve the best growth (results not shown). All of the fermentation trials were carried out at 42°C. The results are shown in Table 21. The medium with no supplemented nitrogen source took the longest to reach the isoelectric point, and the medium supplemented with WPH reached the isoelectric point most rapidly. Therefore, WPH was selected for subsequent experiments.

Table 21 Bacterial count, pH and time of coagulation for *S. thermophilus* production carried out in different media at 42°C (mean of three replicates ± standard deviation)

Medium composition	Bacterial count (log_{10} cfu/mL)	Time of coagulation (h)	pH
10% RSM	8.62 ± 0.04	7.1 ± 0.13	5.08 ± 0.02
10% RSM + 2.0% MPH	8.39 ± 0.05	6.0 ± 0.02	5.03 ± 0.12
10% RSM + 2.0% WPH	8.83 ± 0.01	5.2 ± 0.08	5.13 ± 0.04
10% RSM + 2.0% WPI	8.47 ± 0.08	5.8 ± 0.33	5.25 ± 0.06
10% RSM + 2.0% WPC	8.77 ± 0.01	5.4 ± 0.15	5.19 ± 0.01

In this experiment, it was difficult to draw any conclusion based on the production of visual ropiness. This may have been due to the very low amounts of EPS produced by *S. thermophilus* at an uncontrolled pH. The differences between the rates of cell growth and acid production were not reflected in the relative increases in visual ropiness that occurred; for example, the sample with 2.0% WPI supplementation showed the greatest thickness. EPS–protein interactions play an important role in the thickening of fermented milk. The thickening effect of the medium is due to segregative interactions between EPS and casein micelle aggregates (Petry et al., 2003). Apart from viscosity, EPS quantification was therefore also a prime focus for subsequent experiments. Physical factors, such as temperature and pH, as well as chemical factors, such as medium composition, initial lactose concentration and carbon/nitrogen levels, are known to be important factors that influence EPS production (De Vuyst et al., 1998; Zisu & Shah, 2003).

8.3.2 Effect of pH on EPS production

To elucidate the influence of pH on EPS production, the *S. thermophilus* was cultivated in batch fermenters at different, constant pHs (5.0, 6.0 and 7.0), using the medium containing 10% RSM and at the previously chosen temperature of 37°C.

The results in Table 22 show that the fermentation pH affected the amount of EPS produced and the yield of EPS relative to cell number. The cultures maintained at pH 6.0 showed the greatest EPS production (up to 741 mg/L after 12 h); in contrast, those maintained at pH 5.0 showed maximum EPS production (obtained after 18 h of growth) of 340 mg/L and those maintained at pH 7.0 showed maximum EPS production (measured after 12 h of growth) of 506 mg/L. The yield of EPS relative to cell number also appeared to be highest at pH 6.0, implying a more efficient conversion of substrates to EPS. De Vuyst et al. (1998) also observed pH 6.2 to be optimal for the growth of *S. thermophilus* LY03 and EPS production.

Table 22 Bacterial count and amount of EPS produced during fermentation of RSM by *S. thermophilus* at various pHs at 37°C (mean of three replicates ± standard deviation)

pH	Fermentation time (h)	EPS (mg/L)	Bacterial count (log_{10} cfu/mL)
5.0	0	32 ± 1.0	7.04 ± 0.02
	6	221 ± 7.2	8.93 ± 0.01
	12	296 ± 9.1	9.02 ± 0.04
	18	340 ± 1.9	9.18 ± 0.09
	24	319 ± 1.5	9.36 ± 0.01
6.0	0	21 ± 2.6	7.01 ± 0.01
	6	429 ± 7.8	9.32 ± 0.09
	12	741 ± 16.9	9.58 ± 0.05
	18	697 ± 13.2	9.61 ± 0.01
	24	729 ± 6.4	9.74 ± 0.01
7.0	0	19 ± 2.0	7.04 ± 0.06
	6	342 ± 5.3	9.41 ± 0.03
	12	506 ± 6.8	9.48 ± 0.04
	18	419 ± 1.2	9.54 ± 0.01
	24	453 ± 11.0	9.55 ± 0.09

8.3.3 Effect of pH on viscosity

Figure 46 shows that the fermentation pH markedly affected the viscosity of the sample. The viscosity was higher at fermentation pH 6.0 than at pH 7.0 and was lowest at pH 5.0. Previous studies have revealed that pH has a marked effect on the molecular mass of EPS in medium (Zourari et al., 1992). Viscosity measurements alone are not a reliable indicator of the amount of EPS produced because changes in the viscosity in media could be caused by other factors, such as EPS–protein interactions and different

biopolymer interactions occurring at different environmental pHs (Ayala-Hernández et al., 2009). Viscosity is dependent not only on EPS production but also on the composition of the medium, and physicochemical complexes that form between EPS and proteins in the medium may also play an important role in viscosity changes (Ayala-Hernández et al., 2008). For this study, viable cell counts and EPS concentration were considered to be the most important parameters for tracking the changes in different media.

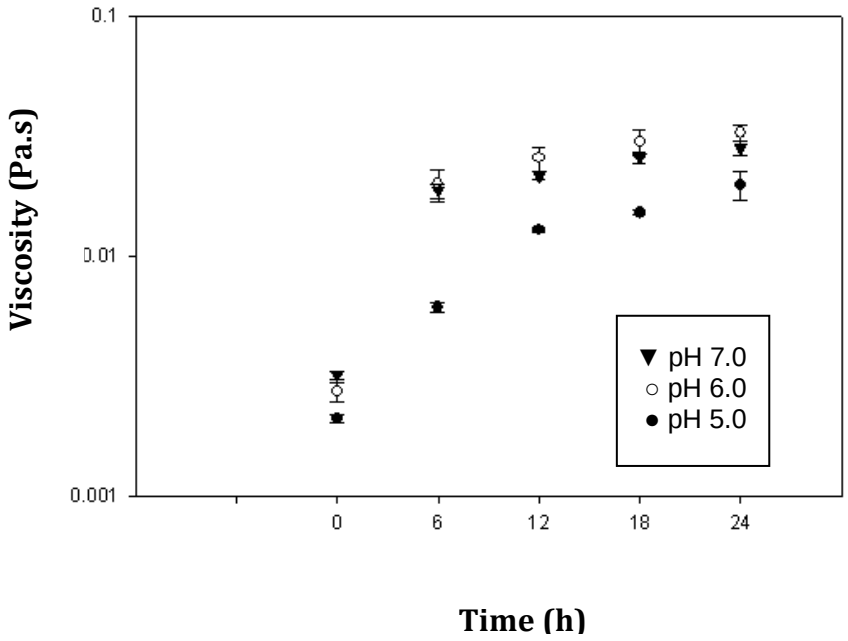

Figure 46 Viscosity measured at 100 s^{-1} in RSM (10% total solids) as a function of pH: ●, 5.0; ○, 6.0; ▼, 7.0. Values are the average of three fermentations carried out in duplicate.

8.3.4 Effect of medium composition on EPS production

The use of whey-based proteins in the fermentation was also one of the objectives of the present research, as whey protein is known to have attractive interactions, commonly referred to as depletion interactions, between aggregated whey protein colloid particles or between casein micelles when they are mixed with EPS (Ayala-Hernández et al., 2008; Tuinier, ten Grotenhuis, Holt, Timmins, & de Kruif, 1999). Dairy protein supplementation was used to study the effect of different nitrogen sources on the yield of EPS and the cell growth. Table 22 shows that EPS production was growth associated, as would be expected (Zisu & Shah, 2003). The growth of *S. thermophilus* at pH 6.0 in media with/without supplementation was followed over time. *S. thermophilus* showed significant variation in its ability to grow in the different media. Growth started at 10^7 cfu/mL and the growth patterns were similar for all fermentations, in that the exponential phase of growth was complete within 18–24 h, as shown by the viable counts in Figure 47. The subsequent decrease in EPS production was probably due to the activation of a degrading enzyme such as glycohydrolase (Cerning et al., 1994).

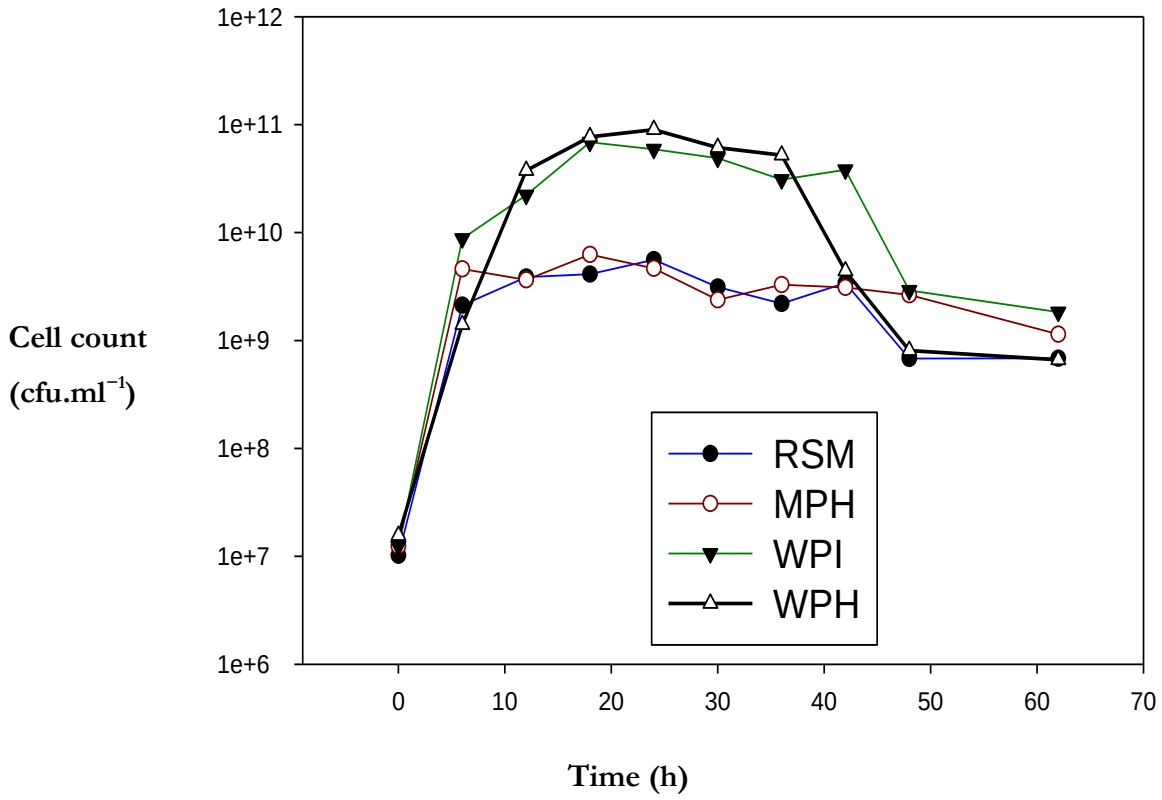

Figure 47 Viable cell count (mean of three replicates) produced during fermentation of RSM by the *S. thermophilus* strain at pH 6 and 37°C.

Supplementation with WPI, WPH and MPH provided additional nitrogen sources for the growth of *S. thermophilus*. As shown in Figure 48, the smallest increase in EPS production was observed in the control fermentation (unsupplemented medium). The experiments demonstrated that increasing the availability of amino acids or peptides in the fermentation medium, apart from MPH supplementation, improved the growth of the *S. thermophilus* strain. In all cases of supplementation, EPS production was also enhanced. The generally greater growth, and greater EPS concentrations relative to viable cells, in the enriched milk media reflected both the limited proteolytic ability of *S.*

thermophilus and the dependence of both growth and EPS production on utilisable nitrogen compounds (Degeest & De Vuyst, 1999). Use of the medium supplemented with MPH resulted in the lowest increase in EPS production and use of the medium supplemented with WPH showed the greatest production of EPS, up to 923 mg/L. The lower molecular weight nitrogen compounds produced by whey protein hydrolysis may have been responsible for the greater EPS production compared with WPI. The simpler peptides and amino acids in whey-based proteins have been proposed as being responsible for an increase in EPS yield (Briczinski & Roberts, 2002).

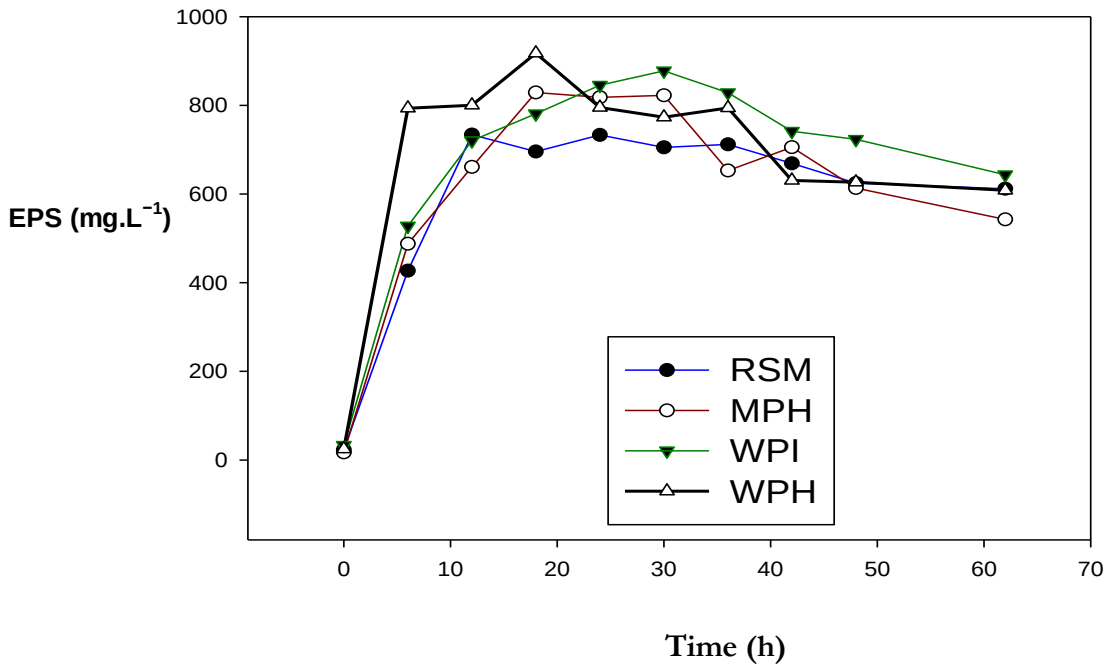

Figure 48 EPS concentration (mean of three replicates) produced during fermentation of RSM by the *S. thermophilus* strain at pH 6 and 37°C.

8.3.5 Effect of freeze drying on bacterial cell count and EPS concentration

The population of *S. thermophilus* decreased approximately 100-fold after freeze drying (Table 23). A similar reduction in viable count has been reported previously (Rybka & Kailasapathy, 1997; Wolff, Delisle, Corrieu, & Gibert, 1990). The reduction in viable count could have been due to metabolic injury during freeze drying as a result of damage to the surface proteins, walls and membranes of the bacteria (Rybka & Kailasapathy, 1997). Freeze drying adversely affects hydrogen bonds involved in binding the surface layer protein to the cell wall. The heat stability of *S. thermophilus* is due to the presence of additional bonds, which provide greater stability to the secondary and tertiary structure of the proteins. These bonds include disulphide linkages, hydrogen bonds, ionised group interactions and hydrophobic bonds. The longer freeze-drying time (72 h) than normal (24 h) for this experiment may have also contributed to the reduction in viable count of the culture. The mortality rate/resistance to freeze drying of *S. thermophilus* species is less than that of other LAB species such as *Lactobacillus bulgaricus* (Kumar & Mishra, 2004).

Table 23 Enumeration of *S. thermophilus* and quantity of EPS in media (supplemented with WPH) before and after freeze drying (mean of three replicates ± standard deviation)

	EPS (mg/L)	**Bacterial count (log_{10} cfu/mL)**
Before freeze drying	936 ± 5.1	10.95 ± 0.03
After freeze drying	889 ± 11.3	8.95 ± 0.02

8.3.6 Viscosity of reconstituted freeze-dried medium

Figure 49 shows the viscosity of fermented medium and reconstituted freeze-dried medium at the same total solids level (9.1%) at two different stages of fermentation. The decrease in viscosity with an increase in shear rate indicates the shear-thinning behaviour of these solutions. Moreover, the higher viscosity of the fermented medium (fresh and reconstituted) at all shear rates compared with the non-fermented medium indicates the texturising effect of EPS in the medium. Such properties are desirable for the use of the medium as a viscosifying agent in food products (De Vuyst & Degeest, 1999). The viscosity plots of fresh fermented medium (after 18 h) and reconstituted freeze-dried fermented medium did not show any major differences. This indicates that the freeze drying and subsequent redissolving had little effect on the viscosifying properties of the medium.

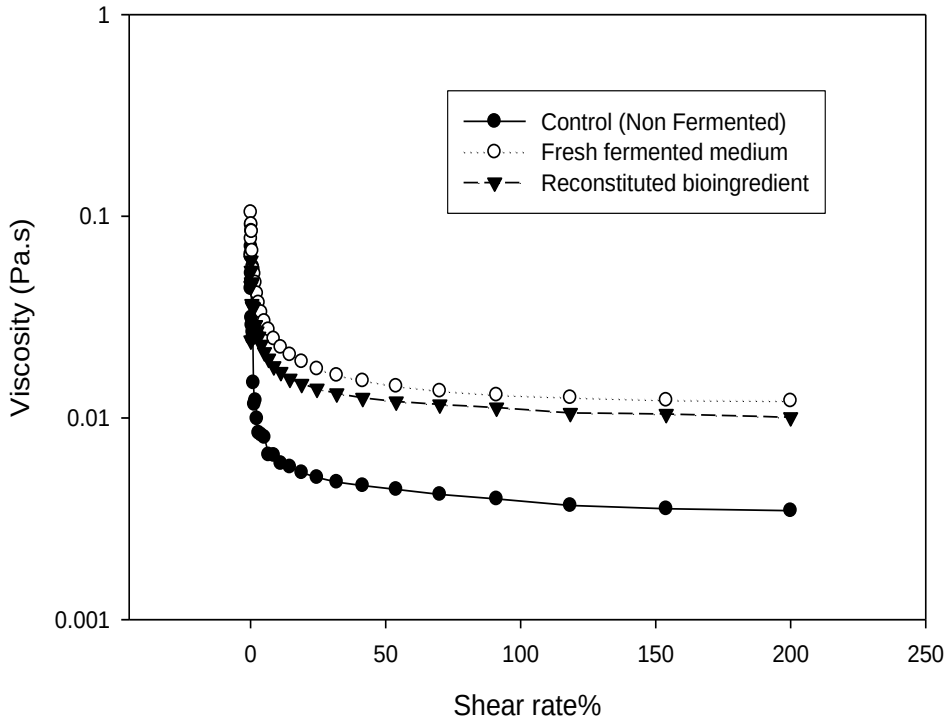

Figure 49 Viscosity of 9.1% total solids RSM with 2.0% WPH fermented at a controlled pH of 6.0 for 18 h (○) and reconstituted freeze-dried powder (▼). The viscosity of non-fermented medium is illustrated as (●).

8.3.7 Effect of temperature on reconstituted powder

EPS in the freeze-dried fermented mixture formed a viscous solution when the mixture was reconstituted in distilled water to 10.0% total solids. Figure 50 shows that temperature had no major effect on the viscosity when the solution was heated to 60°C for 20 min. However, there was a marginal reduction in viscosity at 70°C and a further reduction at 80°C. A pairwise comparison between the viscosity of RSM (10% total solids) and the viscosity of the fermented medium was significant, having $P < 0.0001$ for viscosity at all temperatures except for the viscosity of the medium heated at 80°C (for which $P = 0.0201$). This observation suggests that the effectiveness of reconstituted

freeze-dried EPS medium was unaffected even at temperatures up to 60°C. A further decrease in viscosity at temperatures > 60°C suggests breakdown of the EPS or EPS–protein structure, which results in a reduction in the texturising effect.

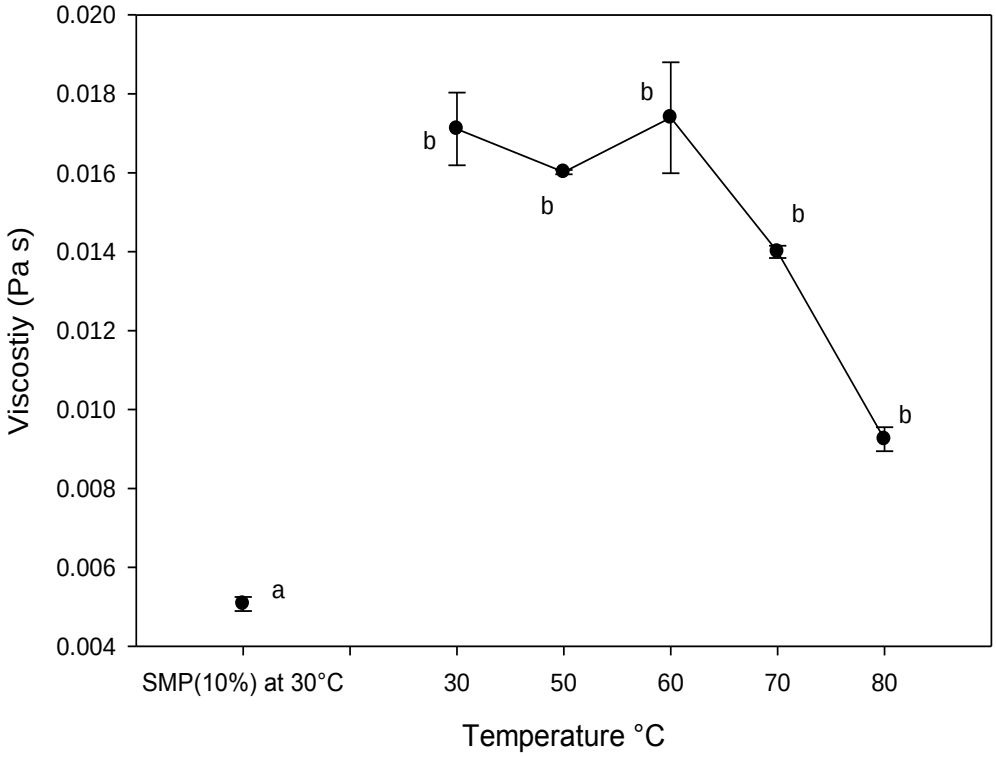

Figure 50 Viscosity of reconstituted freeze-dried powder (10.0% total solids) measured at 100 s^{-1} at different temperatures from 30 to 80°C. Error bars represent the standard error of the mean of viscosity measurements.

8.3.8 Storage conditions

An objective was to reduce the viable count to develop an ingredient with a very low cell count. The data presented in Table 24 show a reduction in cell counts from 8.95 to 7.70 log_{10} cfu/mL when the sample was stored for 30 days under anaerobic conditions. Storage of the ingredient for a further 30 days resulted in a small reduction in cell

counts to 7.54 $\log_{10}$ cfu/mL. The reduction in cell count could have been because of reduced stability of the cells due to unfavourable storage conditions. Cell survival rate in this experiment was used as a strategic criterion in the development of an ingredient that can be used for every type of cheese irrespective of the type of culture used for cheese-making. The method used to reduce the viable count was easy to follow without changing the normal freeze-drying temperature profile. However, increasing the freeze-drying temperature to −5°C (close to the melting temperature of the ice in the product) could be another way of reducing the viable count.

Table 24 Storage trial: enumeration of *S. thermophilus* and quantity of EPS before and after freeze drying (mean of three replicates ± standard deviation)

	Days	Bacterial count ($\log_{10}$ cfu/mL)
Before freeze drying	0	10.95 ± 0.03
After freeze drying	0	8.95 ± 0.02
	30	7.70 ± 0.01
	60	7.54 ± 0.02

8.4 Conclusions

Our results have shown that supplementation of the medium with different milk protein sources stimulated the production of EPS in the ingredient when using a strain of *S. thermophilus*. Supplementation with WPH produced the greatest increase in the

production of EPS in the medium at a pH of 6.0, and over a fermentation time of between 18 and 20 h. The ingredient that was produced maintained its structural integrity during freeze drying and did not significantly diminish the viscosity enhancement caused by the EPS. However, heating the bioingredient above 60°C caused an irreversible reduction in viscosity, suggesting an important contribution of the protein component of the matrix structure to the generation of viscosity. If the overall EPS yield can be increased further by manipulating the medium composition, manipulating process control or introducing a strain with a higher EPS yield, the possibility for a dairy-based EPS ingredient may exist. In subsequent work, we aim to develop the ingredient application further in a Mozzarella cheese model.

Chapter 9 Exopolysaccharide as a bioingredient in skim milk Mozzarella cheese

9.1 Introduction

The use of Mozzarella cheese in the pizza industry has been a major factor in an increasing demand in recent years. The higher demand has also led to the development of the low fat and non-fat cheese market (Perry et al., 1998). The majority of the Mozzarella cheese that is produced must have functional properties that are suitable for pizza production. For use on pizza, Mozzarella cheese should exhibit good melting, stretching and textural properties.

Small vats have been widely used for cheese-making by many researchers. Although cheese-making in small vats has substantial technological differences from commercial manufacture, the same overall effect of elevation of the moisture content was reported when cheese was made using an exopolysaccharide (EPS) culture in small vats as when the same cheese was made using mechanised equipment (Perry et al., 1998).

The rate of whey syneresis has a tremendous practical implication for the final quality of cheese and has been widely reviewed (Johnston, Dunlop, & Lawson, 1991; Johnston, Luckman, Lilley, & Smale, 1998; Walstra, van dijk, & Geurts, 1985; Whitehead & Harkness, 1954). Several processing parameters, such as time, temperature, speed and duration of cutting/agitation and size of the curd particles (Everard et al., 2008; Johnston et al., 1991; Lawrence, 1959), influence the acidity, moisture content, mineral content and lactose content of the curd before ripening, which in turn affect the texture,

colour, flavour and overall quality of the cheese (Castillo, Lucey, & Payne, 2006). Thus, to control variation in the conditions during cheese-making, processing parameters, such as time, temperature and speed and duration of cutting/agitation, were kept constant for all the cheese-making experiments in this study.

It is known that the rheological properties of cheese change with a change in its composition (e.g. casein, fat, water, salt, pH). As reported by many researchers, it is difficult to make cheese that differs only in one component, while keeping the other components constant (Creamer & Olson, 1982; Wium, Kristiansen, & Qvist, 1998).

Consumer demand for reduced-fat products has created interest in the development and manufacture of reduced-fat, low fat and no-fat Mozzarella cheeses. However, fat removal in low fat Mozzarella cheese can result in a cheese that is low in moisture, giving it poor melting and stretching properties (McMahon et al., 1993). Large open channels or columns between protein fibers are occupied by water during Mozzarella cheese-making and this moisture retention in large columns or voids may contribute to the water-holding characteristics and functional properties of Mozzarella cheese. Both fat and unbound water act as lubricants and increase the ability of cheese particles to flow. Therefore, many attempts have been made to achieve the same melting, browning and other functional properties in low fat Mozzarella cheese by increasing the ratio of moisture to protein to be equal to or greater than that of full-fat Mozzarella cheese (McMahon & Oberg, 1998; Perry et al., 1997; Zisu & Shah, 2005b).

The EPS-producing *Streptococcus thermophilus* strain has been used successfully in Mozzarella cheeses as a starter to improve its functional properties and moisture

retention (Perry et al., 1998; Petersen et al., 2000; Zisu & Shah, 2005b). However, the incorporation of EPS as a bioingredient in Mozzarella cheese-making has not been reported in the published literature. Similarly, the effect of such a bioingredient on the functionality of Mozzarella cheeses is not known.

9.1.1 Objectives

The primary objective of this study was to investigate the effect of a functional EPS bioingredient at a high level of addition in Mozzarella cheese-making.

The secondary objective was to compare the effects of the added EPS ingredient and EPS-producing cultures on the functionality of model skim milk Mozzarella cheese.

9.2 Materials and methods

9.2.1 Starter cultures

Streptococcus thermophilus 10 (ST10), *Streptococcus thermophilus* 55 (ST55), *Streptococcus thermophilus* 1 (ST1) and *Lactobacillus helveticus* 1 (LH1) cultures were obtained from the Fonterra Culture Library. The working cultures were propagated using 1.0% (vol/vol) inocula in 10.0% reconstituted skim milk (RSM) at 37°C for 18 h before use.

9.2.2 Cheese-making

Each batch of cheese was made in triplicate using 10 L of milk in small vats of 10 L capacity. These vats were equipped with a jacketed water heating and cooling system. The cutting and mixing operations were facilitated by the agitators mounted on the vats.

All the operations in the vats were programmable and fully controlled by a computer-based control system, which was equipped for manual or automatic operation.

Skim milk (NZMP Meadow Fresh, 0.01% fat, 3.63% protein and 4.83% lactose) pasteurised at 72°C with a holding time of 15 s was used to make the experimental cheeses. The milk was tempered to 37°C for 10 min. Starter bacteria were inoculated at different rates of addition as specified in Table 26 at step 3 of Table 25. The logic behind the culture addition level is explained in section 9.2.8.

Fromase (Fromase® XLG, DSM Food Specialties, New Zealand) was added at a rate of 0.00275% (vol/vol). A setting time of 30 min was used. Cutting was started after carrying out a subjective assessment of the coagulum. After cutting, the curd was cooked for 50 min at 37°C. The whey was drained at pH 5.9 and the curd was cheddared into small slabs before milling at pH 5.3. For each batch, 25 g of salt was sprinkled on the curd, and 0.0132 g of $CaCl_2$ was also added to the curd. The salted curd was hand stretched for 5 min in hot water maintained at 75°C. The cheese balls were placed in a barrier bag and vacuum sealed before storing them at 5°C in a cold room.

Table 25 Processing sequence of Mozzarella cheese-making using 10 L vats

Step No	Step	Time (min)	Temperature (°C)	Speed (rev/min)
1	Pre-equilibrate	10	37	20
2	Mix	1	37	50
3	Prime	30	37	20
4	Mix	1	37	50
5	Set	30	37	–
6	Cut	1	37	10
7	Cut	2	37	15
8	Cut	1	37	25
9	Cut	2	37	35
10	Cut	2	37	45
11	Cook	5	37	20
12	Cook	5	37	20
13	Cook	5	37	20
14	Cook	5	37	20
15	Cook	5	37	20

Table 26 Protocol for very low fat Mozzarella cheese-making

Cheese type	Control (CTR)	In situ EPS-producing culture (EPS-C)	Added EPS powder (EPS-P)
Vat volume (L)	10		
WPH addition (g)	29.8	29.8	Nil
Fat%/protein%/lactose %	0.02/4.02/4.76	0.02/4.04/4.74	0.04/4.05/4.63
Pasteurisation (°C/s)	Standard		
Temperature of milk to vat (°C)	20.0		
Starter characterisation	ST55 + LH1	ST10 + LH1	ST10 freeze dried powder + LH1
Starter (%)	4.0 + 1.0	3.5 + 1.0	10.0% powder + 1.0
Prime pH	6.10		
Set temperature (°C)	37.0		
Coagulant	Fromase		
(mL/100 L)	2.75		
Cook temperature (°C)	37		
Drain pH (whey)	5.90		
Dry stirs	1		
Mill pH	5.30		
Salt addition rate (g)	23.5		
Salt/lactose/calcium (g) added to C/S water	482/482/1		
Mellowing time (min)	20		
Stretching temperature (°C)	60–65		
Cooling water temperature (°C)	8–10		
Storage temperature (°C)	5		

NOTE. The missing values in the cells in this table are the same as for the cells in the column for the control (CTR) cheese.

9.2.3 Cheese yield analysis

The cheese yield, based on the weight of cheese manufactured from a known weight of milk, was calculated and was expressed as kilograms of cheese per 100 kilograms of milk, as described by Metzger et al. (2000).

9.2.4 Moisture analysis

The moisture content was measured using the standard atmospheric oven method. Moisture samples were analysed in duplicate with three repeats on different days.

This method relies on measuring the mass of water in a known mass of sample. The moisture content is determined by measuring the mass of the sample before and after the water is removed by evaporation.

Equation 12

$$\%\text{Moisture} = \frac{M_{\text{INITIAL}} - M_{\text{DRIED}}}{M_{\text{INITIAL}}} \times 100$$

Here, M_{INITIAL} and M_{DRIED} are the masses of the sample before and after drying respectively.

9.2.5 Rheological properties related to melt: elongational viscosity

Cheese blocks were cut into slices of 7 mm thickness with a hand-operated slicer. Then, cylindrical specimens of 30 mm diameter were cut out with a cork borer. The cheese samples, 7 mm in thickness and 30 mm in diameter, were put into plastic bags and

placed in a refrigerator at 5°C until testing. The temperature of the UW melt meter was set to 60°C before the sample was placed in it.

The elongational viscosity was measured as described in Section 3.6.

9.2.6 Rheological and fracture properties related to texture

Refer to Section 3.2.

9.2.7 Proton transverse (T_2) relaxation measurements

Refer to Section 3.7.

9.2.8 Schreiber test

Cheese samples were cut to two 40 mm diameter circles approximately 4 mm thick using a plastic cutter. The cut cheese circles were placed in the middle of the petri dish. . The petridishes were covered and conditioned at 5 °C for 10 minutes. The covered petridises were placed directly in the oven which was set to 232 °C earlier on. The cheese samples were heated for 5 minutes and then taken out of the oven for cooling. They were cooled on a flat surface for 30 minutes. The degree of spreading was measured at the six points indicated on the bulls-eye chart (40 mm centre 2.5 mm graduation).

9.2.9 Experimental approach

9.2.9.1 pH standardisation

The amounts of control starter (CTR), in situ EPS starter (EPS-C) and bioingredient (EPS-P) to achieve the same rate and extent of acid production for each treatment were established. A constant amount of *Lactobacillus helveticus* (LH1) culture was used in each treatment.

In preliminary cheese-making trials in the small vats, the rate and the extent of the pH drop were measured at different stages of cheese-making, i.e. in the milk, the cut whey, the whey after 20 min and the whey after 50 min, as shown in Tables 27, 28 and 29. The amount of starter addition was determined based on these pH results. As shown in the highlighted rows in the tables, 10.0% of ST10 powder, 4.0% of ST55 culture and 3.5% of ST10 culture gave the same extent of pH drop in the experimental cheeses.

Table 27 Effect of ST10 freeze-dried powder addition on the pH drop (mean of two replicates) at different stages of cheese-making

ST10 (powder) (%)	LH 1 (%)	Vat milk	Set milk	Cut whey	Whey after 20 min	Whey after 50 min
2.5	1	6.63±0.01	6.62±0.02	6.02±0.01	5.79±0.02	5.32±0.02
5	1	6.63±0.01	6.62±0.01	5.78±0.01	5.74±0.02	5.34±0.02
10	1	6.61±0.01	6.55±0.03	5.9±0.02	5.62±0.03	5.29±0.02
15	1	6.69±0.01	6.56±0.02	5.77±0.02	5.47±0.01	5.23±0.03

Table 28 Effect of control starters (non-EPS-producing ST1 and ST55) on the pH drop (mean of two replicates) at different stages of cheese-making

ST	ST (%)	LH (%)	Vat milk	Set milk	Cut whey	Whey after 20 min	Whey after 50 min
ST1	0.7	1	6.76±0.01	6.59±0.01	6.42±0.01	6.32±0.01	6.21±0.01
ST1	1.5	1	6.65±0.01	6.45±0.03	6.22±0.03	6.16±0.02	6.04±0.03
ST1	2	1	6.60±0.01	6.49±0.03	6.27±0.04	6.13±0.03	6.00±0.02
ST1	2.5	1	6.64±0.00	6.37±0.01	6.16±0.02	6.05±0.01	5.82±0.01
ST1	3	1	6.65±0.00	6.32±0.03	6.11±0.02	5.98±0.03	5.62±0.03
ST1	4	1	6.64±0.00	6.29±0.03	6.00±0.02	5.86±0.01	5.60±0.02
ST55	2	1	6.64±0.01	6.37±0.02	6.16±0.02	5.86±0.03	5.62±0.02
ST55	3	1	6.67±0.01	6.40±0.02	6.10±0.02	5.75±0.02	5.50±0.02
ST55	4	1	6.65±0.01	6.32±0.01	6.00±0.02	5.59±0.02	5.29±0.02

Table 29 Effect of EPS-producing starter (ST10) on the pH drop (mean of two replicates) at different stages of cheese-making

ST10 (culture) (%)	LH1 (%)	Vat milk	Set milk	Cut whey	Whey after 20 min	Whey after 50 min
2.5	1	6.68±0.01	6.49±0.02	6.14±0.01	5.97±0.02	5.65±0.02
3.5	1	6.68±0.01	6.51±0.01	6.24±0.01	6.07±0.01	5.32±0.00
4.5	1	6.67±0.01	6.46±0.01	6.08±0.02	5.85±0.02	5.12±0.01

9.2.9.2 Standardisation of the cheese milk with in situ EPS starter and added bioingredient to the same composition as for the control

Three 10 L vats were used for manufacturing the cheeses, using a Latin square experimental design, as shown in Table 30.

Table 30 Latin square design for Mozzarella cheese-making experiments

Day	Treatment		
1	CTR	EPS-C	EPS-P
2	EPS-C	EPS-P	CTR
3	EPS-P	CTR	EPS-C

9.2.10 Statistical analysis

Statistical analysis of the texture data was carried out using one-way analysis of variance (ANOVA) with a 95% confidence interval (general linear model procedure, Minitab Version 15.1). A *P* value of < 0.05 was designated as being significant.

9.3 Results and discussion

9.3.1 Determination of the amount of starter

In preliminary cheese-making trials in the small vats, it was observed that the rates and the extents of pH drop at each stage of cheese-making were different when the three different cultures, i.e. (a) control starter ST1+LH1 (CTR), (b) EPS culture ST10+LH1 (EPS-C) and (c) EPS powder (EPS-P), were added at the same rate. This finding was in line with the published literature; that is, the type of culture and the inoculum concentration have a marked effect on the kinetics of syneresis (Castillo et al., 2006), including pH development. Such variations in pH had a marked impact on the cheese manufacturing time, the rate of whey syneresis and the final moisture content of the

current experimental cheeses. The rate of starter addition was established as explained in Section 9.2.8.1.

9.3.2 Composition

Table 31 Composition of experimental cheeses

Mozzarella cheese	Fat (% wt/wt)	Moisture (% wt/wt)	Protein (% wt/wt)	pH (Day 1)
CTR	< 1.0	57.5	36.8	5.27
EPS-C	< 1.0	60.2	34.4	5.20
EPS-P	< 1.0	66.0	28.6	5.22

9.3.3 Rheology

9.3.3.1 Fracture stress

The ANOVA of the means of fracture stress (FSS) for the effect of the three treatments (control, added freeze-dried EPS powder and in situ EPS culture), vats and days showed that each treatment had significant effects on fracture stress (P = 0.014) (Table 32 and Figure 51A). This indicated that the added EPS powder and the EPS culture had marked effects on the reduction in fracture stress compared with the control, and that this reduction was greater for EPS powder than for EPS culture. The main effects plot (Figure 51A) also showed that the changes in fracture stress were not significant and were only slightly influenced by different vats on different days.

Table 32 P values of three repeats for fracture strain, fracture stress and modulus of deformability

Rheological property	Treatment	Day	Vat
Fracture strain	NS	NS	NS
Fracture stress	0.014	NS	NS
Modulus of deformability	0.009	NS	NS

9.3.3.2 Modulus of deformability

The trends observed for modulus of deformability (Mod) were similar to those observed for fracture stress. Figure 51B illustrates the changes in modulus of deformability in Mozzarella cheese for the three different treatments. The cheeses with added EPS powder were the softest, because they had the highest moisture content (Table 31). The cheeses produced using the EPS-producing culture (ST10) were significantly harder than the cheeses with added EPS powder; however, the control cheeses had the greatest hardness, which was possibly because they had the highest protein content and the lowest moisture content. These changes in composition coincided with marked increases in the fracture stress (force required to crack the cheese) and the modulus of deformability.

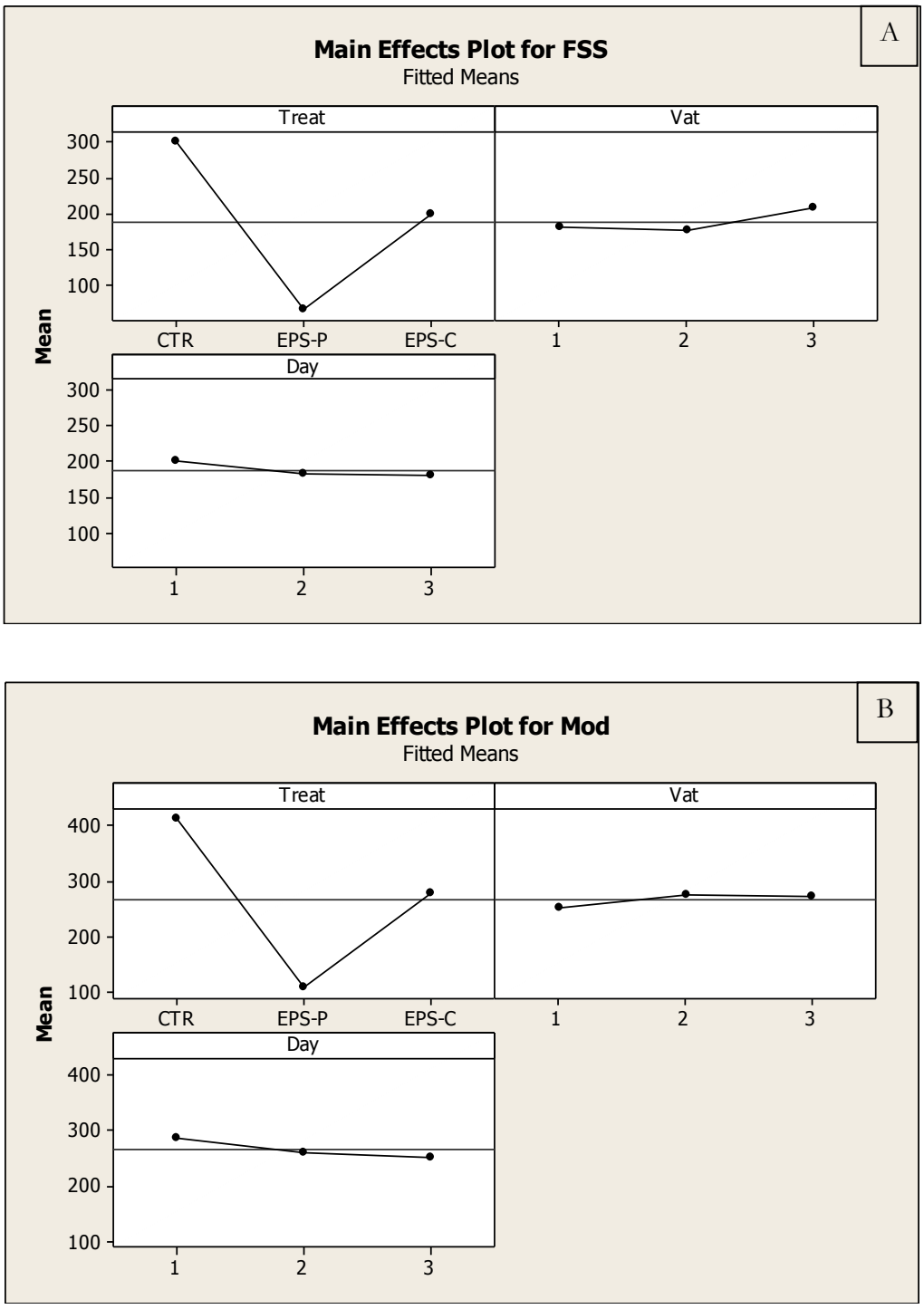

Figure 51 Main effects plots of (A) Fracture stress (FSS) and (B) Modulus of deformability (Mod) for very low fat model Mozzarella cheeses made using three different treatments in three vats on three days.

The lower is the moisture content, the higher is the protein content, the greater is the number of interparticle bonds and the higher are the modulus of deformability and the stress at fracture. The observed changes in rheological and fracture behaviour were in line with the published work by Visser (1991). He reported that the modulus of deformability decreases with increasing moisture content and is almost linearly related to moisture in the non-fat substance (MNFS) for Gouda cheese. Moreover, Visser (1991) also reported a decrease in fracture stress with increasing moisture content.

It was also evident from the results that the amount of EPS had a marked effect on the moisture retention during the cheese-making process. However, unlike commercial polysaccharides, the EPSs were unable to show any strengthening of the cheese structure, which would have been indicated by higher fracture stress or modulus of deformability.

9.3.3.3Fracture strain

The ANOVA results in Table 31 and Figure 52 indicated that the interactions between strain at fracture and treatment, vat and day were not significant. That is, the strain at fracture of the Mozzarella cheese was not affected by the different treatments, vats and days at the 5% level. However, the treatments induced only a small range in fracture strain from 0.9 to 1.15.

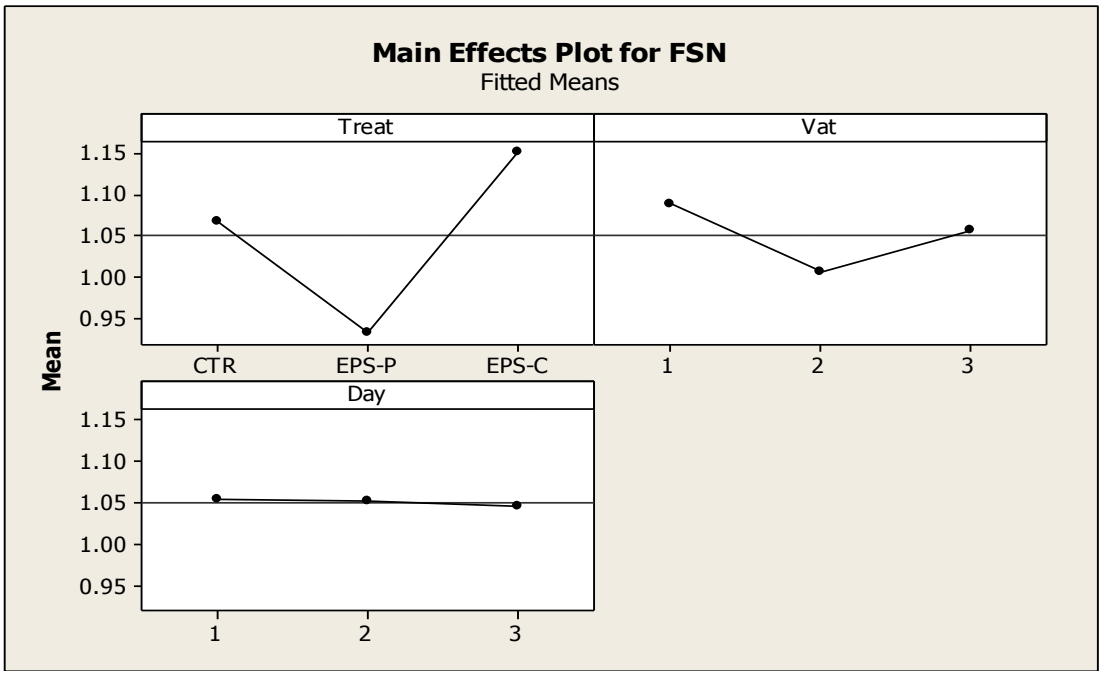

Figure 52 Main effects plot of fracture strain (FSN) for very low fat model Mozzarella cheeses made using three different treatments in three vats on three days.

The strain at fracture did not vary significantly with either added EPS powder or EPS culture in the Mozzarella cheese. This indicated that fracture strain was fairly independent of moisture contents ranging from 57.0 to 66.0% wt/wt in the Mozzarella cheese. It was concluded that the increase in moisture content as a result of the additional EPS present in the structure did not alter the brittleness of the cheese significantly. The results were in line with the published work on Gouda cheese by Visser (1991). He found that the fracture strain was normally independent of a change in the moisture content from 41.4 to 43.9%. However, for a similar range of moisture content change (from 41.2 to 43.2%) in a model cheese system, Watkinson et al. (2001) reported an increase in fracture strain of 0.3 units as a significantly large change.

However, in the current work, no correlation between the fracture strain and the moisture content was observed.

9.3.4 Nuclear magnetic resonance

Changes in water mobility in the very low fat skim milk Mozzarella cheeses as a result of the added EPS powder and the EPS culture were investigated using nuclear magnetic resonance (NMR) spectroscopy and spin relaxation methods. The mobility of the water (that is, roughly the opposite to the degree of "boundness" of the water) in the Mozzarella cheeses with the three treatments was studied. Figure 53 indicated that only one state of water was excited during the spin–spin relaxation in the Mozzarella cheeses. This meant that the relaxation time constants were similar, i.e. 4–5 ms, for all cheeses.

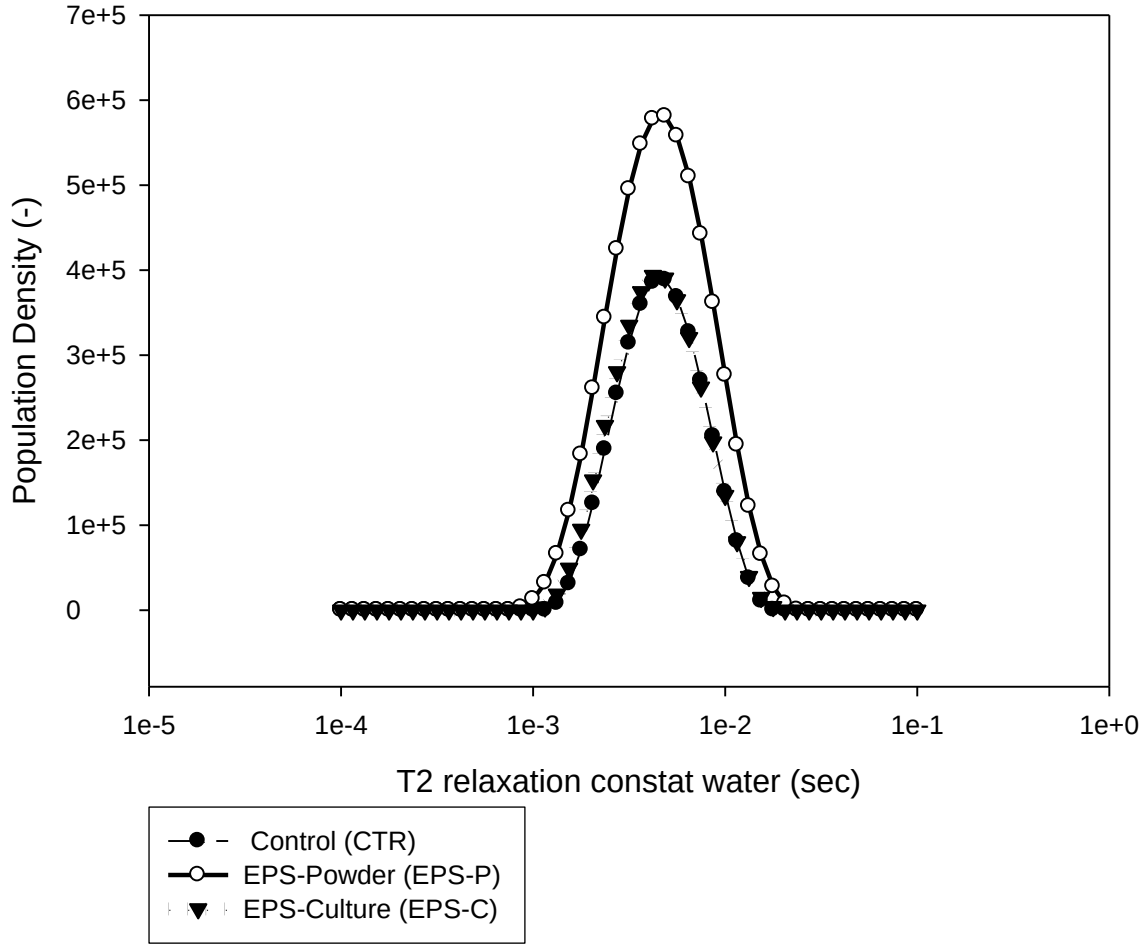

Figure 53 Spin–spin relaxation (T_2) of the water component of Mozzarella cheeses containing CTR, EPS-P and EPS-C. Mean values of repeats were used to plot the graph.

The component in pasta filata Mozzarella cheese with a short relaxation time constant of 7–11 ms corresponds to the protons in the less mobile fraction of water and most of the water molecules in this T_2 range are associated with the cheese protein matrix to a certain degree (Kuo et al., 2001). However, in the current study, the observed relaxation time constant of 4–5 ms was relatively shorter and corresponded to an even less mobile fraction of water in the experimental Mozzarella cheese; this water (T_2 of 4–5 ms) was

far less mobile than pure bulk water (T_2 of 2720 ms)(Mansfield & Morris, 1982). The peak relaxation times for all samples (CTR, EPS-P and EPS-C) were not significantly different in this study. Therefore, it can be inferred that the type of water present in the cheese matrix of all the experimental samples was not different.

The absence of a second more mobile water environment in the current very low fat Mozzarella cheese, which is sometimes found in part skim milk Mozzarella (Kuo et al., 2001), may have been because of the lack of fat. In full fat cheese or part skim milk Mozzarella cheese, much of the water in the microstructure of the open channels between protein fibres is held at the surfaces of the fat globules (McMahon et al., 1993; Oberg, McManus, & McMahon, 1993). Because almost no fat globules were present in the current experimental cheeses, there may have been almost nowhere for more mobile water to accumulate. Although more mobile water is very noticeable in freshly made cheeses (Guo & Kindstedt, 1995), free water is believed to become more "bound" (less mobile) within the cheese structure during maturation.

Therefore, it was inferred that the dominant state of water present in all experimental very low fat cheeses was rotationally immobile and that the water molecules were far less mobile than pure bulk water molecules. Moreover, the water was mostly associated with the protein.

9.3.5 Moisture content and melt

The moisture content of the experimental Mozzarella cheeses varied from 57.5 to 66.0%. The cheeses with added EPS powder had the highest moisture content (66.0%)

and the cheeses made using EPS-containing cultures were higher in moisture content (60.2%) than the control cheeses (57.5%) (Figure 54).

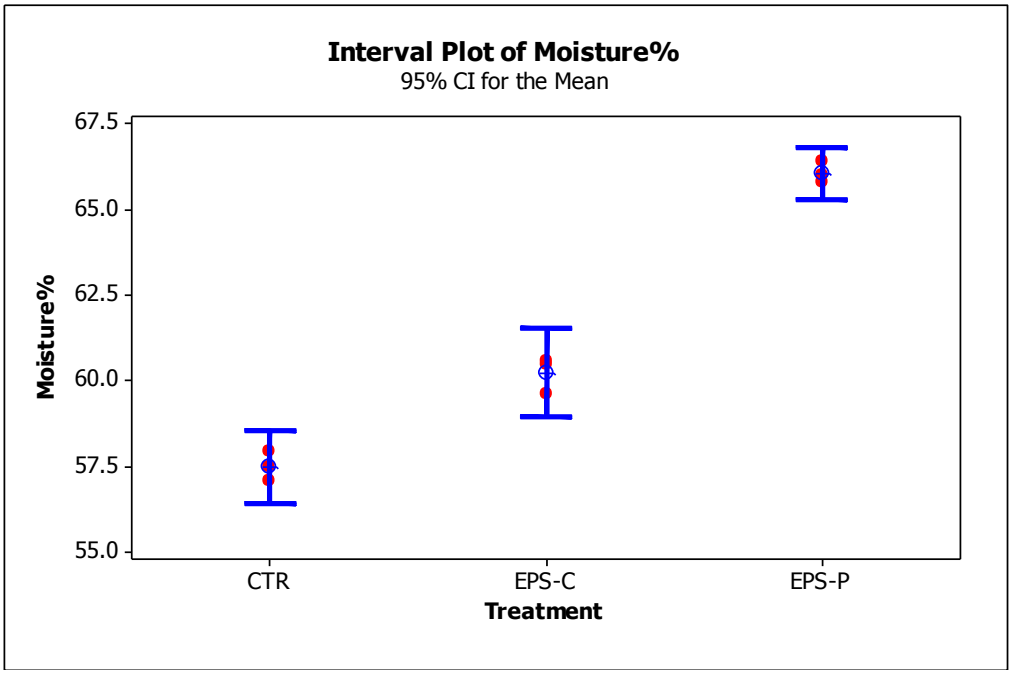

Figure 54 Interval plot of the moisture content for very low fat model Mozzarella cheeses made using three different treatments: control (CTR); EPS culture (EPS-C); EPS powder (EPS-P). The bars show pooled standard deviations and the red dots are the moisture contents for three repeats.

As explained in Section 9.3.3.2, the effect of the moisture content differences on the toughness of the Mozzarella cheeses was clearly evident. Moreover, the melt properties obtained by the Schreiber test for the very low fat cheeses were poor for the control cheese, compared with the cheeses made using added EPS powder and EPS culture (Figure 55A). It has been shown that these melt properties are significantly influenced by the cheese moisture content (McMahon & Oberg, 1998; McMahon et al., 1993). An increase in moisture content gives less resistance to flow at oven melt temperatures;

that is, a higher moisture content means a lower content of the stress-bearing component that flows at oven melt temperatures above 40°C, namely the protein.

Cheeses (with almost no fat) with higher moisture contents and concomitantly lower protein contents are expected to flow or soften more at oven temperatures, which is shown in Figure 55B.

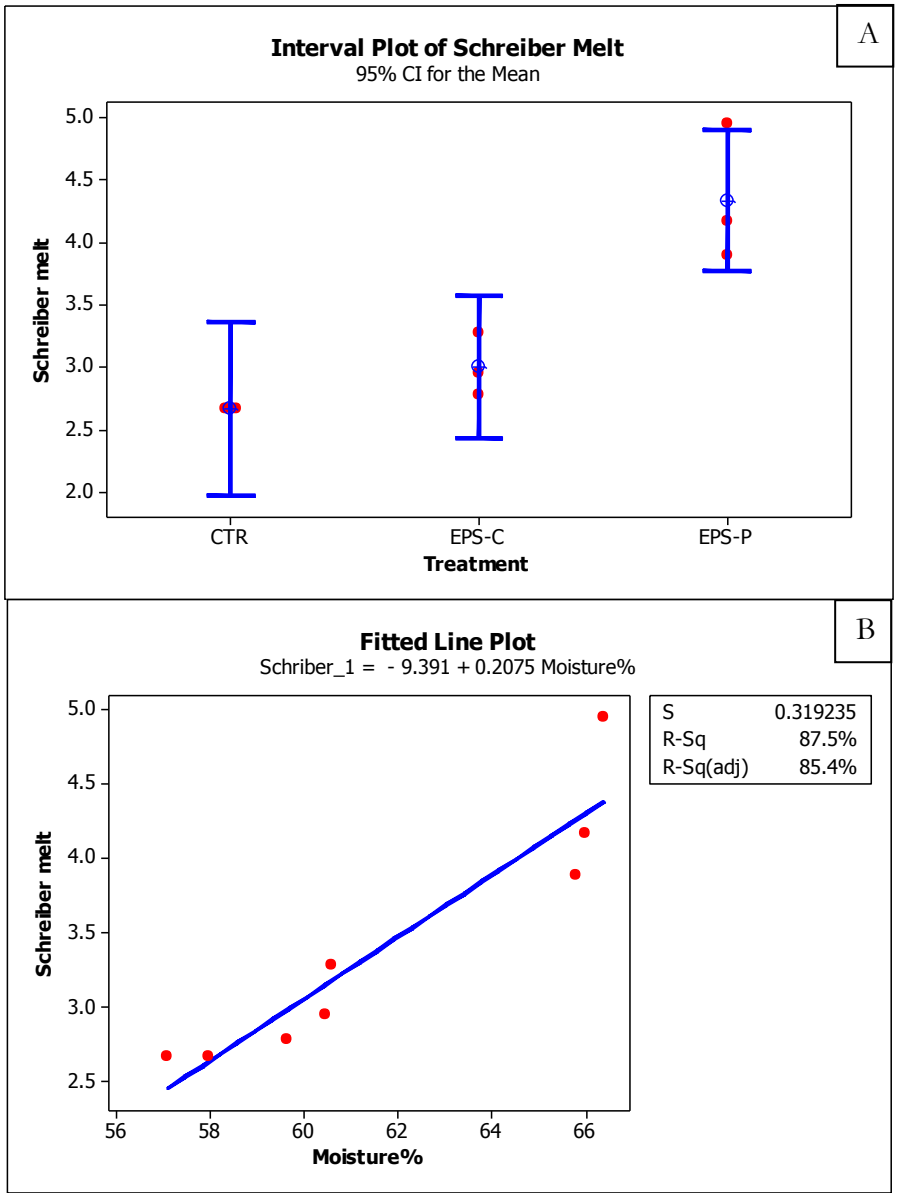

Figure 55 Changes in Schreiber melt of very low fat model Mozzarella cheeses: (A) for the three treatments (control (CTR); EPS culture (EPS-C); EPS powder (EPS-P)); (B) fitted plot showing the correlation between moisture content and melt.

The very low fat Mozzarella cheeses manufactured using EPS culture and added EPS powder retained significantly ($P < 0.05$) more moisture than the control cheeses (Figure 54); consequently, they had better melting properties (flowed more) (Figure 55A) (McMahon & Oberg, 1998). The fitted line plot (Figure 55B) showed that the increase in

the moisture content was linear in the Mozzarella cheeses manufactured using EPS culture and added EPS powder. These findings indicated that the EPS source and the EPS addition level had a significant effect on the increase in moisture retention of very low fat Mozzarella cheese. It is important to recognise that, although EPS production in Mozzarella cheese by ST10 culture (EPS-C) may be expected to be just from the starter, EPS bacteria are less likely to produce as much polymer as when added externally in a large quantity (added EPS powder, EPS-P). Moreover, a significant increase in the moisture content and the melt properties of cheeses made with added EPS powder compared with cheeses made with in situ EPS culture revealed that the additional quantity of EPS in the EPS powder was able to impart larger changes in the melt properties of the experimental Mozzarella cheeses.

9.3.6 Squeezing flow at constant temperature (60°C)

As explained earlier, in qualitative terms, the biaxial stress growth coefficient (BSGC) can be defined as the resistance to flow on heating (melt viscosity). Mean BSGC values at 60°C for the Mozzarella cheeses made using the three treatments are shown in Figures 56A and 56B. These values were measured at two different biaxial elongational strain rates of 3×10^{-3} and 5×10^{-3} s^{-1}. The values at different strain rates were different because of the strain-rate-thinning behaviour of melted Mozzarella cheese.

The observed changes in the BSGC induced by the strain rate (similar to the speed of deformation), as shown in Figures 56A and 56B, were probably a result of thermal softening of the casein network in these cheeses. The resistance of the Mozzarella cheeses to melt at 60°C decreased with an increase in the moisture content. This trend

indicates the important role of moisture content in melting properties, as already mentioned in the literature.

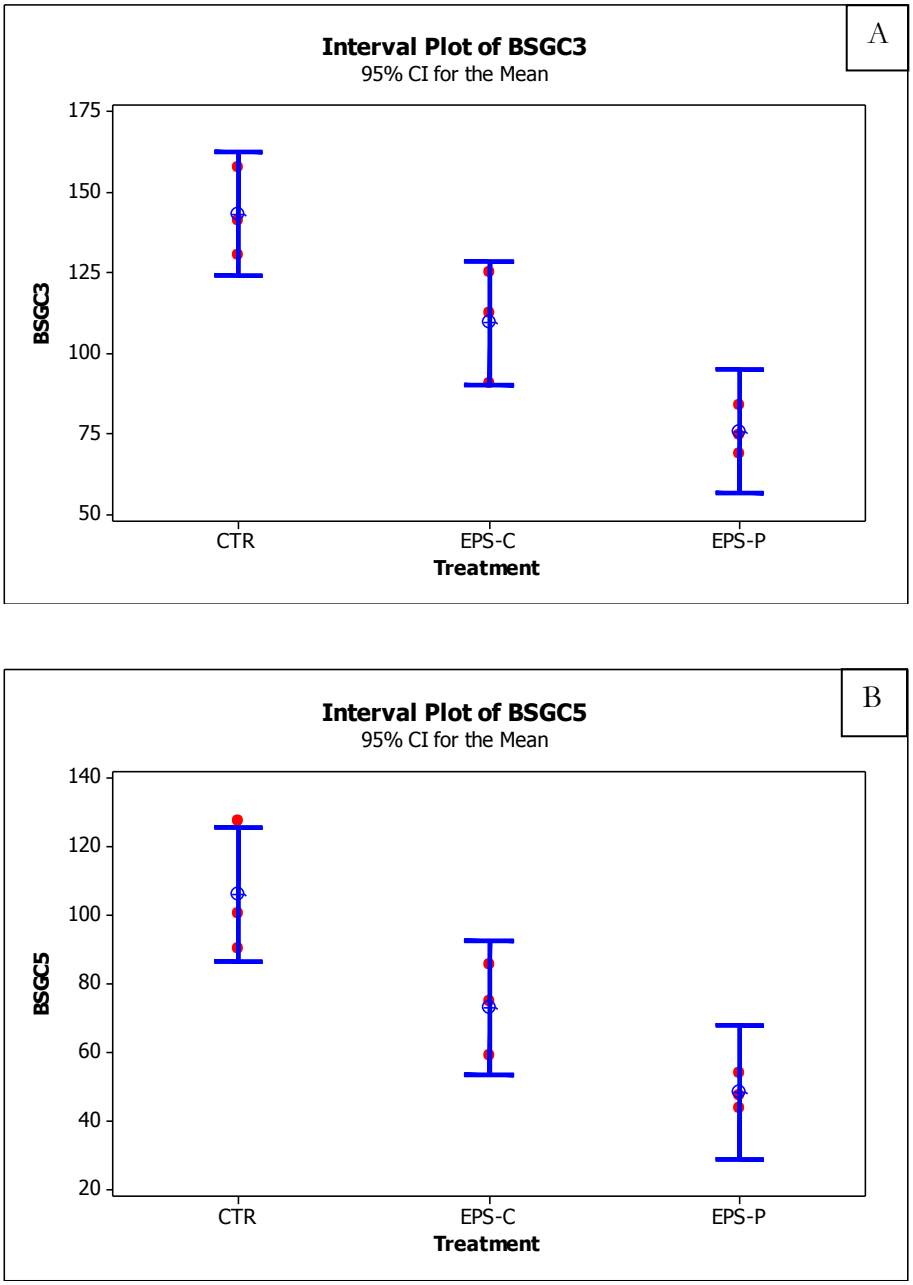

Figure 56 Mean BSGC values at 60°C for very low fat model Mozzarella cheeses made using three different treatments: control (CTR); EPS culture (EPS-C); EPS powder (EPS-P). (A) BSGC3 = BSGC at a strain rate of 3 x 10^{-3} s^{-1}; (B) BSGC5 = BSGC at a strain rate of 5 x 10^{-3} s^{-1}.

As the moisture content increased, the BSGC3 and BSGC5 values decreased. A decrease in the BSGC, a measure of the resistance to flow, can be considered to be an objective indication of increased meltability at 60°C (Walstra et al., 1985). Figures 56A and 56B show that the 95% confidence intervals of the means of the BSGCs for the control Mozzarella cheese and that made with added EPS powder did not overlap (for a given strain rate); thus, to a first approximation, their values may have been different. At higher moisture content, the protein was probably more swollen and consequently more deformable (Visser, 1991). Moreover, at higher moisture content, the protein content was lower. Thus protein–protein interactions within the cheese network may have decreased, lowering the stress (Budiman et al., 2000).

9.4 Conclusions

Pilot-scale model Mozzarella cheese manufacture with added EPS powder and in situ EPS culture gave consistent results after several repeats. Large strain rheology, elongational viscosity, melt and NMR relaxometry were used to determine the effects of the in situ and added EPS on the functionality of the very low fat model Mozzarella cheeses. The inclusion of this bioingredient in Mozzarella cheese resulted in a significant increase in the final moisture content to 66.35%, which was 5.75% higher than in the cheese produced using in situ EPS-producing cultures and 8.90% higher than in the control cheese (non-EPS-producing culture). Moreover, the inclusion of the EPS bioingredient significantly reduced the hardness and increased the melting properties of the cheese.

Added EPS powder caused a significant increase in the moisture content of the final cheese, compared with the Mozzarella cheese containing in situ EPS culture. The general trend in the compositional parameters that determine the rheological properties of cheese was as follows.

An increase in moisture content in the experimental cheese decreased the protein content, which in turn determined the rheological and fracture properties. The modulus of deformability and the fracture stress of the cheeses decreased with increasing moisture content and increased with increasing protein content. Fracture strain was observed to be independent of the moisture and protein contents in the current study. The BSGC ("melt viscosity") and Schreiber melt showed that the meltability (lower "melt viscosity") of the cheese improved when the protein matrix became more hydrated because of the presence of more moisture.

It can also be inferred that the dominant state of water present in these very low fat cheeses was rotationally immobile and that the water molecules were far less mobile than pure bulk water molecules.

Chapter 10 Overall conclusions

The addition of microbial polysaccharides to cheese has the potential to alter the functional properties of cheese, which is partially caused by the higher affinity for water of polysaccharides than the cheese casein phase. The current work measured and gave understanding of the effects of specific polysaccharides in cheeses on the rheological properties related to functionality. The conclusions drawn from the current work on microbial polysaccharides and processed cheese and lactic acid bacteria exopolysaccharides (LAB EPS) in very low fat Mozzarella cheese are as given below.

1) It was concluded from the development study on processed cheese made in a Rapid Visco Analyser (RVA) that the RVA can be used to make experimental processed cheeses. However, to produce a uniform product and to avoid variability in the results, it was important to standardise the moisture variation in the product and to define the limits for protein, polysaccharide and lactose addition. Moisture was added back to compensate for the losses observed in the moisture loss study, thus standardising the composition of the processed cheeses against the targets. It was concluded that lactose contents from 1.0 to 3.5% did not change the cheese structure significantly. Therefore, lactose was used as a filler in the model processed cheese formulation. Moreover, protein limits of 8.0 and 14.0% and polysaccharide limits of 0.0 and 2.5% were established to give homogeneous products with minimum variation for functional measurements.
2) The effects of xanthan gum (xanthan), a xanthan and locust bean gum (xanthan+LBG) blend, high acyl gellan gum (Gellan-H) and low acyl gellan gum

(Gellan-L) on the rheology of the formulated model processed cheese were measured using rheology, syneresis, water mobility and the relative proportions of two states (more mobile and less mobile) of water as measured by proton spin–spin relaxation times from nuclear magnetic resonance (NMR). The results from the rheological study indicated that the affinity of polysaccharide for water, compared with the affinity of casein for water, was very high for Gellan-H and was moderate for Gellan–L. The degree of phase separation (measured qualitatively by microscopy techniques) of polysaccharide compared with casein also showed the same sequence, from very high for Gellan-H to moderate for Gellan-L. It was revealed from the NMR proton transverse relaxation study that Gellan-H and Gellan-L significantly reduced the water mobility in the experimental processed cheese but that the reduction was greater for Gellan-H. The mechanism possibly includes immobilisation of water because charged polysaccharide attracts water in the cheese matrix.

3) It was concluded from the study that the affinity of polysaccharide for water, the degree of phase separation and the mobility of water have significant effects on the functionality of processed cheese. In this study, the increase in cheese stiffness (modulus) and firmness (fracture stress) was high for Gellan-H, high to moderate for xanthan and the xanthan+LBG blend and moderate for Gellan-L. The reduction in the flow of the cheese at the melt temperature (phase angle) was high for Gellan-H, moderate to low for xanthan and the xanthan+LBG blend and low for Gellan-L. The following mechanism was hypothesised for these rheological changes: the greater affinity of the polysaccharides for water,

compared with the proteins, together with phase separation, led to an effective concentration of the continuous protein phase. Moreover, it was evident from the water mobility study that the reduction in cheese flow at ambient temperature (elongational viscosity at 25°C) was caused by the increase in the effective concentration of protein in the continuous phase. This effective increase in protein concentration was shown by the change from more mobile water to less mobile water in the cheese matrix. The reduction in the mobility (of the less mobile fraction of water) as the polysaccharide concentration was increased was evidence that there was reduced mobility of the water in the cheese. Therefore, the relative spreadability of processed cheese containing Gellan-H reduced (i.e. its viscosity increased) because of the reduced availability of water in the protein (aqueous) phase.

The transmission electron microscopy study suggested that dispersibility of the polysaccharide could be an issue at higher levels of polysaccharide addition in the model processed cheese system. Therefore, an extensive range of polysaccharide addition and dispersibility of the polysaccharide could be an area of future work.

4) An "all-dairy" ingredient with an increased content of EPS can be produced at laboratory scale by using an EPS-producing *Streptococcus thermophilus*. However, the extent of increase in the effective polysaccharide content is limited without extensive commercial separation and purification processes. An intrinsically lower concentration of LAB EPS (compared with commercially

available microbial polysaccharide), in this study, resulted in different changes in the functional properties of Mozzarella cheese (compared with microbial polysaccharides in processed cheese). The LAB EPS in Mozzarella cheese had the beneficial functional property of reducing serum exudation. Cheeses made with the EPS ingredient retained about 6.0% more moisture in the cheese matrix, without any visible serum exudation, than cheeses made using EPS-producing cultures. This greater moisture retention in the cheeses was reflected in their rheological properties. The cheeses made with the EPS ingredient exhibited greater meltability, lower elongational viscosity and lower modulus of deformability (stiffness) and fracture stress than the cheeses made with EPS-producing cultures and non-EPS-producing cultures. It was also evident that the amount of EPS had a marked effect on the moisture retention during the cheese-making process. However, unlike commercial polysaccharides, the EPSs were unable to show any strengthening of the cheese structure, which would have been indicated by a higher fracture stress or modulus of deformability.

Note: **General summary of estimated effects of polysaccharides on cheese functionality and generalised polysaccharide properties relevant to cheese are shown in appendix F.**

10.1 Future work

This experimental work showed that great understanding of the interactions between microbial polysaccharides and the cheese matrix can be achieved by using rheology and syneresis and by lowering the water mobility (proton transverse relaxation by nuclear magnetic resonance). Moreover, the clarity and the accuracy of the results from this work make such techniques useful for product development and process control. Further work to substantiate the findings reported here and to expand the clarity between the physical and chemical relationship of these microbial polysaccharides with cheese includes the following.

1. Development of more direct measurement techniques that measure the direct relative affinity of polysaccharide for water, compared with protein. This relative affinity for water probably has a large influence on the effects of the addition of polysaccharide on the functional properties of cheese.
2. The protein–polysaccharide interactions would be easier to study in a food system that is simpler than that used in the current work. This would help in studying the physico-chemical nature of the interactions between the individual components of the processed cheese.
3. Development of a more appropriate methodology for microstructure evaluation of the processed cheese containing polysaccharides in which the water phase, along with the protein, polysaccharide and fat phase, could be stained successfully. This would be followed by development of a methodology for

generating quantitative data from image analysis to study the water mobility of individual components in a food matrix of a complex food emulsion.

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Appendix A Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) is widely used to study food microstructure (Auty, Twomey, Guinee, & Mulvihill, 2001). This technique also gives information about the shape and the composition of the particles. However, as only a small portion of a sample is measured using this technique, good sampling techniques and good sample preparation are essential (Allen, 1990). Food components such as fat and protein can be stained selectively prior to the processing of the product, or by diffusion into the product. Specific stains are available for this purpose (Blonk & van Aalst, 1993).

Multiple labeling

Three or more different probes can be imaged simultaneously by using multiple labeling techniques. Vodovotz et al.(1996), however, reported that bleeding from one fluorescence channel to another may lead to interference. Therefore, it is always to have good knowledgeof working with dyes that have narrow absorbance and of using more restrictive excitation wavelengths and filters. It is also important that dyes being used in multiple lablelling have a minimum of overlapping, so that emission can be separated (Herbert, Bouchet, Riaublanc, Dufour, & Gallant, 1999)

Principle

A confocal microscope consists of a fluorescence microscope, multiple laser sources, a confocal box or scan head with optical and electronic equipment, a computer monitor for display, and software for acquiring, processing and analysing images (Murphy,

2001). A schematic diagram of a confocal laser scanning microscope is shown in Figure 57.

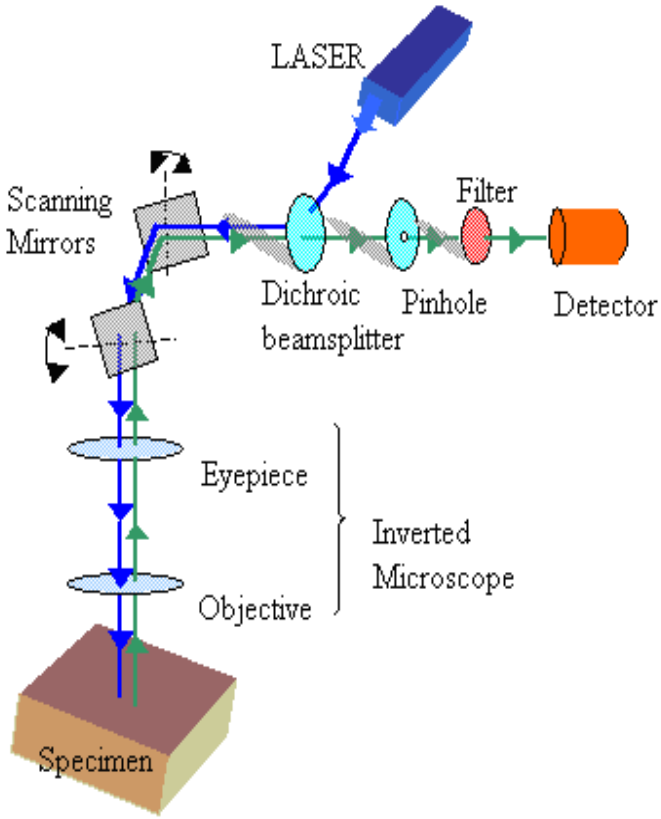

Figure 57Schematic diagram of a confocal laser scanning microscope (Institute of Food Research, 2007).

A laser beam forms an intense diffraction-limited spot that is scanned across the specimen (Murphy, 2001). Fluorescent light emitted from an excited point on the specimen is collected by the objective. The fluorescent light then passes through a dichroic mirror and becomes focused on the pinhole aperture. The pinhole aperture ensures that only light from the point on the sample is collected (Blonk & van Aalst, 1993). The focused spot varies in intensity with time as the laser excites different locations in the specimen. Fluctuations in light intensity are converted into a

continuously changing voltage and are then digitised by a computer to generate pixels, and an image is displayed on the monitor (Murphy, 2001).

Appendix B Particle size measurement

Application of the Malvern Mastersizer to measurement of fat globule size distributions

Light scattering techniques have been used in a number of studies to measure the fat globule size distribution (FGSD) of dairy products. Walstra (1965) developed a method for diluting a milk sample for analysis of the FGSD using a light scattering technique. With this method, an emulsion without clusters of fat globules was obtained. The milk was diluted with water to nearly the desired volume. Sufficient solution A (0.375% disodium ethylenediamine tetra-acetate (EDTA) and 0.125% polyoxyethylene sorbitan monolaurate (Tween 20) in water, adjusted to pH 10 with NaOH) was added to give a final pH of the dilution that was not much higher than 8. The emulsion was made up to volume with water and was mixed without stirring or shaking more than necessary to obtain homogeneity.

In a study by Michalski, Briard and Michel (2001), a Malvern Mastersizer 2000 was used to measure the FGSD in milk. The sample was diluted 1:1 with 35 mol/L of EDTA/NaOH, pH 7.0 buffer to dissociate casein micelles and aggregates. A small volume was then dispensed into a sample unit containing 100 mL of 0.1% sodium dodecyl sulphate (SDS) solution in Milli-Q water. The FGSD was then measured by the Malvern Mastersizer 2000.

Lee, Anema and Klostermeyer (2004) investigated the effect of moisture on the pH, rheological properties and fat particle size of a model processed cheese spread system.

The fat particle size was measured using a Beckman Coulter laser scattering particle sizer (similar to a Malvern Mastersizer). To measure the FGSD, 0.5 g of the processed cheese spread was dispersed in 50 mL of solution A, developed by Walstra (1965). The samples were left at 5°C overnight in a refrigerator and were allowed to stand at room temperature for 1 h before measurement. Approximately 3 mL of the solution was added to the cell of the Beckman Coulter laser scattering particle sizer and the fat particle size and the FGSD were obtained. This same sample preparation method was used by Trivedi (2006) to measure, using a Malvern Mastersizer, the FGSD in individually wrapped slice (IWS) processed cheese containing different starches. The processed cheese samples used in the present study had higher solids contents than the spreads used in the study of Lee, Anema and Klostermeyer (2004); thus, it was suggested that a sample size of 0.2 g of processed cheese for dispersion would be more appropriate.

In the present study, the method used by Lee et al. (2004) and Trivedi (2006) was the base method that was used for preparing samples of processed cheese for analysis of their FGSD using the Malvern Mastersizer 2000.

Safety: Mastersizer S

HAZARD	**RISK**	**EFFECTS OF HAZARD**	**MANAGEMENT OF HAZARD**
Noise	Very High	Hearing damage	Wear earmuffs
Laser light	Moderate	Damage to eyes	Avoid exposure

Principle

The light from a low power helium-neon laser is used to form a collimated and monochromatic beam of light. Sample particles presented to this beam will scatter the light at low angles. The scattered light is incident on to a receiver lens. This operates as a fourier transform lens forming the far field diffraction pattern of the scattered light at its focal plane. Here the detector, in the form of a series of 31 concentric annular sectors, gathers the scattered light over a range of solid angles of scatter. The range lens configuration has the interesting and useful property that wherever the particle is in the analyser beam, its diffraction pattern is stationary and centered on the lens optical axis.

Appendix C Transmission Electron Microscopy

The transmission electron microscope (TEM) typically use high-energy (100-400 KeV) electron to form images of the internal structure of materials. A TEM has several advantages over a light microscope, but principally it is a higher resolution instrument that provides much more than simple images. The resolution of a microscope (δ, the smallest observable distance between two points in an image) is controlled by the wavelength (λ) of the radiation used. The value of λ for 100KeV electron is 0.0037 nm, which compares with 0.5μm for visible light. Therefore, the resolution limit of the TEM is theoretically, several orders of magnitude smaller than that of the light microscope although, in practice, electron lens defects limit the resolution to 0.1 nm in the highest resolution instruments. The TEM is available commercially in several versions such as high-resolution.

A cross-sectional view of a typical 200keV TEM is shown in Figure 48. The electron gun sits above the condenser lenses, which control the beam that interacts with the specimen in the goniometer holder. Imaging lenses take the electrons scattered by the specimen and form images or diffraction patterns, which are observed through the viewing port and recorded in the camera chamber.

Electrons are produced either from a lanthanum hexaboride single crystal heated to about 200K or by a field-emission gun (FEG), which consists of a sharp tungsten needle to which an intense electric field is applied. Electron are emitted from the source and are accelerated through 100-200kV and enter the illumination system. The whole instrument is evacuated to $<10^{-6}$Pa to prevent electron scatter by the atmosphere. The

illumination system produces a demagnified image of the source at the plane of the specimen by using two or more electromagnetic condenser lenses to restrict the electron paths. In combination with limiting diaphragms within the lens, approximately parallel, coherent electron beams of 2-60μm diameter is produced at the specimen or, if analysis is to be performed, fine electron probes down to 0.1 nm are obtainable from a FEG.

Limitations

A significant limitation of all TEM studies is that the specimen must be transparent to the electron beam and this means that drastic thinning process are often necessary to produce suitable specimens. The thinning method can change the structure of the specimen if not cut carefully especially in a soft product such as processed cheese.

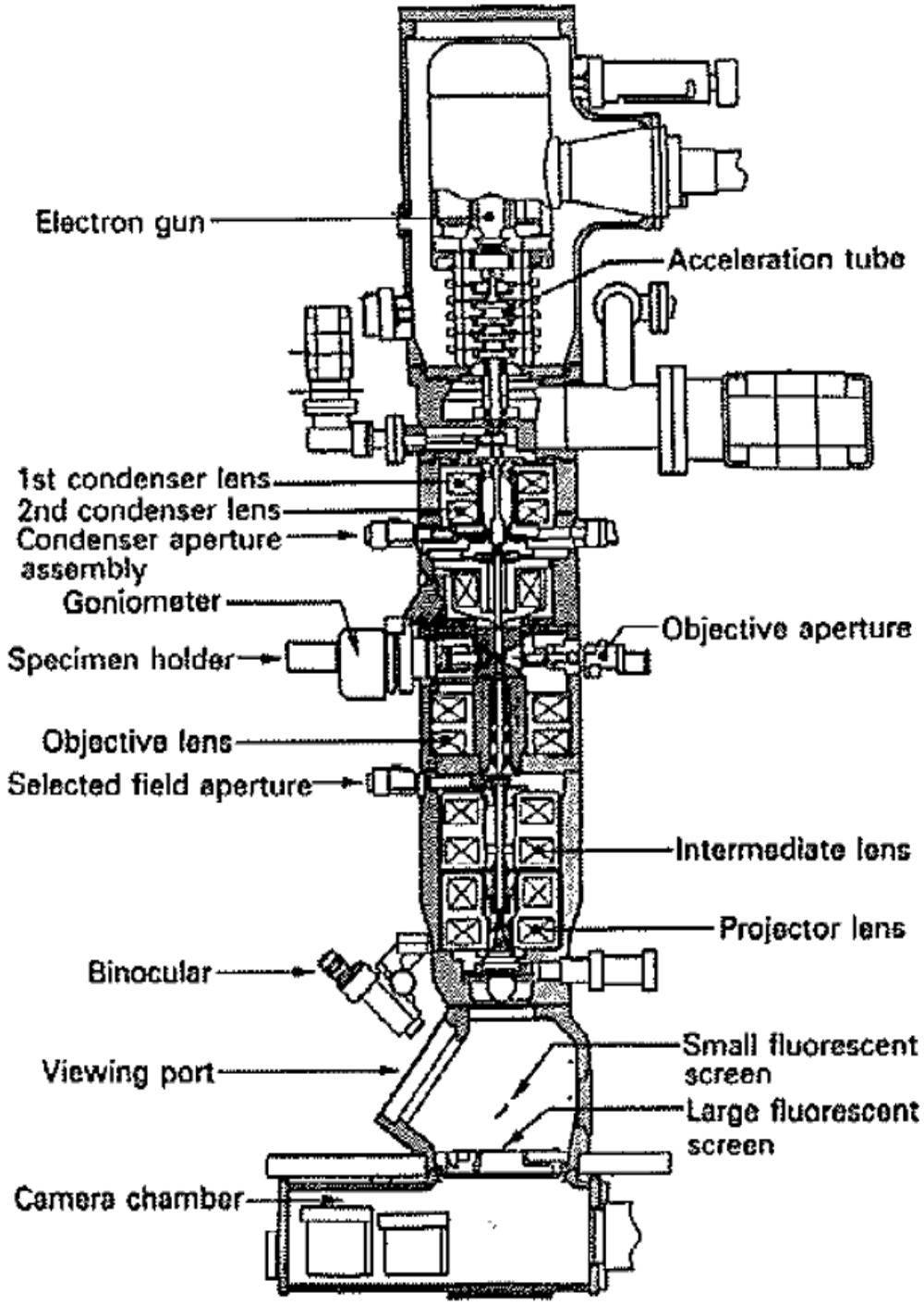

Figure 58 Cross section of column of a 200kV FEG TEM.

Appendix D Texture Expert Exceed texture analysis

Standard Double Compression test (strain controlled): ISO/TS 17996:2006 (E) IDF/RM 205:2006(E) (2006)

Technical Specification. *Cheese – Determination of rheological properties by uniaxial compression at constant displacement rate*. Geneva.

Abbreviations

<> press the enter key

Point to point the cursor using the arrow keys or mouse (windows)

- a normal instruction

➔ an instruction requiring manual writing an output to a lab book

Overview

- 0 Scope
- 1 Sampling (Cut up samples to get 20mm diameter, 25mm high cylinders)
- 2 Equilibrate samples overnight at test temperature in texture fridge usually
- 3 Lab book preparation
- 4 Set texture room Air temp (with TAHD load cell in) on at least 1 h before doing tests
- 5 Set peltier on & test jig in peltier or fridge at least 1 h before test
- 6 Test set up
- 7 Running test - sample
- 8 Running test - software

0 Scope

The principles of most of the following methods apply to any semisolid that is not too different in rheological and fracture properties to cheeses. Sample details listed are for natural cheese or processed cheese.

1 Sampling

The aim is to get samples, each being a cylinder 20mm diameter 25mm high. In general sample for rheology before any other test. Sample preparation is as explained in section 3.2.1

2 Equilibrate samples overnight at test temperature

3 Lab book preparation

- Set up lab book with headings for test and data analysis.
- Headings left to right taking up left hand page face (right facing page is left spare for later , second rheology software information.)

Filename label diameter temperature time parallel force vs time picture archive check distance at 80%strain force 80%str. adhesion area springback distance export file

4 Set texture room Air temp (with TAHD load cell in) on at least 1h before test

- **(1) texture room air/load cell**
- turn on power outside room
- set room air fan to full speed
- set room to desired temperature
  - room = 13°C for normal tests with peltier 5°C or 10°C or 2 °C
  - rarely room = 20°C for full sensory consumer tests at 20°C

5 Set peltier on (if <13°C test temp) & test jig in peltier or fridge at least 1 h before test

- Turn on power for peltier, ALWAYS TURN ON BOTH heat exchanger AND PELTIER
- Set temperature of peltier by
- Make sure paraffin oil is in room equilibrating to room air temp.

Extra notes

TAHD load cell needs 1 to 2 hours to get thermal equilibrium when changing 5°C or more.

Load cell equilibration usually takes longer than any air temp. equilibration

If TAHD off then TAHD needs 0.5hour warm up before using – but normally left on.

6 Test set up

Test jig assembly

- Select peltier cabinet if required as follows
- Sample Temperature 13°C to 20°C – do not need peltier cabinet – have heavy duty platform only Teflon top
- Sample temperature 2°C to 10°C –require peltier cabinet and then screw on heavy duty platform
- Select test assembly
- Standard compression test ;
- Screw standard teflon plate onto load cell knurled aluminium fixing
- Move plate to be 28 mm above baseplate
- Put 2 top covers over peltier

Other – penetration test

- Put 3mm thick Teflon cover (put ring shaped stainless steel weights on top of sample)
- REMINDER you already have sample overnight at test temperature normally in texture room fridge 10°C to 2°C or 13 - 20°C elsewhere

Start Software (assumes v2.64a software Texture Expert Exceed)

- make sure intranet is operational (if not copy onto c drive temporarily)
- Always leave computer on
    - to avoid condensation forming inside the computer and destroying it
- Initial Log on window... type Ctrl Alt Del
- Security window. Click on OK
- Log on window.
    - Preferable option....Click on Shutdown. then on RESTART option
    - (If no intranet.. use standalone option)
- Security window (2nd time).. Click on OK
- Double click on icon "shortcut to Texture Expert Exceed v2.64a (UK)
- Select username from list type in password (click OK on Hints window)

TAHD Load cell calibration

- It is good practice to always calibrate the load cell prior to test, but a minimum is:
- Calibrate load cell if it has been More than 1 month since last calibration OR

- If load frame/load cell has been moved OR
- If the temperature of the load cell has changed (by 5°C or more)
- Remove any test plate or probe
- And if peltier is on.. put top plate/rod back into peltier cabinet
- Always have key on TAHD keyboard at "Run"
- Move load cell up or down to position using keyboard up or down arrows.
- Screw calibration hanger onto standard (knurled aluminium) top fixing

Software use

- Click T.A. / calibrate force / click OK
- The computer screen should prompt you for a 10kg weight.
- Place two 5kg weights on the hanger slowly
- Wait till hanger stops moving
- Click OK button a second time on the PC screen
- The computer screen should now indicate that the calibration was successful
- Remove weights and hanger
- Put plates back on load cell (if peltier used make sure its back inside the peltier

Plate height calibration

- Use if test method requires strain or %compression measurement
- Click T.A. / Control probe
- Click on tare (sets probe mass to zero wrt force output)
- Click & hold mouse on slider bar to move up and down to position the plate to within about 5mm of baseplate.
- Click T.A. / calibrate force /
- (check return distance is set to 28.00 mm = default)
- (check trigger force is set to 10 g = default)

Plate should move down, touch the base plate and move up again by return distance = 28.0mm.

Open an existing Project for texture expert exceed project

- Click on lower left button called project wizard
- Click on button Open an existing Project
- Select under Title ... the title of type of test you require (do NOT click on file)
- NOTES ON MOST USED TESTS
- Double compress. 80% std = standard texture / shred tests
- double click on selected test type

Get test project setup window with all requirements to run a test, get graph & save

- Click on Test settings button
- Type in box **File Id:** the filename FOR THIS BATCH – 6 digits eg. c0214a
- Click OK on this message box .. You can get .. max. of 100 files....for File Id..
- Type in box **File No:** 1
- This autosaves after every test and adds 1 to the previous file
    - eg. C0214a01 then next test is saved as c0214a02
- Click OK

Default file path uses data network drive ..

7 Running test - sample (v2.64a TextureExpertExceed software)

- Only for very first sample of the day measure temp. of sample with thermocouple, while keeping sample in the fridge. rewrap

If peltier (<13℃ sample) then timing is an issue, then timing is important as follows:

- Check peltier temp. is near (within 1°C set temp. (press *for set temp.)
- Max. of 30seconds Open front cover of peltier to clean plates with tissue and put lubricating oil on using oiled tissue paper
- Wait until peltier returns to set point
    - 1 min for 5°C
    - While waiting set up files in software ready to test
    - While waiting max. of 60 seconds Open texture fridge unwrap sample, measure sample diameter inside fridge (or every 2nd sample)
- Max. of 5 seconds open peltier and put sample into peltier
- Run test straight away

If NO peltier (13, 20℃ sample) then peltier timing is an NOT an issue,

- clean plates with tissue & put lubricating oil on using oiled tissue paper
- Max. of 60 seconds open texture fridge unwrap sample, measure sample diameter inside fridge (or every 2nd sample)
- put sample into peltier
- Run test straight away

DETAILS

Lubrication and positioning of compression plates

- Bottom of plate should automatically be 28mm above the base plate.
- (Briefly touch arrowed button on the TA keyboard and note distance display = 28.0.)
- Saturate a sheet of white tissue paper with high density (0.87 g/ml) paraffin oil.
- Place about 5 drops of this oil on the base plate.
- Spread the oil using the saturated tissue into about a 80mm disc.
- Place about 5 drops of oil on the tissue (do not rub in to tissue).
- Spread the drops of oil on the tissue onto the underside of the top plate.

Sample measurements prior to test

➔ Record under *label* heading the label number
- make sure any imperfection in the sample is noted in the lab book.

➔ Record under ***Temperature*** heading (when at test temperature),
- the temperature of a dummy sample or a standard sample, putting a small thermocouple into the side of the cylinder no more than about 2mm.
- Measure at least one sample per batch just prior to test, using a thermocouple put in the center of the cylindrical side of a sample.

➔ Record under ***Diameter*** heading (when samples are at test temperature), the diameter of at least one sample per batch at a given test temperature using a caliper.
- Average the diameter of two measurements at right angles for each sample.
- Typically measure the diameter of 1 or 2 samples from one core at a given test temp.
- (For tapered samples - older mozzarella - also record the minimum and maximum diameter of every sample.)

Sample preparation for Test start

- Place sample in midpoint of baseplate
- At the start of the test see if the top sample surface is parallel to the top plate.

➔ If the the plate is not parallel to the sample, record this.
- If there is a crumb on the top of the sample or the bottom of the top plate where it will compress the sample, then record this fact (It affects initial rheological properties).

8 Running test

Check test and recording comments during first compression

- Initially the top-plate moves down a few millimetres and compresses the sample face until the trigger force (0.1N) is reached.

- The TAHD then sets this point as the start of the test.
- The cursor on the computer should start at the origin and immediately move to a positive value of force and time.
- Thereafter, the top plate moves at a constant speed of 0.83 mm/s (49.8 mm/min) compressing the sample to 80% strain (standard value is 80% sometimes set to 90%).,
- decompression or movement in tension
- wait 0.1seconds
- A second compression to 80% strain (standard value is 80% sometimes set to 90%).
- This corresponds to an initial set of positive peaks for the 1st compression,
- a negative peak for the decompression or tension
- and a final positive peak for the 2nd compression.
- After the test, the plate should move quickly (5 mm/s) back to its original position.

- Keep looking at the sample until there is a significant deviation from a cylinder shape.

➔ Record under *Time parallel* heading the approx. time on the on-line data output where the sample is significantly different from a cylinder (eg. barrel). Repeat for at least 2 per batch

➔ Record any recognizable fracture pattern (eg. lines 45deg to baseplate or 90deg to baseplate)

➔ Record under *force v time picture* heading the approx shape of first compression noting if there is an inflection, local max. in force, or neither feature.

- NOTE IF SAMPLE OVERLOADS(>500N) OR UNDERLOADS(< -50N)
- message appears on TA keyboard.

Press Reset on TA keyboard and after archiving & clean up, you will need to repeat the

Appendix E: Moisture loss data for the model formulation with a polysaccharide content from 0.5 to 2.5% and a protein content from 8.0% to 14.0%

Protein %	Polysaccharide %	Added Moisture %	Moisture Loss %
7.98	1.5	52.13	4.532
7.98	1.5	52.13	4.478
8.88	1	50.6	4.855
8.88	1	50.6	4.856
8.85	1	51.82	4.416
8.85	1	51.82	4.421
8.88	2	49.17	4.481
8.88	2	49.17	4.465
8.83	2	51.62	4.642
8.83	2	51.62	4.917
10.11	0.5	50.85	4.97
10.11	0.5	50.85	5.14
10	1.5	47.39	4.713
10	1.5	47.39	4.6932
9.98	1.5	49.92	4.9767
9.98	1.5	49.92	4.7808
10	1.5	52.41	4.877
10	1.5	52.41	4.5953
9.94	2.5	49.59	4.283
9.94	2.5	49.59	4.298
11.17	1	47.22	5.344
11.17	1	47.22	5.644
11	1	49.34	5.127
11	1	49.34	5.153
11	2	49.34	4.488
11	2	49.34	4.492
11.17	2	50.16	4.144
11.17	2	50.16	3.776
12	1.5	47.78	4.279
12	1.5	47.78	4.265
14	1	46.52	4.325
14	1	46.52	4.319
14	2	46.5	4.213
14	2	46.6	4.221

Appendix F General summary of estimated effects of polysaccharides on cheese functionality and generalised polysaccharide properties relevant to cheese

SCOPE: Omitting effects of protein–polysaccharide interactions.

Polysaccharide in cheese	**Polysaccharide affinity for water compared with casein affinity for water**	**Polysaccharide degree of phase separation compared with casein degree of phase separation**	**Cheese functionality improvement Reduction in water exudation (% water)**
Gellan-H	Very high	Very high	Very high
Xanthan gum/LBG 50/50	High?	High	High
Gellan-L	High to moderate	Low	High
LAB EPS	Moderate	No	Moderate
Comment	Inferred from rheology and syneresis and lowering of water mobility (T_2 by NMR) **Future work could include measurement of this by direct measurement techniques** Can obtain LAB EPS only in low concentrations	Measured qualitatively by visual separation of phases from confocal laser microscopy and transmission electron microscopy no direct measure	no direct measure, but inferred from syneresis and lowering of water mobility (T_2 by NMR) Mechanism possibly includes immobilisation of water by charged polysaccharide attracting water
Polysaccharide in cheese	**Cheese functionality**	**Cheese functionality**	**Cheese functionality**

	improvement Increase in cheese stiffness (modulus) or firmness (fracture stress)	**improvement Reduction in cheese adhesiveness (adhesion area)**	**improvement Reduction in cheese flow at melt temperature (phase angle)**
Gellan-H	High	Negligible(Data not presented in the thesis)	High
Xanthan gum/LBG 50/50	High to moderate	Moderate	Moderate to small
Gellan-L	Moderate	Negligible(Data not presented in the thesis)	Small
LAB EPS	Low	Negligible(Data not presented in the thesis)	High to moderate for EPS powder (mainly due to more moisture retention)
Comment	Measured from rheological tests No direct measure, but inferred from intrinsically low concentration of LAB EPS Mechanism possibly both phase separation and water moving out of protein into polysaccharide, giving higher protein concentration in continuous phase	Measured from rheological tests (Data not presented in the thesis) No direct measure, but inferred from measurements	Measured from rheological tests. Inferred from elongational viscosity Inferred from intrinsically low concentration of LAB EPS

H = high acyl; L = low acyl; LBG = locust bean gum; LAB = lactic acid bacteria, EPS = exopolysaccharide.