INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
MASSEY UNIVERSITY, PALMERSTON NORTH, NEW ZEALAND

INCORPORATION OF EXTRACELLULAR POLYSACCHARIDE PRODUCED BY *XANTHOMONAS CAMPESTRIS* INTO MILK POWDERS

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY

HAMISH SHARPE
2003

ABSTRACT

The purpose of the research was to investigate the functional properties of milk powders following exopolysaccharide (EPS) addition to milk solutions and their subsequent spray-drying. The aim was to replace some of the milk proteins with polysaccharide in dairy products while maintaining or improving the functional characteristics. Both commercial xanthan EPS and ferment xanthan EPS were incorporated into whole milk powder (WMP), skim milk powder (SMP), and milk protein concentrate (MPC).

Ferment EPS was produced from a by-product of the dairy industry, milk permeate, through the hydrolysis of the lactose and fermentation with a strain of *Xanthomonas campestris*. Ferment EPS had a characteristic and unpleasant odour. The main compound responsible for this odour was p-cresol which, in milk, is largely bound in the conjugate form. *Xanthomonas campestris* hydrolyses these conjugates releasing the odour compounds. Ultrafiltration (UF) of the ferment or passing the ferment through a bed of activated carbon was effective in reducing the odour. UF was proven to reduce the levels of p-cresol in the ferment from 138ppb to less than 5ppb after 98 concentration factors. Milk powders made with UF ferment were more acceptable to the consumer sensory panel than those made with untreated ferment.

The incorporation of EPS into milk powders has beneficial effects on the product with small additions increasing the viscosity of reconstituted SMP and WMP considerably. The EPS addition could result in a thickened milk product or alternatively, substitute for some of the milk solids. Sensory testing showed that 13.3% WMP solution, containing 0.02% commercial EPS, was not detectably different from a 15% WMP solution.

The addition of both commercial and ferment EPS into milk powders leads to the formation of separate flocculated casein and polysaccharide phases with reconstituted milk. Confocal microscopy showed that casein flocculation occurred at all EPS concentrations tested, but this only resulted in sedimentation at intermediate EPS concentrations. At high EPS concentrations of approximately 0.2% the high

viscosity limited flocculation and prevented sedimentation. At low EPS concentrations of approximately 0.05% flocculation was insufficient to overcome Brownian motion.

Fresh cheese (Panela) made from MPC containing either ferment or commercial EPS showed greatly decreased whey loss. This was attributed to (i) the increased viscosity of the continuous phase limiting the flow of liquid through the pores of the cheese, and (ii) diminished casein interaction in the presence of EPS leading to a looser curd and lower contraction forces. For example the incorporation of 0.161% ferment EPS decreased the whey lost by approximately 75%. Negative effects were also apparent. The addition of EPS led to a granular appearance, which became more apparent with increasing EPS concentration. Cheese firmness was also decreased by approximately 40% by the addition of the ferment EPS at 0.161%. This could also be attributed to the localised aggregation of protein during renneting and the increased heterogeneity of the network. Sensory testing of cheeses made with 15.6% MPC + 0.045% commercial EPS compared with cheese made with 17.37% MPC alone showed that the consumers had no significant preference for one cheese over the other, but did notice a difference in texture.

For reasons of safety and health, the sensory testing of milk and cheese in this research was confined to commercial xanthan. Future sensory testing of milk and cheese should be conducted with ferment EPS after odour removal rather than commercial EPS, and use consumers familiar with these cheese and milk products.

For commercial production of dairy powders containing UF ferment EPS it is vital that either the xanthan or casein micelle structure be altered to prevent casein flocculation. If this is not feasible then an alternative use of the product may need to be found. A potential option involves the addition of the powder containing UF ferment EPS into food products as a minor food constituent. This may limit the occurrence of phase separation while improving the functionality of the product. Commercialisation is also limited by the increasing costs caused by ferment EPS purification and the lower solids concentrations required for spray-drying. As such the viability of the powder production must be determined.

ACKNOWLEDGEMENTS

Firstly I would like to acknowledge and thank my chief supervisor Dr. Derek Haisman for his guidance and support during the experimental work and write up and for helping to solve the problems that have arisen throughout. I am grateful for all the effort and encouragement provided.

I also gratefully acknowledge the contribution of the other supervisors Dr. Richard Archer and Professor Harjinder Singh for their help interpreting the results and the guidance regarding the direction of the project. My appreciation also goes to the other people working on the project including Kelvin Goh, Peter Zhu and Dr. Ian Maddox for their input and especially to Dr. Alan Welman for providing the samples throughout the year.

A special thanks the people at the Fonterra Research Centre who have helped me over the last year. This includes Richard Vautier and Bing Soo with regards to the fresh cheese and milk powder production and Dr. Justin Bendall with his expertise with the GC/MS facilities.

I have also appreciated the support of the staff at Massey University including Gary Radford, Steve Glasgow, John Palmer, Liz Nickless, Warwick Johnson, and Chris Hall. Thanks for allowing me to use the food laboratories and confocal microscopy facilities at Massey University and for all the technical expertise that was provided.

Finally, I would like to thank my family and friends who have encouraged me along the way including my Mother and Father, Nigel, Kurt, and Kelly. It has been tough but your encouragement and support has helped me throughout.

CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Fonterra Co-operative Group Limited produces and exports a large proportion of its dried powdered milk products to overseas countries for both consumer and commercial applications. Consumers can reconstitute powders when fresh milk is unavailable or the powders can be used for incorporation into products such as chocolate or infant formulas to improve functionality, taste and texture. While whole milk powder (WMP) is used for both consumer and commercial applications, other speciality powders such as milk protein concentrate (MPC) are often used commercially in food manufacture such as cheese making or to increase the nutritional content of food products.

Polysaccharides such as carrageenan, xanthan, and gellan have a large molecular size. They are added to food products for their beneficial effect on the rheological and textural properties (Dickinson, 1998). Xanthan is an extracellular polysaccharide (EPS) produced from microorganisms and imparts a high viscosity with a pseudoplastic nature (Pastor et al. 1994). The incorporation of xanthan polysaccharide into milk solutions and the subsequent spray-drying would yield products with different functional properties to existing powders. If the functionality of the milk protein could be extended by the incorporation of a polysaccharide such as xanthan, the profitability of milk powder manufacture would be markedly increased.

Two xanthan sources were available for the present research. The first is termed 'commercial EPS' which is food grade xanthan produced, purified, and dried by a company. The second is termed 'ferment EPS' and was produced from inoculation of *Xanthomonas campestris* into milk permeate containing hydrolysed lactose which has been shown to produce the xanthan EPS (Yang and Silva, 1995). The properties of xanthan have been shown to differ depending on the growth conditions and processing (Callet et al. 1987; Papagianni et al. 2001). It was expected that the ferment EPS would be different to the commercial EPS in some ways.

The practical application of commercial and ferment EPS into milk powders has not been investigated. Two products specifically targeted in this research were cheese and milk from reconstituted powders. Cheese can be made from reconstituted powder, such as MPC, and milk fat. The process has been used extensively overseas and many different cheeses, such as Camembert and Panela, can be produced. Increasing the yield of cheese by the addition of food polymers is already practiced and can be termed 'cheese extension'. It usually involves the addition of a polysaccharide or denatured protein to the dairy product to increase the yield (Singh et al. 1988; Kailasapathy, 1996). Thus incorporation of the ferment EPS into MPC would be expected to increase cheese yield. In milk drinks made from reconstituted powders, the milk protein can be substituted with polysaccharide to obtain a desired mouth feel or alternatively a thickened milk product can be produced. However, the effect of the incorporation of commercial EPS into reconstituted milk powders has been investigated by Hemar et al (2001), and was found to cause phase separation. Thus it will be necessary to examine whether this still occurs in powders made from milk spray dried in conjunction with a ferment EPS. Ideally the EPS incorporation would produce a powder with reduced protein levels, which upon reconstitution would retain the same, or have improved, functionality for both the cheese and milk product.

The aims of the project were:

1. Determine the functionality of spray dried powders containing commercial EPS and ferment EPS.
2. Produce a milk product with the same textural and sensory attributes as a 15% WMP solution with some of the protein replaced with EPS and to produce a thickened milk product containing EPS at 15% solids with increased viscosity.
3. Investigate the incorporation of EPS into MPC for recombined cheese making to improve whey retention and yield.

1.2 Xanthan Production

Production of the exopolysaccharide, known as xanthan, involves the organisms of the *Xanthomonas* species, namely *Xanthomonas campestris.* The organism produces xanthan provided the right conditions and nutrients are present. For commercial production (Figure 1.1) carbohydrates such as glucose, a nitrogen source and other nutrients are required. The organism is inoculated into the sterilised fermentation medium, aerated and agitated to produce the optimum amount of xanthan. The fermentation medium containing xanthan is heat treated to kill any viable bacteria. The xanthan is then isolated from medium with alcohol precipitation before being dried and crushed to a powder with the desired particle size (Satia, 1982).

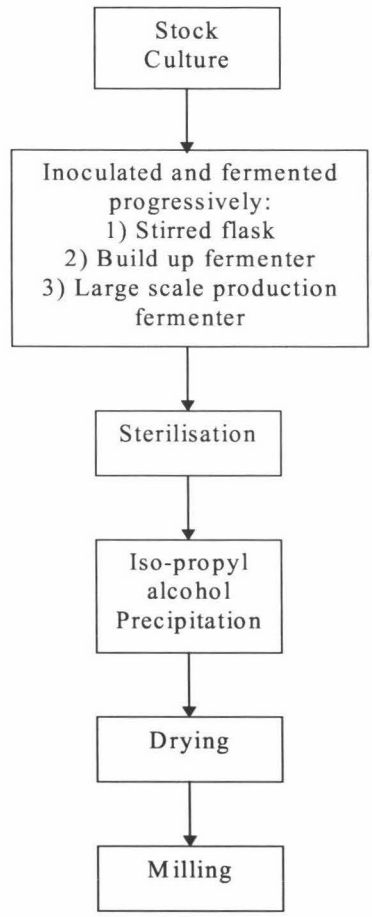


Figure 1.1 Commercial production of Xanthan from *Xanthomonas campestris* (Satia, 1982)

The carbohydrate, lactose, is unsuitable for xanthan production as many strains of *Xanthomonas campestris* poorly utilise lactose (Yang and Silva, 1995). The reason behind the low yield is the low substrate affinity of the β-galactosidase produced by the bacteria for hydrolysis. A potential way to counteract the low affinity is through hydrolysis of lactose before or during inoculation of the organism for xanthan production or the use of a genetically modified strain of *Xanthomonas* (Walsh et al. 1984; Thorne et al. 1988). A readily available source of lactose for xanthan production is milk or whey permeates. The media used influences the product, both compositionally and through interactions of xanthan with media components. Thorne et al (1988) found that a substance present in clarified whey decreased the viscosity of the isolated xanthan. The component was not identified but was precipitated along with the xanthan during isopropyl alcohol precipitation.

1.3 Xanthan Structure

Xanthan is a repeating pentasaccharide structure based on a 1-4 linked β-D-glucose backbone with side chains, comprising β-D-Mannopyranosyl – (1-4) -β-D-glucopyranosyl uronic acid – (1-2) - α-D Mannopyranosyl trisaccharides (Figure 1.2). The side chains can be acetylated at the C-6 portion on the inner mannosic residue. The terminal mannose can be pyruvated, to various degrees, as a sugar ketal at the O-4 and O-6 positions (Jansson et al. 1975; Melton et al. 1976)

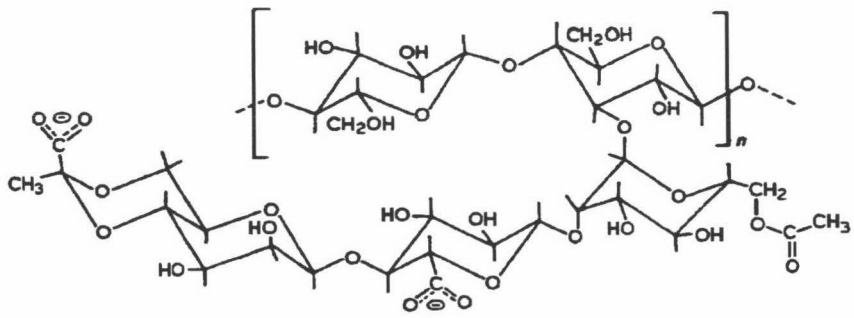


Figure 1.2 Xanthan molecular structure with repeating pentasaccharide unit

Xanthan can exist as a single or double helix with a diameter of 2.4nm and a pitch of 4.7nm (Sato et al. 1984). Xanthan is a polyelectrolyte, allowing for both intra- and

intermolecular interactions. This structure underlies the molecular characteristics including the physical properties of xanthan.

The molecular weight Mw of xanthan has been shown to vary considerably and is dependent upon the method of measurement used. Association between molecules along with molecular composition complicate the determination of Mw. The most commonly found value for the Mw lies within the range 2.0 - 5.0 x 10^6g/mol (Berth et al. 1996; Lecoutier et al. 1986). Xanthan can exist as single or double helices and the relationship of mass to molecular length is twice for double stranded xanthan compared to single stranded, 2000D/nm and 1000D/nm respectively (Stokke et al. 1986)

The stability of xanthan to temperature fluctuations and hydrolysis by acids, alkalis and enzymes is largely due to the orientation of the side chains (figure 1.3). Xanthan in the ordered (native or renatured) conformation has the side chains aligned with the backbone, with the inner mannosyl residue binding to the helical backbone via hydrogen bonds. As xanthan changes to the disordered state, the side chains are released from the inner mannosyl with the side chains being stabilized in the disordered form by steric effects (Morris, 1995). The increase in the mobility of the side chains makes the molecule more prone to acid or enzymic hydrolysis as the side chains no longer shield the backbone of the structure to the same extent (Rinaudo and Milas, 1980; Sutherland, 1984; Sutherland, 1987; Cheetham and Mashimba, 1991; Christesen and Smidsrod, 1996).

Figure 1.3 Xanthan double helical structure with side chains (from Satia 1982)

Pyruvate and acetyl groups are found on the side chains of the xanthan molecule with the proportions changing due to changes to production factors, strain of organism and by chemical means. Irregularities in the side chain sequence may also occur with the occasional one being absent (Sutherland, 1984). The number of pyruvate and glucuronic acid groups present on xanthan affect the charge state and stability of the molecule and hence the repulsion and attractive forces (Callet et al. 1987; Shatwell et al. 1990). Pyruvate groups have a negative charge and as such repulse other pyruvate and negatively charged groups creating a destabilizing effect. Increasing the number of acetyl groups provides a stabilizing effect (Shatwell et al. 1990).

1.4 Xanthan Conformation and Association

1.4.1. Dilute System

While the primary structure of xanthan has been determined there is debate as to the conformation that the molecule adopts in solution. In the solid form xanthan takes a five-fold helical structure (Moorhouse et al. 1977) and with the aid of molecular modelling studies it is evident that the molecule forms a helical structure in solution. The helix is 'hollow' with the folded down side chains partially filling the 'hollow' areas. Non-covalent interactions and hydrogen bonding stabilize the structure (Nussinovitch, 1997; Powell, 1979).

Xanthan potentially exists in 3 conformations native, renatured, and disordered and in the ordered states (native/renatured) is considered to be a semi-flexible molecule (Rodd et al 2000). Native can be defined as the conformation produced during fermentation. While largely in the single stranded form, native xanthan can exist as a double helix or a combination of both (Stokke et al. 1986; Milas et al 1996). Heating above the transition temperature (Tm), which is the temperature where conformational change is observed, or removal of sufficient levels of ions, changes the conformation to the disordered form through increasing steric repulsions. This conformational change is irreversible due to the reorganization of hydrogen bonds (Lecoutier et al. 1986; Muller et al. 1986; Milas et al. 1996). Subsequent cooling below Tm or ion addition transforms the xanthan to a double helical structure known as renatured (Milas et al. 1996).

Once in the renatured form heating above Tm or removal of ions leads to the disordered conformation and a decrease in molecular weight. This was attributed to partial or total dissociation of the double helices in the disordered form caused by electrostatic repulsions. An example is shown in figure 1.4. Cooling below Tm is thought to cause the dissociated molecule to fold back on itself making a double helical conformation. The net result is a halving of the Mw (Milas et al. 1996).

There is a considerable difference between xanthan produced during fermentation and that of powdered commercial xanthan, as most commercial xanthan has undergone a heat treatment and purification process, potentially increasing the viscosity by altering the conformation (Satia, 1982; Rinaudo 2001). The conformation adopted, therefore depends upon the treatment undergone and also the ionic strength, pH and pyruvate content, which may explain the variability in results obtained by researchers (Shatwell, 1990; Christensen and Smidsrod, 1991).

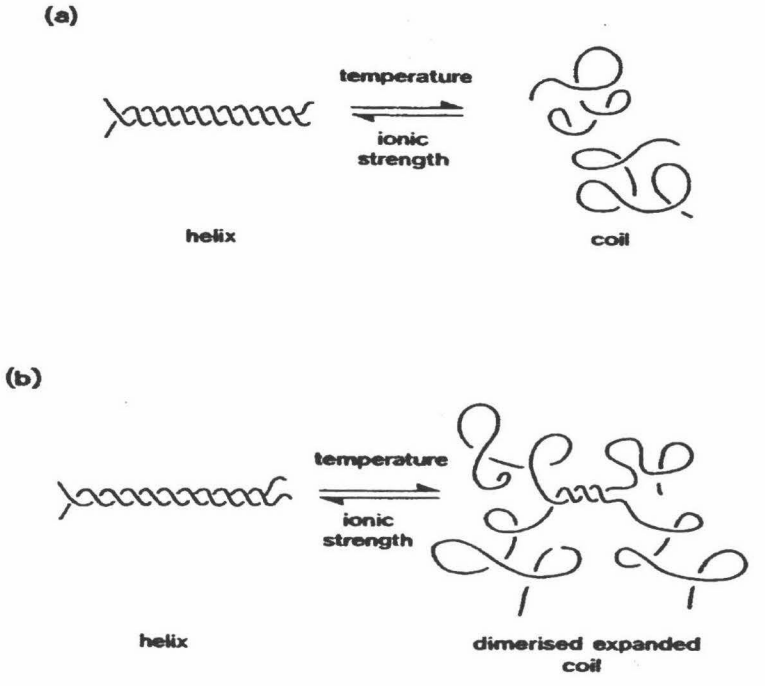


Figure 1.4 Xanthan transition from helix to a) Randomised coil b) Dimerised coil (from Morris, 1995)

When salt is added to xanthan solutions the molecular repulsive forces within and between xanthan molecules are reduced. This stabilises the helical conformation (Young and Torres 1989). A reduction in salinity reduces the Tm for xanthan. However the salt dilution required to obtain the disordered state is very low, approximately 10^{-5}M NaCl at room temperature. As NaCl concentration decreases from 10^{-1} to 10^{-4} M xanthan double strands get progressively elongated. A transition point is reached between 10^{-4} and 10^{-5}M NaCl where the double stranded xanthan dissociates to a single stranded form. Physiological factors such as temperature and pH, along with the pyruvate content can alter the ion concentration required for this conformational change (Lecourtier et al. 1986). Low acetyl contents lead to a decrease in the salinity required for transition as acetyl groups stabilize the ordered form, whereas high pyruvate levels have the opposite effect (Lecourtier et al. 1986; Muller et al. 1986; Shatwell, 1990).

1.4.2. Concentrated System

Xanthan can aggregate in both the ordered and disordered states (Morris, 1995). Aggregation is one of the main reasons for the high viscosities of xanthan solutions, with the degree of aggregation dependent upon shear and temperature (Rodd et al. 2000). How the molecules aggregate depends upon the conformation adopted by the xanthan molecules. Two theories explaining aggregation are shown in figure 1.5 with figure 1.5a showing aggregation of single stranded xanthan associating side by side and figure 1.5b showing a double helical molecule. Partial dissociation of the helices can increase the aggregation (Morris 1995).

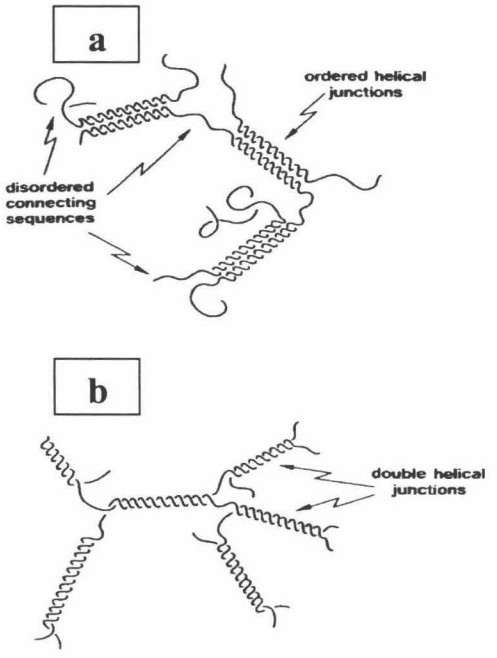


Figure 1.5 Possible aggregating tendencies of xanthan molecules a) single helix, b) double helix (from Morris, 1995)

The degree of association is dependent upon the concentration of solution. Three concentration regions exist; the dilute, semi dilute and the concentrated domain. The concentration required for the transition from the dilute to the semi-dilute region is designated as c*, while c** defines the transition from the semi-dilute to the concentrated domain. Below c* the molecules can diffuse freely and are far apart. At c*, the concentration where molecules begin to physically interact and overlap, there is a sharp increase in shear viscosity due to interaction and aggregation. Increasing the concentration further to c** yields another sharp increase in viscosity

as the molecules arrange to form a uniform distribution due to an increase in interactions and associations (Southwick et al. 1981; Rodd et al. 2000).

The values obtained for c* range from 0.02% and higher and are dependent upon the molecular weight; values of 0.07% were obtained for low molecular weight xanthan (Southwick et al. 1981, Milas et al. 1995; Rodd et al. 2000). The addition of salt decreases the c* for the solution which is thought to be caused by the screening of biopolymer charges (Launay et al. 1997). A value of 0.07% was found for c**, and is also dependent upon the conditions of the system (Southwick et al. 1981; Rodd et al. 2000).

While xanthan is often classified as non-gelling it can exhibit a yield value due to associations between molecules and has been considered gel-like. This gel-like property is evident during oscillatory deformation measurements. The storage modulus tends to dominate the loss modulus indicating that the solid aspects dominate the solution properties for the system (Ma and Barbosa-Canovas, 1997).

Increasing the temperature of solutions containing xanthan has been found to alter the aggregation and rheological properties of solutions in terms of storage and loss moduli. However the degree of change is dependent upon the temperature, the concentration of solution and salinity (Capron et al. 1998). Xanthan undergoing an annealing process can form hydrogels. The storage modulus increases during both the heating (40^{o}C in this case) and cooling process. Increased annealing temperature causes increased dissociation leading to more overlapping and hence a greater number of junction points for hydrogen bonding. Upon cooling a three dimensional network is formed comprising more junctions than were originally present. The storage modulus increases to a lesser extent at lower annealing temperatures, when there is insufficient dissociation between molecules and a weaker overlap (figure 1.6) (Iseki et al. 2001). Salt also impacts upon the storage modulus affecting the Tm point and hence the gel type structure produced (Capron et al. 1998).

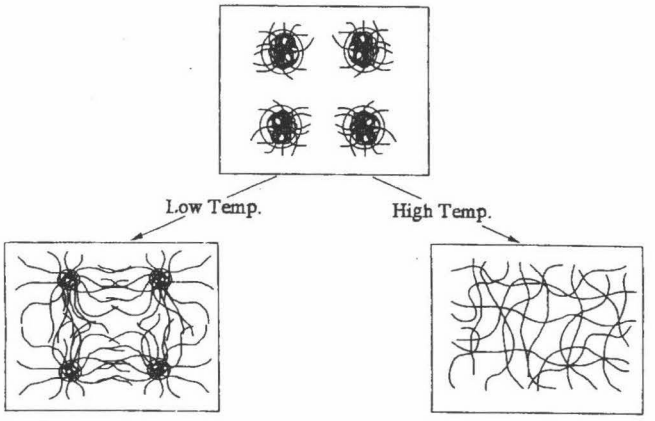


Figure 1.6 Effect of high and low annealing temperatures on the interaction of xanthan molecules (from Iseki et al. 2001).

1.5 Viscosity of Xanthan Solutions

The viscosity of xanthan solutions increases sharply with concentration. At concentrations greater than 0.2% the flow is considered to be pseudoplastic with a yield stress greater than 1Pa (Pastor et al. 1994). Viscosity is dependent upon concentration, temperature and shear. Speers and Tung (1986) developed a power law function to determine the viscosity of a solution for a commercial xanthan EPS sample named Keltrol. The equation was found to be applicable between shear rates of 0.5-3000s^{-1}, concentrations between 0.05-1.00% w/w and temperatures between 5-45°C:

$$\eta = 396.\gamma^{-0.642} C^{1.22} e^{668/T}$$

where η = Apparent viscosity (mPa.s), γ = shear rate, C = Concentration (w/w%) and T = Temperature (°K)

Xanthan tends to aggregate but the aggregation is disrupted by the application of shear, which is partly the reason for the pseudoplastic behaviour of the molecule. The pseudoplasticity is caused by the disruption of molecular interactions between molecules and once sufficient shear is applied to overcome the yield value further reorganizing of the network occurs. Increasing shear leads to the progressive alignment of molecules, decreasing the viscosity until the point where the molecules are fully aligned and a Newtonian domain is obtained. Upon resting, the interactions

can reform and increase the viscosity of the solution (Kang and Pettitt, 1993; Pastor et al 1994, BeMiller and Whistler, 1996). Shear therefore has a large influence upon the viscosity of the xanthan solution over various concentrations as shown in figure 1.7. Low concentration solutions show greater Newtonian tendencies while higher concentration solutions exhibit a greater yield stress (Southwick et al. 1981; Pastor et al. 1994). The size of these domains relies heavily upon the sensitivity of the rheological equipment used for the measurements. The pseudoplastic properties of xanthan are also dependent upon the molecular weight of the molecule as higher molecular weight molecules align more easily under shear than do the lower molecular weight xanthan molecules (Lee and Brant, 2002a; Lee and Brant, 2002b).

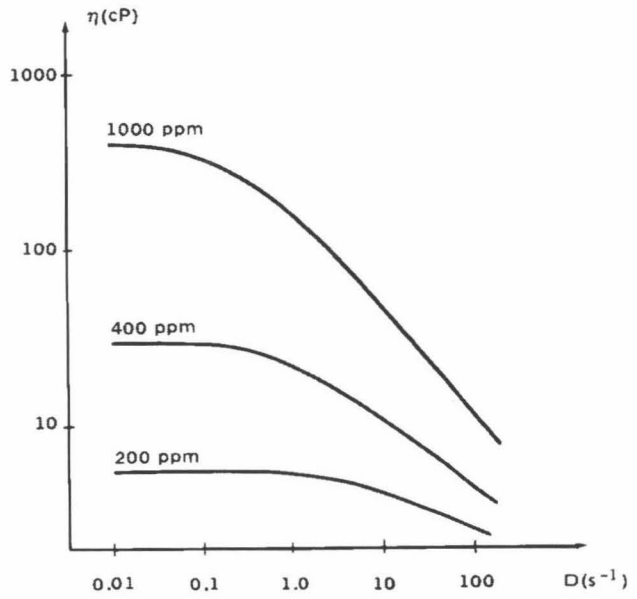


Figure 1.7 The effect of shear rate on the viscosity of various concentrations of xanthan in 0.5% sodium chloride (from Satia 1982)

Xanthan is a polyelectrolyte and as such has a charge dependent upon the pH and salinity. Increasing the ionic strength reduces the repulsive forces between molecules, and tends to decrease the hydrodynamic volume. This leads to a decrease in viscosity of xanthan solutions (<0.2% xanthan) but only up to a certain ion concentration as shown in figure 1.8. This concentration came to approximately 0.1% (Satia, 1982; Lecourtier et al. 1986; Kang and Pettitt, 1993). The effect that salt has on the viscosity of xanthan solutions is also dependent upon the xanthan

concentration. In very dilute xanthan solutions there is minimal interaction between molecules and intermolecular associations are not involved significantly. At higher concentrations of xanthan there appears to be some intermolecular association in the presence of salt. The ions added interact with the charged groups of xanthan decreasing the repulsive charge and promoting interactions between molecules. There is an increase in the pseudoplasticity of the ≥0.25% xanthan solutions with salt (Pastor et al. 1994).

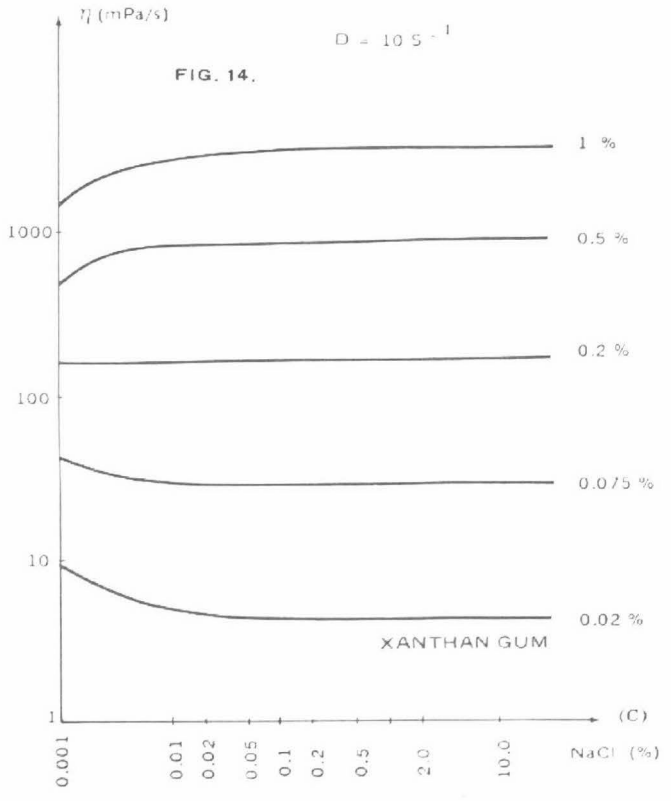


Figure 1.8 Effect of NaCl addition on viscosity for xanthan solutions at a shear rate of $10s^{-1}$ (from Satia, 1982).

The effect of temperature on viscosity is dependent upon the salt and xanthan concentrations. In salt-free, dilute xanthan solutions, increasing the temperature increased the viscosity up to and past the Tm. This can be attributed to a gradual increase in hydrodynamic volume as the ordered structure breaks down. In very dilute salt systems, like salt-free solutions, the viscosity slowly increased, however,

decreased considerably at a temperature just before Tm indicating a complete breakdown of the structure (Holzwath, 1976).

In industrial processes, the heating of xanthan solutions has been found to increase the shear viscosity (Rinaudo 2001). The heating of concentrated xanthan solutions (with 0.1M NaCl) leads to dissociation of xanthan structure once a sufficient temperature is reached. A greater degree of aggregation is apparent upon cooling due to the increase in interactions and junctions of molecules and this leads to a permanent increase in the viscous properties of the xanthan (Oviatt and Brant, 1993).

1.6 Milk Proteins

The major components of milk are water, 87%; fat, 3.9%; lactose, 4.9%; proteins, 3.5%; and ash, 0.7%, with slight compositional variations. The milk proteins can be split into two categories caseins (80%) and whey proteins (20%) (Corbin and Whittier 1965).

The milk proteins known as casein are categorized by their tendency to precipitate at pH 4.6. Caseins are classed into four main groups that include the α_{s1}-, α_{s2}-, β-, and κ-caseins. Casein molecules have hydrophobic and hydrophilic regions and various charged groups (such as phosphate), so that interactions with water, casein, ions and other molecules can occur. One important interaction is between the casein molecules and Ca^{2+} ions. Calcium exists in four forms within milk systems including soluble, ionic, casein bound and colloidal. Ca^{2+} regulates the molecular charge, affecting solubility, and the ability to self-associate and associate with other casein molecules. Association is important as it stabilizes casein within the milk system, ultimately leading to the formation of casein micelles (Walstra and Jenness, 1984a, b & c).

In the presence of Ca^{2+} casein associates forming aggregates with a hydrophobic centre and hydrophilic exterior. These aggregates are termed 'sub-micelles'. Due to the very hydrophilic charged group (carboxylic acid) on κ-casein, this molecule predominately exists at the exterior of the sub-micelle with other caseins occupying both the exterior and interior. κ-Casein is not always present on sub-micelles. The

sub-micelle size is still unknown, however diameters of 0.010-0.020μm are estimated (Walstra and Jenness, 1984b).

In the presence of calcium phosphate the sub-micelles aggregate to form micelles. The sub-micelles arrange themselves so that the more hydrophobic sub-micelles are near the centre while the hydrophilic micelles are near the exterior. The exterior sub-micelles contain a higher proportion of k-casein, which protrudes from the exterior forming a charged 'hairy' outer layer. This structure provides stability for the micelles, minimising the number of hydrophobic groups exposed to the aqueous phase, and repulsing other casein micelles. The micelles are estimated to range in size from 0.02 to 0.3μm in diameter, however larger diameters of 0.8μm have been reported. Casein micelles have a high voluminosity and a high proportion of calcium phosphate (~8g/100g casein) (Walstra and Jenness, 1984b).

The micelle structure has yet to be conclusively determined. The micelle formation described is considered to best-fit observations of casein micelles (Walstra and Jenness (1984b). Other explanations are discussed by Rose (1969), Slattery and Evard (1973) and Schmidt (1980).

While casein precipitates at pH 4.6 whey proteins are still quite soluble at this pH. Whey proteins comprise mainly β-lactoglobulin, α-lactalbumin, serum albumin and proteose-peptones (largely proteolytic products from caseins). In the native state, most whey proteins exist as globular molecules with hydrophobic components folded towards the centre. These proteins are affected by the heat treatments which cause denaturing and unfolding of the protein structure. Some whey proteins do not exist in the globular form and, therefore, are not affected in the same way by heat treatments (Walstra and Jenness, 1984a).

1.7 Milk Powders

Milk powders are classed according to the whey protein nitrogen index, which varies according to the heat treatment given to the powders. Three categories exist: low-, medium- and high-heat powders. All milk before spray-drying undergoes pasteurization while milk for high heat treatment undergoes a considerably larger

degree of heating including 110-120°C for 1-3 minutes. This increases the amount of denatured protein present in the final powder (Caric, 2003). Pasteurised milk is evaporated to approximately to 40-50% solids before spray-drying to minimize the costs (Caric, 1994). The sample to be dried passes to an atomizer that allows the milk to be broken up into extremely small droplets. Surface tension causes the formation of a spherical shape, which the droplet generally holds upon drying. The particles are sprayed into a drying chamber where they hit air heated to approximately 180-200°C under very high convection. Loss of moisture in the form of vapour from the surface of the particle removes much of the heat energy adsorbed. This increases the salt concentration at the surface of the particle, so water diffuses from the centre under the osmotic gradient. Particles ranging between 10-250μm in size are produced depending upon the conditions. The drying of the surface and the increase in dry matter content reduce the ease of evaporation. After drying the powder is collected and packaged (Caric, 1994).

The optimum heat pattern in the spray drier depends upon the droplet size, with problems ensuing if incorrect treatments are used. Excess heating in the later stages causes fat migration to the surface of the dried particle and denaturation of the whey protein, which can affect solubility. Insufficient heating and retention of more than 5.7% moisture can cause lactose crystallization and caking (Caric, 1994). Maillard reactions can occur in powders with the right conditions (BeMiller and Whistler; 1996). There is some product variability between spray dryers due to the differences in the physical nature of the equipment. Scale up from laboratory to industrial equipment can result in a different product (Masters, 1976). Masters (1976) stated 'the important powder characteristics are flowability, wettability, sinkability, dispersability and solubility'.

Milk protein concentrate (MPC) is a powder produced by the ultrafiltration (UF)/diafiltration of skim milk and subsequent spray-drying (figure 1.9). The UF process concentrates the casein and whey protein present to the desired protein concentration relative to the other solid constituents, namely lactose and minerals. Following UF the concentrated solution is evaporated to the desired solids concentration and spray dried producing powders with protein concentrations usually

ranging from 56 - 85%. As casein micelles are relatively undamaged MPC can be used for the manufacture of cheese or for the incorporation into other products to increase their nutritional properties. The casein levels of the MPC powders can also be changed via ion exchange after UF.

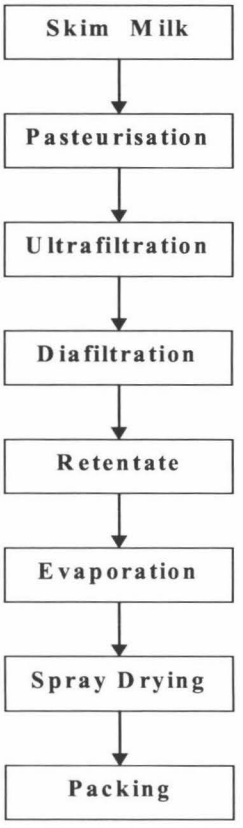


Figure 1.9 Flow diagram of MPC production

1.8 Recombined/UF Fresh Cheese Manufacture

Fresh cheese is commonly consumed in Latin America and other parts of the world. Fresh cheeses such as Panela have minimum lactose fermentation as no starter organism is added and no ripening period is needed with proteolytic enzymes being used to coagulate the casein. The cheeses are characterized by their high pH, high moisture and high water activity compared with mature cheeses. These attributes yield a short product life, which is prolonged by the addition of salt and a pasteurisation step before manufacture. In the case of Panela, skim or whole milk is commonly used (Phelan et al. 1993). Fresh cheese, traditionally made from milk, has considerable whey loss with syneresis being controlled by first order kinetics (Castillo et al 2000).

Certain fresh cheeses made from recombined milk with added anhydrous milk fat have poor organoleptic properties compared to cheese made from fresh milk, largely due to the anhydrous fat flavours or the spray-drying process. Unpleasant flavours are less apparent with recombined milk containing no anhydrous milk fat. However, in the production of Queso Blanco 'acetic acid' flavours were present to a higher degree when produced from recombined milk rather than fresh milk (Gilles and Lawrence, 1981). When fresh cheese is made by UF or the use of recombined milk protein concentrates, whey loss can be reduced or eliminated, thus retaining the whey proteins and other solid constituents. Heating of milk before acidification leads to denaturation of whey proteins which alters the structure of the cheese produced due to incorporation of whey proteins into the cheese matrix (Marshall, 1986). Retention of the whey protein is often desirable for cheese as the increase in yield may surpass the ill effects of a decrease in curd firmness and the influence on rennet coagulation (Singh et al. 1988; Singh and Waungana, 2001). The addition of $CaCl_2$ can reduce these ill effects (Singh et al. 1988; Wolfschoon-Pombo, 1997)

A difference also exists in the casein matrix. Upon heating, spray-drying and reconstitution the casein structure can be changed which affects the fat and casein interaction tendencies with the fat potentially limiting the extent of casein coagulation. Quality can be improved by the use of low-heat treated powders for cheese making as the reduction in heating reduces deterioration of organoleptic properties and rennetability. These powders contain less denatured whey proteins, and the micelles contain a greater amount of ionic calcium, which are essential attributes for cheese quality (Muldoon and Liska, 1972; Walstra and Jenness 1984b, Shaker and Gilles, 1990).

Calcium addition increases interactions between casein micelles due to Ca^{2+} binding and a decrease in repulsive charges, ultimately increasing aggregation tendencies and changing the rheological properties of cheese and gel systems (Walstra and Jenness, 1984b & c; Wolfschoon-Pombo, 1997). Heating of milk during spray-drying or other treatments affects the calcium distribution throughout the system with high heat treatment reducing the levels of ionic calcium hence addition of calcium is often required for cheese making (Muldoon and Liska, 1972; Singh et al. 1988).

1.8.1. Coagulation by Rennet

Coagulation is a process required for cheese making and involves enzymic and flocculation stages. κ-Casein is susceptible to hydrolysis by endopeptidases, such as chymosin which is commonly used for cheese making. Rapid hydrolysis by chymosin has been shown to occur at the Phe (105)-Met (106) bond forming two molecules; insoluble para-κ-casein (1-105) and soluble caseinomacropeptide (CMP 106-169). This hydrolysis largely occurs on the 'hairy' layer at the exterior of the casein micelle. CMP 106-169 disperses into the solution while the para-κ-casein 1-105 remains attached to the micelle (Walstra and Jenness, 1984b). Flocculation of aggregation begins once approximately 85% of κ-casein has been hydrolysed (Dalgleish, 1983). The casein micelles form a network. This network gradually rearranges itself over time so the micelle structure changes and the calcium phosphate is redistributed throughout the network (Walstra and Jenness, 1984b).

1.8.2. Cheese Syneresis

The casein network rearranges itself over time, increasing strand thickness, and causing syneresis through aggregation and contraction. Syneresis is the loss of the aqueous phase and solid components including whey proteins, lactose, and salts. The syneresis mechanism however is not fully understood (Lomholt and Qvist, 1999; Ramet, 2000). The degree and rate of syneresis is determined by the composition and porous structure. Aqueous loss is dependent upon the physical ability of the whey to vacate the network while contraction pressure provides the driving force for the process (Castillo et al. 2000). The reduction of the aqueous phase and the strengthening of the casein network are accompanied by an increase in gel firmness and hence the gradual hardening of the cheese.

The fat content of the cheese, and also the pH affects syneresis. The effect of fat is largely physical. The fat emulsion droplets limit the aqueous flow through pores, and by interfering with the casein interactions, reduce the contraction pressure (Parnell-Clunies and Bullock, 1985). The repulsive forces between caseins are reduced as the pH decreases toward the casein isoelectric point of 4.6. This increases the degree of aggregation and expels more of the aqueous phase (Walstra and Jennes 1984b). The decreasing pH decreases the hydration of casein and increases the solubility of

calcium and phosphate that were previously present within micelles. The combined effect is an increase in syneresis through increasing contraction pressure (Patel et al. 1971). The effect of $CaCl_2$ addition to renneted milk gels is partially due to the effect of a decreasing pH (Hill et al. 1982). However, $CaCl_2$ addition, under certain conditions, can increase the aggregation tendencies of the cheese and hence the contraction pressure (Lucey and Fox, 1993)

The heat treatment of the cheese milk can also affect syneresis. Although native whey proteins present in milk have minimal impact on the rate of syneresis, heat treatment can denature these whey proteins and allow them to interact with other proteins affecting syneresis. Denatured β-lactoglobulin and α-lactalalbumin interact with κ-casein via the disulfide bonds on the micelle surface forming a complex. This complex formation increases the rennet coagulation time and interferes with casein contraction (Wheelock and Kirk, 1973; Mohammed and Fox, 1987; Singh et al. 1988; Singh and Waungana, 2001). This interference decreases the extent of syneresis.

The temperature during the aggregation and the setting stages also effects syneresis. Higher temperatures increase both the permeability of the network while decreasing the viscosity of the aqueous phase. This causes an increase in syneresis (Lomholt and Qvist, 1999).

1.8.3. Cheese Rheology

Cheese firmness is dependent upon the constituents and degree of syneresis with curd/cheese continuing to firm with the loss of aqueous phase (Daviau et al. 2000). Formation of the casein network is very important with interference in the aggregation and coagulation process potentially producing a softer cheese. Pastorino et al (2003) stated that 'anything that changes the ability of the proteins to interact with water or other proteins can also influence cheese adhesiveness' and found that diminishing interactions decrease the elasticity and cohesiveness of Muenster cheese. Fat and denatured whey proteins can interfere with aggregation tendencies of casein, an effect that is more pronounced upon homogenisation due to the increase in the number and surface area of fat agglomerates. The complex formation of whey proteins with κ-casein limits casein aggregation and also produces a weaker cheese (Parnell-Clunies et al. 1985, Schreiber and Hinrichs, 2000). The final cheese strength has been found

to be very dependent upon the protein content with an increasing protein concentration increasing the firmness of the cheese (Garnot et al. 1982; Mietton, 1990).

pH and the ionic strength are critical for cheese firmness affecting the degree of interactions. $CaCl_2$ addition has been shown to increase the firmness at low levels by increasing the aggregation tendencies of caseins. This is especially noticeable with cheese made with recombined milk due to the reduction in ionic calcium (Muldoon and Liska, 1972; Shaker and Gilles, 1990; Lucey and Fox, 1993). NaCl increases the hardness of the cheese up to a certain level (~100mM) (Lomholt and Qvist, 1999), which is thought to be due to the promotion of interactions between proteins. Recent research, however, has found that the interactions between proteins decrease and protein/water interactions increase upon the addition of salt. Therefore, the increase in hardness is most likely caused by the swelling of the protein matrix due to increased hydration producing thicker strands, counteracting the adverse effect of the increasing protein solubility (Pastorino et al. 2003).

The crucial factor with regard to cheese firmness is the consistency of the cheese. A uniform cheese dissipates the stress throughout the sample compared to an uneven cheese. It, therefore, is vitally important to ensure cheese consistency throughout production as disruption to the matrix and formation of an uneven curd caused by stresses, cutting, pressing and sample removal can lead to variability in test results and consumer perception (Marchesseau et al. 1997).

1.9 Xanthan Incorporation

1.9.1. Milk

Reconstituted dairy powders such as SMP, MPC and WMP contain whey proteins and casein micelles. The addition of even low levels of xanthan dominates the rheological characteristics of the solution. The rheological properties of milk/xanthan systems mimic those of pure xanthan with a Newtonian domain at low shear stress with pseudoplastic tendencies becoming more evident as shear stress is increased (Schmidt and Smith, 1992; Hemar et al. 2001).

In solutions containing casein micelles and xanthan, phase separation occurs which involves the formation of spherical protein-rich aggregates surrounded by the xanthan phase. The degree of protein aggregation is reliant upon casein being relatively undamaged, as is the case with standard pasteurisation and spray-drying processes. The extent of phase separation is dependent upon the viscosity and concentration of both xanthan and casein micelles. The mechanism responsible is thought to be depletion flocculation in which xanthan particles are excluded from the space between casein particles. This causes a polymer concentration difference between the internal and outside particle regions creating an osmotic pressure difference between the two regions, the exodus of water from between the micelles and the flocculation of casein micelles (Hemar et al. 2001).

The polysaccharide, carrageenan, has been studied extensively in dairy systems and is used as a stabiliser and thickener in dairy products. Carrageenan interactions differ from xanthan in dairy systems due to the presence of $-OSO_3-$ groups (sulphate) which have stronger attraction for the NH_3^+ groups on protein, compared to the $-CO_2$ groups (carboxylate) present on xanthan (Dickinson, 1998). Xanthan as a milk stabiliser or thickener is not as effective as carrageenan because the weaker interactions lead to phase separation at lower polysaccharide levels (Hemar et al 2001). Carrageenan/casein systems can also experience phase separation as at relatively high temperatures depletion flocculation was apparent when sufficient levels of carrageenan were added (0.2%) independent of protein concentration (Langendorff et al. 1997).

Phase separation also occurs in denatured whey protein/xanthan solutions through thermodynamic incompatibility, a different mechanism to the casein/xanthan systems. The mechanism results in two layers with a high proportion of xanthan in the upper layer. The separation is not apparent, or occurs to a far lesser extent with native protein/xanthan solutions (Bryant and Mclements, 2000).

1.9.2. Fresh Cheese

With a majority of cheeses a culturing time is required to allow the development of flavours and textures of cheese. Microorganisms and enzymes have an important role in this development assisting in syneresis, breaking down constituents and

reordering networks. Losses occur during the cheese-making and culturing process and increasing cheese yield through whey and polysaccharide incorporation is important for recouping some of these losses. The benefits of increasing cheese yield must outweigh any negative effect on the consumer's appreciation.

The addition of polysaccharide can reduce the loss of whey proteins, water and other constituents from cheese through syneresis and potentially make a product more appealing to the consumer (Keogh and O'Kennedy, 1998). The reduction of syneresis is likely caused by the ability of the polysaccharide to bind water and solid constituents and to limit the flow of the aqueous phase through the cheese. Kailasapathy (1996) found that the addition of the polysaccharides, carrageenan and gellan to cheddar and cottage cheese increased the yield during the cheese making process. A decrease in syneresis and the formation of a matrix preventing the loss of solid components were thought to be responsible.

Consumers can detect the addition of polysaccharides to milk products at low concentrations due to the physical properties of water binding and markedly increased viscosity, caused by the formation of ordered networks (Nussinovitch, 1997). These networks lead to an increase in residence times of product constituents in the mouth and alter the texture and flavour of cheeses produced, however this is dependent upon the type of polysaccharide or cheese and the polysaccharide concentration (Duboc and Mollet, 2001). Work conducted by Kailasapathy (1996) found, from sensory trials, that the flavour of cheddar cheese containing 500ppm of carrageenan was significantly different to control cheddar cheese. Cheese containing gellan at the same levels, however, rated better than the control. Cottage cheese containing carrageenan tested at 1000ppm was rated less liked overall compared to the control. Flavour is also altered by volatile retention through the encapsulation or binding of volatile compounds by polysaccharide (Ramirez-Figueroa et al. 2002).

The effect of xanthan incorporation on the consumer appreciation of cheese is dependent upon how the polysaccharide interacts with protein, cell debris, water and other constituents. Xanthan alters the homogeneity and morphology of the casein network affecting both the textural properties and the perceived flavour (Walkenstrom et al. 1998). The addition to cheese can decrease the firmness through an increased

moisture content and interference with casein coagulation. With a skim milk/xanthan acid induced gel, separation between casein and xanthan was apparent, and caused a more fibrous and porous structure with increasing xanthan concentration (Sanchez et al 2000). The incorporation of xanthan evidently interfered with the interaction and homogeneity of the casein network. Thus the improvement of cheese yield may be offset by phase separation and rheological changes in the system.

1.10 Conclusion

This project is focused on spray-drying milk solutions containing xanthan EPS produced from the action of a microorganism on milk permeate. The aim is to produce cheese and milk products from the spray dried powders with improved functionality and a decreased protein content. Important aspects found in literature included:

- Inoculation of *Xanthomonas campestris* into milk permeate containing hydrolysed lactose will produce a xanthan EPS.

- Properties of the xanthan produced are dependent on production methods including media constituents, fermentation conditions, and the heat treatment given. As such the properties of the xanthan produced from milk permeate will likely differ to commercially produced xanthan.

- Incorporation of xanthan into milk systems imparts a pseudoplastic nature and a substantial increase in viscosity. The addition of low levels of EPS can therefore substitute for a reduction of milk protein while retaining or increasing the viscosity.

- Phase separation occurs in casein/xanthan systems and will likely occur in the reconstituted milk powders containing xanthan. As phase separation is driven by an osmotic pressure difference the extent of phase separation will be dependent upon casein and xanthan concentrations.

- Xanthan incorporation into fresh cheese will increase the yield. This will decrease the amount of protein required for cheese production.

- Organoleptic attributes of fresh cheese may be affected by the incorporation of xanthan. The disruption of the casein matrix through phase separation could also affect the texture.

CHAPTER 2 MATERIALS AND METHODS

2.1 Materials

2.1.1. EPS sources

Food grade xanthan (Keltrol) was purchased from CP Kelco, San Diego, USA and was termed 'commercial EPS'.

Broths produced from the fermentation of *Xanthomonas campestris* strain 13951 on lactase hydrolysed milk permeate were produced by Dr. Alan Welman at the Fonterra Research Centre, Palmerston North, New Zealand. The lactase hydrolysed milk permeate medium contained K_2PO_4 (0.20% w/v), $MgSO_4.7H_20$ (0.01% w/v), and either urea (0.1% to 1.0% w/v) or yeast extract (1.0% w/v) depending upon growth trials. Yeast malt peptone agar was inoculated with the organism, and added to the milk medium at the 5% level. The fermentation broth produced was termed 'Ferment EPS'.

Dr. Alan Welman also produced the broth to mimic milk permeate medium for determination of p-cresol evolution. The broth was produced from the fermentation of *Xanthomonas campestris* strain 13951 on medium containing glucose (1% w/v), galactose (1% w/v), K_2PO_4 (0.24% w/v), $MgSO_4.7H_20$ (0.012% w/v), and urea (0.12% w/v). Yeast Malt peptone agar was inoculated with the organism, and added to the medium at the 10% level.

2.1.2. Food Grade Ingredients

Milk protein concentrate, containing approximately 70% protein (ALAPRO 4700), skim milk powder and whole milk powder were supplied by Fonterra Co-operative Group Ltd. New Zealand.

Fresh frozen milk fat for recombining (FFMR) contained 99.95% milk fat and 0.05% water and was obtained from Fonterra Co-operative Ltd. New Zealand.

Rennet contained 540 IMCU/ml and was provided by Christian Hansen. L - Calcium lactate was provided by Purac Biochem bv, Netherlands. Lactic acid containing 88% lactic acid was supplied by Archer Daniels Midland Company, Illinois, USA. Sodium Chloride (NaCl) was provided by Pacific Salt, Mt Maunganui, New Zealand and contained anticaking agent 536.

2.1.3. Chemicals

Glucose, calcium lactate, concentrated sulphuric acid, lactic acid, and phenol were all of analytical grade and supplied by BDH Laboratory Supplies, Poole, England. Silicone Antifoam Agent (30% w/w silicone) was supplied by BDH Chemicals Ltd., Poole, England. Ethanol comprised special denatured alcohol SDA-3A containing 20ml/L methanol and was supplied by Australian Solvent and Chemicals Co. Pty Ltd. Flavourzyme was supplied by Chemcolour Industries (NZ) Ltd, Auckland, New Zealand. Fast green was supplied by FCF Merck, Darmstadt, Germany and Nile Blue by BDH Chemicals Ltd., Poole, England. The 2,3,5,6-tetradeutero-4-methylphenol standard was provided by the Fonterra Research Centre, Palmerston North. Norit Granular Activated Carbon 1240 was produced by Norit Nederland B.V. Amersfoort, Netherlands.

2.2 Preparation of Milk Powders Containing EPS

Reverse Osmosis (RO) water was heated to 50°C. Under constant agitation with a Heidolph RZR 50 overhead stirrer, MPC, SMP or WMP was added to the desired solids concentration and mixed for 30 minutes. At this point, if required, reconstituted commercial EPS or ferment EPS was added to produce the powders containing the desired EPS content and was mixed for 10 minutes before proceeding. The sample was homogenised for 5 minutes with a Silverson laboratory mixer/emulsifier at low speed. The sample was further mixed for 20 minutes at 50°C with a Heidolph mixer, and homogenised with the Silverson mixer for 10 minutes. A 300μm mesh sieve was used to remove any lumps. Any lumps present were crushed using a pestle and mortar and mixed into the solution, which was sieved again.

The solution was maintained at 50°C in a water bath before passing through an Anhydro Lab S1 Spray Dryer (Denmark) with the following parameters:

Inlet temperature = 180°C

Outlet temperature = 80-90°C

The feed rate was altered to ensure the outlet temperature was kept constant. The powder was vacuum sealed in light- and moisture-impermeable bags.

2.3 Powder Analysis

2.3.1. Determination of Moisture Content

The method used was that of Winger at al (1997). Moisture dishes were placed in an oven at 100 ± 2°C for 1 hour. The dishes were removed and placed in a desiccator containing silica gel for one hour and allowed to cool. 2-5g of sample was weighed into the dishes using Mettler AE 200 scales to 4 decimal places in triplicate, then placed in an Eyela vacuum oven-300SD at 70°C for 5 hours. The samples were reweighed and the percentage moisture was calculated using the formula.

$$\text{Moisture Content (\%)} = \frac{\text{(original total weight - total dry weight)}}{\text{original sample weight}} \times 100$$

2.3.2. Solubility Analysis

(Adapted from International Dairy Federation 1989)

1. Three 50ml centrifuge tubes were dried for 1 hour in 100 ± 2°C oven, placed in desiccator for 1 hour to cool and then weighed (***Wc***).
2. Portions of 5g MPC, 10g SMP or 13g WMP were weighed into beakers (***Wp***)
3. RO water (10ml) was added to a Waring blender followed by the powder. RO water (85ml for MPC, 80ml for SMP or 77ml for WMP) was used to rinse the beaker that contained the powder before being poured into the blender followed by three drops of prepared antifoam solution (5% antifoam agent, 95% RO water - Mixed in waring blender for 2 minutes).
4. The solution was blended for 2 minutes on high speed and poured in beaker. The sample was left 1 minute then sprayed with ethanol to reduce foam using a spray bottle, left to rest one minute then sprayed again.
5. The sample was left a further 13 minutes, stirred with spatula and 30g was poured into 50ml centrifuge tubes in triplicate.

6. Centrifugation of the samples was carried out at 2,600 relative centrifugal force (RCF) for 10 minutes, using a BHG Hermule Z320 centrifuge.
7. The supernatant was removed by vacuum suction until the 5ml mark was reached or until 3ml above the sediment. The sample was made up to the 45ml with RO water and stirred with wire to redisperse the sediment.
8. Centrifugation was carried out at 2,600 RCF for 10 minutes.
9. Using vacuum suction, the supernatant was removed until the sediment was reached (without removing any sediment).
10. The centrifuge tubes were dried in a 100°C oven for 12 hours, left in desiccator for 1 hour and reweighed (***Ws***)

Weight of centrifuge tube = ***Wc***
Weight of Dry Sample and tube = ***Ws***
Weight of powder = ***Wp***

$$\text{Solubility Index (g/100g dry powder)} = \frac{Ws - Wc}{Wp} \times 100$$

2.3.3. Determination of EPS

2.3.3.1 Preparation of standard curves for xanthan and glucose

Glucose and commercial xanthan (Keltrol) standard curves were prepared for EPS determination. Solutions were made containing 0.1g/L glucose and 0.1g/L xanthan in RO water with the samples being diluted to produce varying concentrations. Phenol/sulphuric assay was conducted and the plots of absorbance at 485nm vs. concentration were made. The multiplication factor was determined from the gradient and was used for the EPS concentration determination. For each EPS determination glucose standards were included.

2.3.3.2 Preparation of powder for phenol/sulphuric assay

1. Milk powder (4g, $\mathbf{P_w}$) was homogenised with RO water (50ml, $\mathbf{P_d}$) in a Laboratory tissue homogeniser. The control powder was processed at the same time for use in the calculations.

2. The homogenised sample (5g, S_w) was added to a round bottom centrifuge tube
3. Flavourzyme was added (0.05g for SMP and WMP, 0.15g for MPC) and incubated at 51°C for 2 hours.
4. The samples were heated to 95°C for 10 minutes.
5. Ethanol (10ml) was added, mixed thoroughly and centrifuged at 23,900 RCF for 20 minutes using the RC5C- Sorvall Instruments centrifuge.
6. The tubes were decanted, inverted and left to drain.
7. RO water (5ml) was added to dissolve the pellet and the sample was heated if required to 95°C.
8. Ethanol (10ml) was added, mixed thoroughly and centrifuged for 15 minutes at 23,900 RCF.
9. The sample was decanted, inverted and left to drain.
10. RO water was added to dissolve the pellet to the required dilution factor (D_f) (usually 100 or 200).
11. Phenol/sulphuric assay was carried out and the control was subtracted from the readings for the calculation.

2.3.3.3 Phenol/sulphuric assay

Triplicate aliquots of diluted EPS (1ml) were added to clean test tubes (for control 1ml RO water was used). Phenol (1ml of 5%) solution was added and vortexed, followed by addition of 5ml concentrated H_2SO_4 and vortexed. The solution was left for 30 minutes for colour to develop. Absorbance was read at a wavelength of 485nm with the control as the reference. The EPS concentration was calculated by:

P_d =Powder Dilution

P_w =Powder Weight

D_f =Final dilution Factor

S_w =Solution weight

A =Absorbance

y =Standard curve gradient:

Glucose = 0.0092, Xanthan = 0.0078.

$$\text{EPS Concentration (g/kg)} = \frac{A \times P_d \times D_f \times 1000}{y \times P_w \times S_w \times 1000000}$$

2.4 Determination of EPS in the Ferment

1. Ferment (1g, **Fw**) was weighed into a 10ml centrifuge tube and heated to 95°C for 10 minutes.
2. Ethanol (2ml) was added and vigorously mixed.
3. Centrifugation of the sample was carried out at 2,600 RCF for 20 minutes.
4. The tube was decanted then inverted and left to drain.
5. RO water (1.5ml) was added to dissolve the pellet, heating to 95°C if required.
6. Ethanol (3.5ml) was added and mixed thoroughly.
7. Centrifugation was carried out at 2,600 RCF for 15 minutes.
8. The sample was decanted, inverted and left to drain.
9. The pellet was dissolved in RO water to the required dilution (**Df**) (usually 100-200ml)
10. Phenol sulphuric assay was carried out on the diluted sample.

D_f = Dilution Factor

F_w = Ferment Weight

A = Absorbance

y = Standard curve gradient:

Glucose = 0.0092, Xanthan = 0.0078

$$\text{EPS Concentration (g/kg)} = \frac{A \times D_f \times 1000}{y \times F_w \times 1000000}$$

2.5 Powder Functionality

2.5.1. Phase Separation

WMP and SMP containing commercial and ferment EPS were reconstituted for one hour to 10, 15 and 20% total solids at 20°C. WMP and SMP controls were made to the same concentrations and the solutions were mixed for two minutes with a Heidolph DIAX 600 homogeniser at 8000rpm. Control and EPS containing solutions of the same total solids concentration were mixed to obtain the desired EPS concentration while maintaining the total solids content. The EPS concentrations in the final solutions were: 0%, 0.002, 0.008, 0.013, 0.018, 0.023, 0.075, 0.127, 0.18, and 0.231%. 10ml of solution was placed in graduated test tubes and homogenised

again for 40 seconds at 8000rpm. The samples were sealed, held at 5°C, and monitored for phase separation and flocculation every 24 hours minimum, for a total of 72 hours.

2.5.2. Viscosity Measurements

2.5.2.1 Preparation of solutions

The control and EPS containing powders were mixed to attain varied EPS concentrations. The measured powder was gradually added to RO water to obtain the desired total solids concentration and was mixed for 1 hour. The viscosities were measured using either the Rheometric Scientific SR-5000 viscometer with a cone and plate attachment or a Ferranti-Shirley Viscometer.

2.5.2.2 Ferranti-Shirley

A Ferranti-Shirley Viscometer system with a cone (diameter = 100mm, angle = 0.3°) and plate at 25°C was used. Viscosities were plotted as a function of concentration at the desired shear rate.

2.5.2.3 Rheometric Scientific SR-5000

SMP and WMP were reconstituted at EPS and total solids concentrations where phase separation was visible with ferment EPS but not with commercial EPS. A Rheometric Scientific SR-5000 instrument with a plate and cone with a 40mm diameter and an angle of 0.04 radians was used. Steady stress sweeps were performed with a 60 second delay before the test with an initial stress of 0.06Pa and a final stress dependent upon the viscosity of the sample. A gap of 0.05mm was used with the temperature being held at 5°C to mimic the temperatures for phase separation trials. Pure ferment and commercial EPS and control WMP and SMP were also tested. Viscosities were plotted as a function of shear rate for the EPS concentration tested.

2.5.2.4 Dynamic frequency sweep (DFS) test

DFS were carried out with a Rheometric Scientific SR-5000 instrument with a plate and a cone with a 40mm diameter and a 0.04 radian angle. Initial tests were preformed with a 60 second delay with the strain controlled at 2% and a frequency

range of 10 - 0.01Hz. Pure ferment and commercial EPS of the same concentration were measured at a temperature of 20°C.

2.6 Cheese

2.6.1. Manufacture of Recombined Panela

Panela is a fresh cheese eaten in Mexico. While often made from fresh milk Panela can be made from reconstituted milk powders such as MPC. Panela made from reconstituted MPC containing commercial or ferment EPS formed the basis of the work conducted. The Panela recipe used was obtained and adapted from the Fonterra Research Centre, Palmerston North.

The cheese making process involved a series of steps (figure 2.1). The milk fat (emulsion) and reconstituted MPC (milk base) were prepared separately to conserve the powders containing EPS otherwise, homogenisation of the final sample would have caused substantial losses from the small batch sizes produced. Homogenisation of the emulsion before combining with the milk base eliminated this loss.

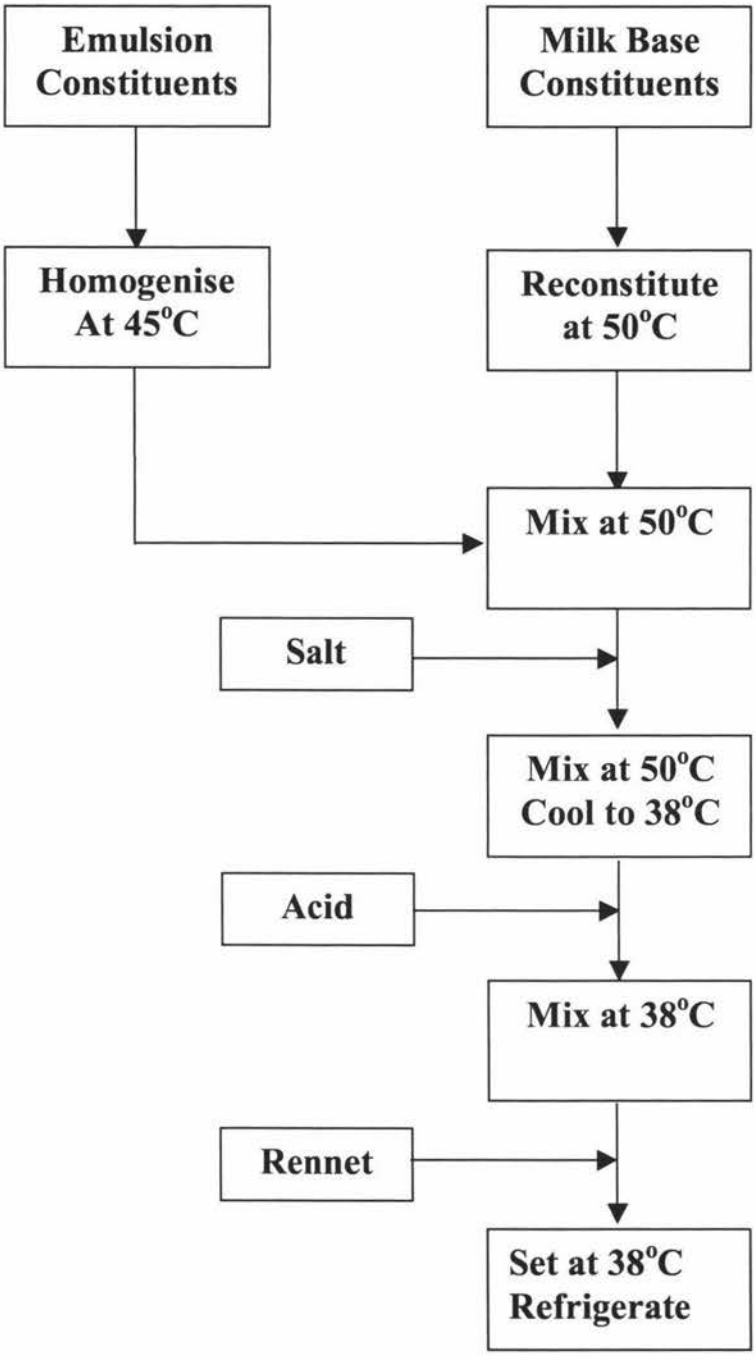


Figure 2.1 Adapted Panela Cheese Making Process

For emulsion preparation 480g RO water was heated to 50°C. Control MPC (20g) was added and mixed for 30 minutes. FFMR (500g) was melted and added to the MPC solution and homogenisation was carried out in a Silverson mixer at low speed until the mixture appeared homogeneous. The sample was then passed through a two-stage Rannie homogeniser at 45°C. The pressures used were 70 and 50 bar for the first and second stages respectively.

For the milk base RO water was heated to 50°C. With a pestle and mortar (to remove air and ensure minimal lumps) the MPC and water were ground to a paste making a solid concentration of 25.6% (The quantity varied depending on the amount of cheese required). The MPC solution was mixed with a Heidolph RZR 50 overhead stirrer for one hour at 50°C. The sample was passed through a 300μm sieve to remove any lumps or foam. Any lumps present were ground with a pestle and mortar and added to the solution, which was mixed and sieved again with a 300μm sieve.

The emulsion, milk base and other cheese constituents were combined using the cheese making method detailed below. While batch sizes varied, the proportions of different constituents were kept constant. To obtain varied amounts of EPS in the cheese the emulsion was mixed with different proportions of the two milk bases, one with EPS and one without EPS. A typical batch preparation is shown in Table 2.1.

2.6.2. Cheese Making Method

- The milk bases and emulsion were weighed into a beaker, warmed to 50°C and mixed with a Heidolph RZR 50 overhead stirrer for 5 minutes.
- Salts were added to the solution and mixed for 5 minutes.
- The solution was cooled to 38°C at which point the lactic acid solution was added to obtain a pH of approximately 6.0 and mixed for a further 2 minutes. The sample was covered and set aside until the other cheese solutions had been prepared.
- The samples were warmed to 38°C, mixed for 5 minutes, 0.015% Chymax rennet added and poured into pottles to the desired weight of cheese
- The pottles were placed in a water bath at 38°C for 40 minutes then the cheese was left to cool overnight at 4°C.

Table 2.1 Proportions of ingredients used in making up Panela cheese containing various levels of EPS and two levels of MPC – 17.37% (a) and 14.37% (b).

MPC Conc.- xanthan %	Control Milk Base (g)	Xanthan Milk Base (g)	Emulsion (g)	NaCl (g)	Calcium Lactate (g)	Lactic Acid Solution 1..39% (g)	Water (ml)
a - 0.161	-	170	50	3.0	0.375	30	-
b - 0.133	-	140	50	3.0	0.375	30	30
a - 0.080	85	85	50	3.0	0.375	30	-
b - 0.067	70	70	50	3.0	0.375	30	30
a - 0%	170	-	50	3.0	0.375	30	-
b - 0%	140	-	50	3.0	0.375	30	30

2.7 Cheese Analysis

2.7.1. Texture Measurements

Texture profile analysis theoretically mimics the chewing action in the mouth and was conducted on cheese samples a day after samples were made. A scalpel was used to cut away the plastic pottle and 1cm^3 cheese cubes were cut. Measurements were conducted using a TA-XT2 texture analyser at 5°C. Cheese cubes were used rather than cylinders as they were easier to prepare and showed similar results on preliminary tests. The cubed samples were placed between two plates and the force applied. The test speed was 5mm/s to a distance of 7mm followed by removal of the force at the same speed. A rest period of 2 seconds was given then the same force applied. The area under the force vs. time graph for the first compression was calculated and used for analysis. Two other methods for determining texture were tested including a compression test using the Instron 4500 and a probe compression test, however, TPA yielded the most reproducible results.

2.7.2. Moisture Determination of Cheese

Method used was from Winger at al (1997). Moisture dishes were left in an oven at 100°C $\pm$ 2°C for 1 hour. The dishes were removed and left to cool in a desiccator containing moisture-adsorbing material for 1 hour before being weighed. Cheese samples (2-5g) were added and weighed on Mettler AE 200 scales to 4 decimal places

in triplicate. The samples were left in an oven overnight at 100 ± 2°C then removed and placed in the desiccator for one hour to cool before being reweighed. The percentage moisture was calculated by the following equation:

$$\text{Moisture Content (\%)} = \frac{(\text{original weight - total dry weight})}{\text{original sample weight}} \times 100$$

2.7.3. Measurement of Whey Loss

Cheeses (25g) were formed in plastic pottles with analysis being conducted a day after the cheese was made. The cheese was cut into sections (figure 2.2) at 5°C and sections were removed and weighed into a 50ml conical centrifuge tube until a minimum of 5g had been added. Measurements were carried out in quadruplicate. The cheese was left in an incubator at 21°C for 3 hours then centrifuged with a BHG Hermle Z320 centrifuge at 162g for 10 minutes. The whey was poured off and weighed. The centrifuge tube was then inverted at 30° for 2 minutes and the whey poured off and weighed. The percentage whey loss was then calculated by the following formula:

$$\text{Whey Loss (\%)} = \frac{\text{total weight of whey}}{\text{original sample weight}} \times 100$$

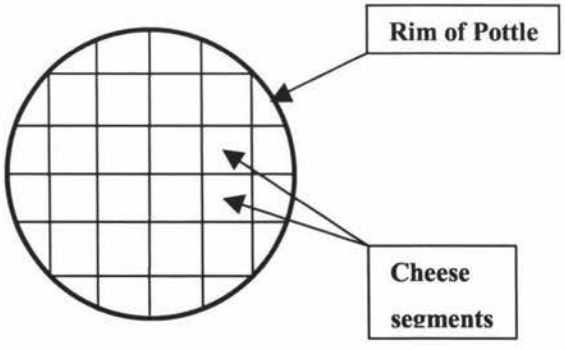


Figure 2.2 Topographic view of cheese showing how cheese was cut from the round pottle.

2.7.4. pH Measurement

Measurements were conducted with an Orion SA 720 pH meter at 5°C.

2.8 Odour Removal Methods

2.8.1. Ultrafiltration

A 10,000 M_w cut off Koch spiral wound membrane was used for UF of the *Xanthomonas campestris* ferment. The ferment was weighed and RO water added (conducting discontinuous diafiltration) before commencing UF. Once the UF system reached the lowest point (approximately 1500ml) a 15g sample was removed. At this point run one had been completed. RO water was added and this process continued until the odour in the retentate had been reduced considerably. Concentration factors (Cf) were used to determine the amount of purification given and represented 'the ratio of the volume of the feed to the final volume of the concentrate' (Bennet, 2003).

The concentration ratio (Cr) was calculated for each UF run by:

$$\mathrm{Cr} = \frac{Vf + Wf}{Vt - Vp}$$

Vf = Volume of feed/retentate, *Wf* = Volume of water added, *Vt* =*(Wf + Vf)*, *Vp* = Volume of permeate removed.

The Cf was calculated for UF run x by:

$$\mathrm{Cf} = Cr_1 \times Cr_2 \times Cr_{\ldots\ldots} \times Cr_x$$

2.8.2. Granular Activated Carbon (GAC) Treatment

GAC was added to a bed with a diameter of 125mm and a depth of 18mm, making a bed volume of 2.209×10^{-4} m^3. The system comprised of a pressure tank with a capacity of 3 litres and a tube connecting the pressure tank to the bed. Nine litres of water was flushed through the bed to remove carbon fines. The ferment was passed through the bed with the flow rate being calculated by hourly space volumes (HSV), which is the volume of ferment passed through the bed in 1 hour divided by the bed volume. A sample was collected after each pass. Once the solution had passed through the GAC the pressure tank was cleaned and dried and the ferment was put back into the pressure tank for the next pass. This continued for a set number of passes.

2.8.3. Odour Sensory Analysis

5g of the samples obtained from GAC and UF trials were mixed with 0.5g SMP. The control consisted of 5g RO water and 0.5g SMP. UF trial 2 (repeat) was the exception having 0.975g SMP, 4.81g Ferment 9g RO water for each sample. The samples were poured into 50ml containers with screw top lids.

Control samples were compared with GAC and UF treated samples. At least 30 panellists were provided with four unknown samples at room temperature in randomised order and asked two consecutive questions with regards to the odour. The first question involved a 9-point hedonic scale asking the panellist the degree to which they liked or disliked the odour. The second question was a 7-point scale regarding the intensity of the odour. Results were statistically analysed using one-way ANOVA and Tukey's pairwise comparison.

2.8.4. Quantification of Odour Compounds

2.8.4.1 Gas chromatography (GC) / Mass spectrometry (MS)

The solid phase microextraction (SPME) fibres used included: Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) of thickness 65μm, which was used for general screening; Polyacrylate of thickness 85μm for phenolic compounds; and Carboxen/Polydimethylsiloxane at 75μm for volatile and sulphur compounds.

GC-MS was preformed on a Fisons 8060 instrument. All chromatography used EC-1000 columns (equivalent to FFAP, 30m x 0.25mm; 0.25μm stationary phase thickness; Alltech, Auckland, New Zealand). The samples were applied by split/splitless injector at 230°C.

General analysis

SPME fibres were exposed to the headspace of 20mL UF batch two samples in a 50mL round bottom flask under vortex at 1000rev/min and 21°C. Exposure time was 5 minutes for very volatile compound scanning and 30 minutes for general scanning. The fibre was retracted and exposed to the injection port of the gas chromatograph for 5 minutes. The oven temperature was increased from 35°C at 5°C/min to 230°C. Helium, at an inlet pressure of 50kPa, was used as the carrier gas.

Selected ion monitoring (SIM)

UF batch 2 samples (20ml) containing 0.5ppm 2,3,5,6-tetradeutero-4-methylphenol (D_4 p-cresol) were vortexed at 1000rev/min in a 50mL round bottom flask and was heated at 47°C via a water bath. The Polyacrylate SPME fibre was exposed for 15 minutes, retracted then injected into the injection port of the gas chromatograph for 5 minutes. SIM mode was tuned to m/z 108 and m/z 112 for p-cresol quantification. The oven temperature was maintained at 150°C for 15 minutes. The D_4-p-cresol internal standard was measured from the separate 'mass chromatograms' of the m/z 108 and m/z 112 ions. The relative peak area of p-cresol compared to the D_4-p-cresol internal standard was determined and obtained a relative response factor of 1.84. p-Cresol concentration could then be calculated by the peak areas obtained for each sample.

2.9 Confocal Scanning Laser Microscopy (CSLM)

Confocal microscopy was carried out on a Leica TCS 4D confocal microscope (Leica Lasertechnik GmbH, Germany).

2.9.1. Cheese

Cheese samples were made from MPC containing commercial EPS, ferment EPS, and no EPS (control). Samples of diameter 10mm were cryogenically cut to a thickness of 60μm. Samples were dyed with Fast Green and Nile Blue and left overnight in a sealed moist environment at 20°C. CSLM was carried out with a 40x objective 1.0NA oil immersion lens. Images were 250 x 250μm square and of resolution 512 x 512 pixels. Nile Blue and Fast Green dyes were used to stain the lipid and protein, respectively. Nile Blue fluorescence was gathered using 488nm excitation with a LongPass filter of 515nm to capture the emission wavelengths. Fast green fluorescence was gathered using 568nm excitation with a LongPass filter of 590nm to capture the emission wavelengths.

2.9.2. Milk

SMP and WMP containing commercial EPS, ferment EPS and no EPS (control) were reconstituted for one hour at 10% solids. The EPS solutions were mixed with the control solutions to obtain varied levels of EPS including: 0, 0.046, 0.093, 0.14, and

0.23%. Reconstituted SMP samples were dyed with fast green, while reconstituted WMP samples were dyed with fast green and Nile blue. CSLM was carried out one hour after preparation with the samples being agitated slightly to ensure no sedimentation. Samples were measured with a 100x objective 1.4NA oil immersion lens. The pixel resolution, area, and dye excitation and emission wave lengths were the same as the cheese CSLM settings.

2.10 Final Product Sensory Testing

2.10.1. Cheese

The cheese samples were made as per the method stated in the cheese section. The control cheese consisted of 17.37% MPC while the commercial EPS containing cheese consisted of 15.6% MPC + 0.045% commercial EPS. The samples were prepared overnight and tested the next day. Thirty consumers were tested at Massey University, Palmerston North, and were asked to rate the flavour, overall liking, and texture using a hedonic like/dislike scale and the saltiness using an intensity scale. The consumers were asked to comment on each attribute tested. Samples were held at 4°C before testing.

2.10.2. Milk

Milk samples were prepared by mixing a WMP containing commercial EPS and control WMP to obtain varied concentrations of EPS in the powder. A triangle test comprising reconstituted 15% WMP and 13.3% WMP + 0.02% EPS was used to test for any differences caused the addition. Both were evenly used as the odd sample. The thickened milk test comprised four samples. The control consisted of a 20% WMP solution while the test samples were reconstituted to 15% WMP solids and contained 0.015%, 0.040% and 0.079% commercial EPS. They were asked to rate the flavour, overall liking, thickness and creaminess using a hedonic like/dislike scale. Intensity scales were also used for the thickness and creaminess. The consumers were asked to comment on each attribute tested. Thirty two consumers were tested at Massey University, Palmerston North for both trials with a randomised order of presentation. Results from the sensory testing for the cheese and milk sensory testing were statistically analysed with one way ANOVA and Tukey's pairwise comparison.

CHAPTER 3 REMOVAL OF ODOUR FROM THE FERMENT

3.1 Introduction

The ferment EPS produced from milk permeate contained a potent undesirable odour which was retained in the cheese and milk products after spray-drying and reconstitution. This odour was not expected and as such odour removal was paramount to producing a product that would be liked by the consumer. The volatile compounds found in milk are often derived from bovine feed and metabolism. Microbial activity and dairy processing such as pasteurisation, spray-drying, fermentation and product storage can also produce these compounds (O'Connell and Fox, 2001). Volatile compounds however, such as methyl sulphide, are often removed from dairy products during processing due to their high volatility and vapour pressure (Gordon and Morgan, 1972), however this was not the case for this odour. Removal or reduction of the odour was essential for the successful application of the ferment EPS into the dairy products. This odour was not noticed with the incorporation of the commercial EPS.

Three methods were utilised in an attempt remove the odour present including air stripping, granular activated carbon (GAC) and UF. Air stripping involves the bubbling of air or an inert gas through a sinter into solutions to remove the volatile compounds. GAC is effective in removing both colour and low Mw organic compounds with adsorption usually taking place at active sites on the surface of the GAC. A minimum of GAC fouling and the maintenance of product quality are required for this process to be effective (Wilson, 1977; How and Morr, 1982). UF separates compounds based on their molecular weight with low molecular weight molecules being separated from larger ones. The effectiveness of odour compound removal is dependent upon on their size and affinity for the EPS (Ramirez-Figueroa et al. 2002). The use of UF is dependent upon the cost of operation and the amount of UF required for effective odour removal.

3.2 Results and Discussion

3.2.1. Air Stripping

Air stripping was unsuccessful in removing the odour from the ferment. Preliminary trials indicated that the odour could not be removed after 5 hours of bubbling with inert nitrogen gas and 18 hours with compressed air at room temperature.

Two possible reasons exist for the unsuccessful removal of odour. The first is the inability of the free compounds to be released from the ferment. Polysaccharides can encapsulate and retain volatile compounds, which limit their release (Ramirez-Figueroa et al. 2002). However the large volume of gas passed would be expected to remove a considerable amount of these volatile compounds. The continued release of free compounds from conjugates is the second reason. While air stripping may remove some volatile components due to the low volatility the conjugates may continue to release the volatile compounds into solution (Lopez and Lindsey, 1993). Air stripping may not remove the conjugates in the solution and this is where the three methods differ. UF could potentially remove all compounds under a specified molecular size including the volatiles and small conjugates. GAC could also remove both free and conjugated odour compounds (How and Morr, 1982).

3.2.2. Granular Activated Carbon

3.2.2.1 Viscosity decrease with GAC

The small amount of ferment EPS available limited the GAC experiments that could be conducted. Ferment EPS was passed through a bed of GAC in an attempt to remove the odour causing compounds. The viscosity of the ferment decreased after passing through the bed of GAC as shown in figure 3.1. The viscosity decreased by 21% at $100s^{-1}$ after four passes. The reasons for the drop may include either EPS dilution or retention of EPS by active sites on the carbon. The dilution effect would be due to the retention of the wash water in the carbon bed before the addition of the ferment EPS. This is a minor problem and can be solved with the removal of the first bed volume of product. If the decrease in viscosity is a reflection of EPS retention by

GAC the carbon would be fouled quickly hindering volatile removal and posing problems for cleaning (Wilson, 1977).

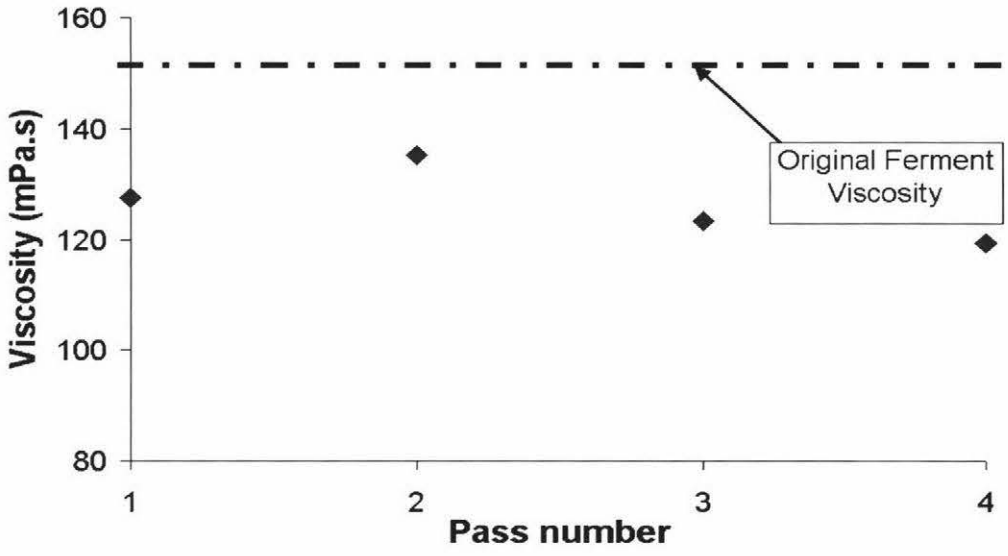


Figure 3.1 Viscosity reduction of ferment EPS passing through bed of GAC for four consecutive passes (shear rate = $100s^{-1}$).

3.2.2.2 Residence times for ferment/carbon fines

Carbon fines were present in the ferment after passing through a bed of GAC. As the number of passes increased the amount of carbon coming through decreased, however there was still carbon present after the final pass. The amount of fines was greater in more viscous solutions. The presence of carbon fines posed a problem and the fines must be removed before incorporation into milk powders. Methods such as filtration and centrifugation were ineffective in separating the fines from EPS due to the high viscosity. The amount of fines could be reduced by dilution of the ferment before GAC treatment; however, the sample may have to be concentrated again at a later stage. An increased flushing of water before using the column would also reduce the fines present (Norit, 1995)

The effectiveness of the GAC odour removal was dependent upon the residence times. The residence times for each pass (Table 3.1) were within those used commercially of 0.1-3.0 Hourly Space Volumes (H.S.V) and varied due to the variability of pressure and viscosity. A lower H.S.V. is commonly used for high viscosity solutions (Norit, 1995) and would remove odour more effectively then a higher H.S.V.

Table 3.1 Flowrate of ferment EPS through GAC in Hourly Space Volumes (H.S.V).

Pass Number	Flow rate (H.S.V)
1	1.78
2	1.81
3	1.53
4	0.70

3.2.2.3 Sensory analysis of GAC treated ferment

Sensory analysis was used to determine the effectiveness of GAC for odour removal. A consumer test was used to gauge the odour, as a trained panel was not warranted. Both a hedonic scale of like/dislike and a rating of the intensity were asked with regard to the odour present. The samples were prepared by mixing GAC treated ferment EPS with SMP. SMP reconstituted in water was used as the control.

The results for the hedonic test are shown in figure 3.2. Before GAC, the samples were extremely offensive. GAC effectively removed the odour to appreciable levels with one pass. This means that a flow rate of 1.78 HSV is enough to make the samples not significantly different from the control. With subsequent passes the odour remained not significantly different from the control. The averages for the samples tested including the control lay below the 'neither like nor dislike' rating (5 mark) and so were in the dislike section. This is probably a reflection of the SMP used for the sensory and the use of whole milk for sensory testing rather than SMP may have yielded different results. The mean intensity ratings shown in figure 3.3 are similar to the results of the hedonic scale. This may be due to bias, in particular a halo effect, as the intensity question followed the liking question for each sample (Larmond, 1977). While this is plausible, another reason may be that the intensity attribute is crucial in determining the overall liking of the sample odour.

The samples tested were not the same as the intended final product, as the ferment EPS is to be spray dried onto WMP and MPC. The spray-drying process could yield different sensory results as heating and processing could remove volatile components, while also potentially producing more odour compounds (Lopez and Lindsay, 1993; Gordon and Morgan, 1972; O'Connell and Fox, 2001). The proportion of ferment

added and hence the amount of volatile components was also higher than what would be present in the final powder.

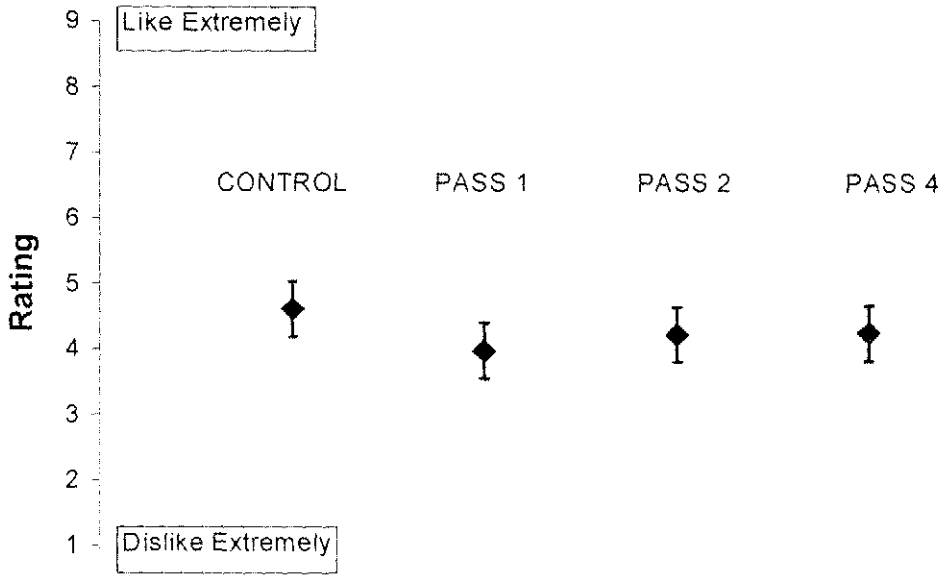


Figure 3.2 **Hedonic like/dislike rating for odour sensory tests of GAC treated ferment EPS with 95% confidence intervals.**

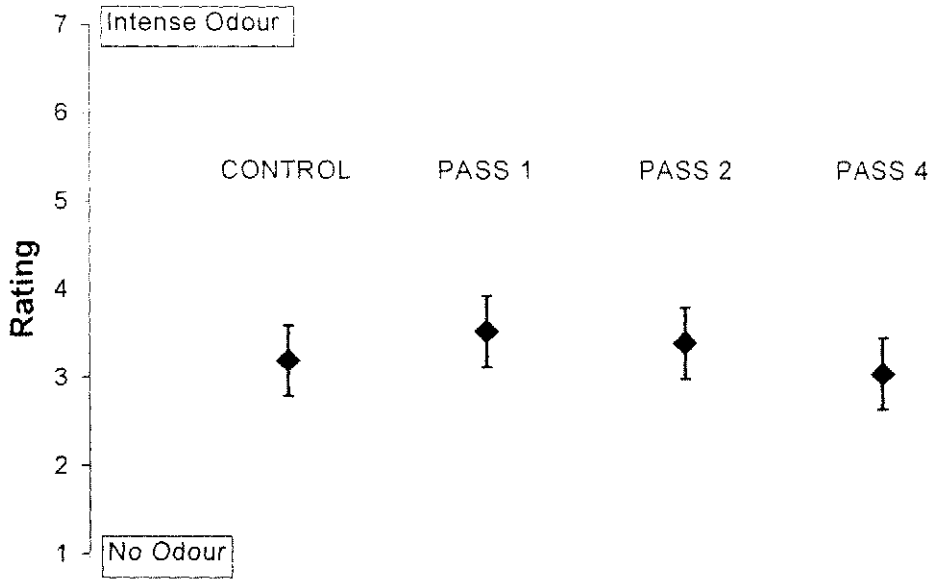


Figure 3.3 **Intensity rating for GAC odour sensory tests of GAC treated ferment EPS with 95% confidence intervals**

3.2.3. UF - Batch One Sensory Analysis

UF and diafiltration removed odour and colour as both were apparent in the permeate and diminished within the retentate. Clogging of the membrane occurred and has to be taken into account during large-scale production to ensure that the permeate flow rate does not diminish. The final product was not contaminated with carbon fines unlike GAC and therefore could be incorporated directly into milk for spray-drying.

The samples used for odour testing were prepared in the same way as GAC samples and panellists were asked the same questions. Figure 3.4 shows the hedonic scores for odour sensory testing of UF samples. After 17.1 concentration factors (Cf) a significant difference existed between the sample and the control while no significant difference existed between the control and the ferment after 430.1 Cf. This indicates that the number of Cf required to minimise the odour to appreciable levels lies between 17.1 and 430.1.

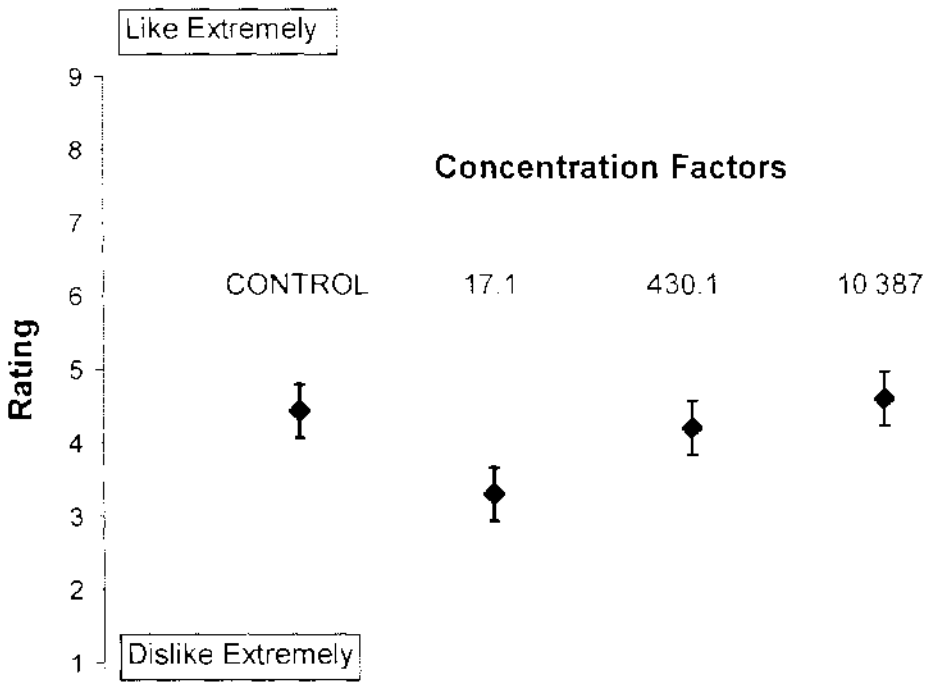


Figure 3.4 Hedonic like/dislike rating for odour sensory tests of the UF treated ferment EPS samples with 95% confidence intervals.

The intensity results shown in figure 3.5 reflected the hedonic results. A halo effect may have caused these similarities or they may be due to the intensity rating being a

major indicator of the liking of the product. Again there was a significant difference occurring between the 17.1 Cf sample and the control. 430.1 Cf was required to make the sample not significantly different from the control. The minimum number of Cf required to make the sample acceptable to the consumer therefore lay between 17.1 and 430.1. The control sample received identical ratings with regard to overall liking and intensity for both the GAC and UF questionnaires, indicating that the experiment was consistent.

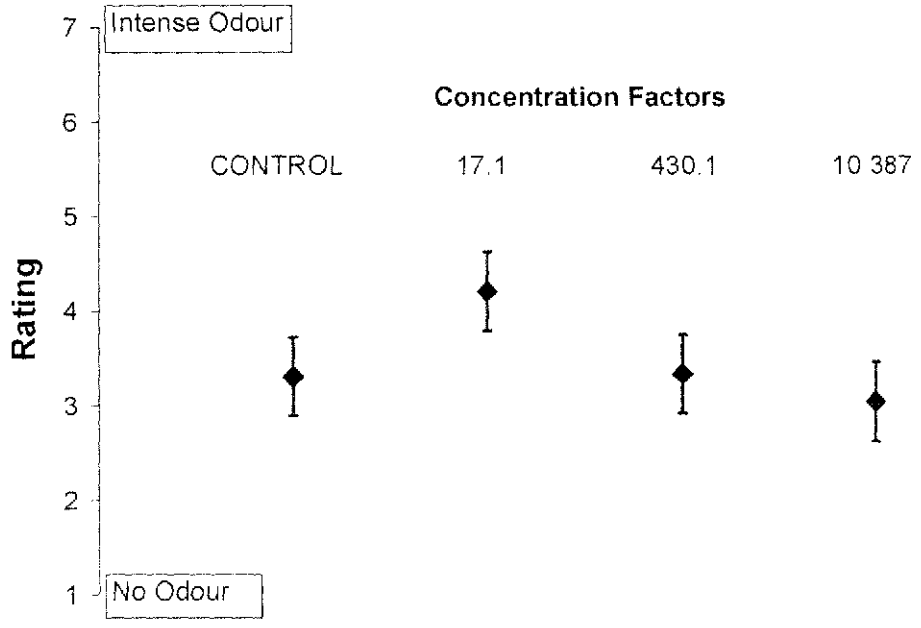


Figure 3.5 Intensity rating for odour sensory tests for UF treated ferment EPS samples with 95% confidence intervals

3.2.4. UF - Batch Two Sensory Analysis

A second batch of ferment EPS was ultrafiltered, to approximately the same extent as the previous batch. The initial odour was considerably different in this batch, compared to the first batch of ferment, and had a characteristic cow urine smell. Using the same sensory sample preparation as UF Batch one, all of the samples tested (from 98 to 7034 Cf) were found to be significantly different from the control. Both intensity and overall liking rated lower than the control, meaning that UF was not as effective at removing the odour as from the previous sample. A different compound or a larger initial quantity of odour compound may have been responsible.

In this test, the ferment EPS used for odour sensory analysis were more concentrated than would be used in spray dried powders. In subsequent testing the quantity of the ferment EPS was reduced and partially replaced with RO water to match the amount of EPS that would be present in the powder. Figure 3.6 shows the hedonic results for the odour sensory trial with the decreased ferment content. There was no significant difference between the control and all samples. This implies that, at this level of ferment EPS, 98 or less Cf is required to obtain a product not significantly different from the control.

The occurrence of the undesirable odour in the UF ferment was a major problem, and the cause of the smell and the effectiveness of the removal process were investigated further. The Fonterra Research Centre provided the detection equipment incorporating SPME, GC and MS to determine the nature of the volatile compounds and the quantities present in samples from UF batch two.

3.2.5. Quantitative Analysis of UF Ferment

3.2.5.1 Determination of p-cresol

Two components were identified in the ferment pre-UF. These were 4-methylphenol (p-cresol) and 3-methylphenol (m-cresol). Of these components p-cresol gives the characteristic smell of old milk and is partially the reason for the cow urine smell (Suemitsu et al. 1965; Bendall, 2001). Other components, such as sulphates and their conjugates, were also likely to be present however would not be adsorbed by the SPME fibres in sufficient quantity to detect.

The level of reduction of p-cresol by UF was an indication of the removal of low Mw odour compounds. The samples collected during UF were analysed to determine the quantity of p-cresol present after a number of Cf (figure 3.6). The initial concentration in the untreated ferment was 138 parts per billion (ppb). As the number of Cf increased the p-cresol concentration decreased until, at 98 CF, the p-cresol concentration had levelled out to between 1.7 - 4.7ppb. These low levels were at the minimum level of detectability. The decrease in p-cresol concentration during UF would be through the permeate stream along with some evaporation of volatiles at the operation temperature of 45°C. The continuing low level of p-cresol in the

solution indicated that some conjugates were still present, continuing to release free volatiles.

Odour sensory results (figure 3.6) showed that all sensory samples tested contained less than 5ppb p-cresol. There was no significant difference between the sensory samples and therefore ensuring less than 5ppb p-cresol is present is essential to make sample more acceptable to the consumer. The samples containing higher levels of p-cresol were not used in the sensory testing as the odour was too potent. Incorporation of these samples would dominate the results and interfere with the values obtained for the other samples.

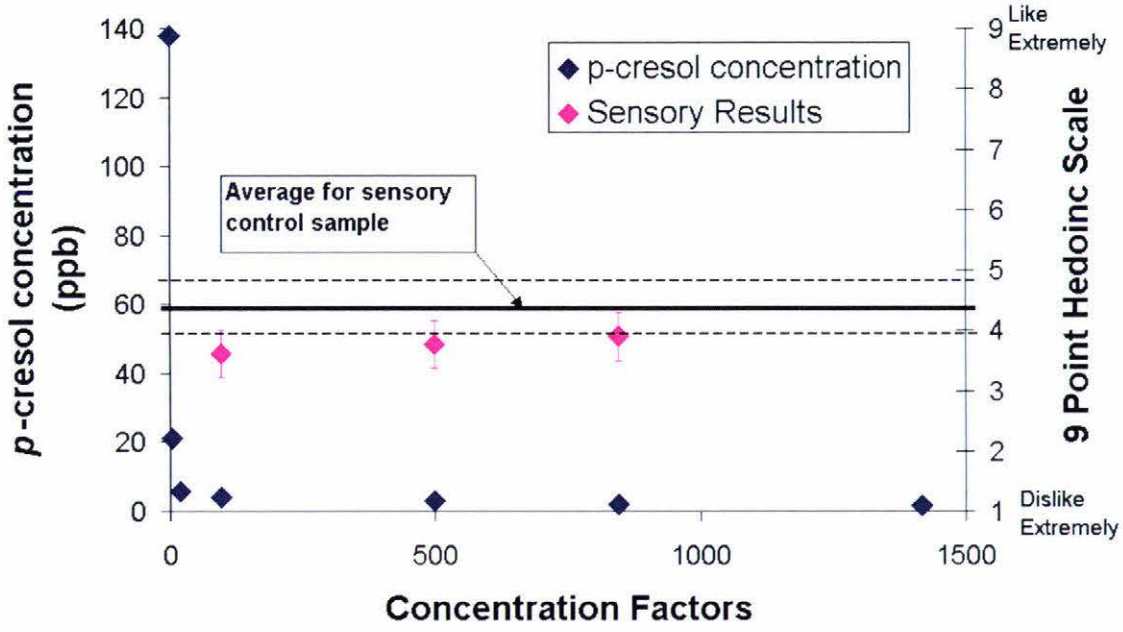


Figure 3.6 **p-Cresol concentration reduction in ferment EPS by UF plotted alongside hedonic results from sensory testing. Sensory results are averages with 95% confidence intervals (control - - -).**

3.2.5.2 Determination of odour production

The mechanism of p-cresol evolution was investigated in an attempt to limit or prevent the production of the compound. Firstly milk permeate was analysed to determine the compounds present. Milk permeate contained acetaldehyde, dimethyl sulfide, acetone and ethanol. These four compounds all have low boiling points and

were not detected in the fermented milk permeate, indicating that the agitation and elevated temperatures used for fermentation removed these volatile components.

Milk permeate was also found to contain low levels of 'free' p-cresol, approximately 4-5 ppb, which is much lower than that in the ferment EPS which contained 138ppb. p-Cresol is largely bound as a conjugate with sulphate, and, to a lesser extent, glucuronides. The hydrolysis and release of p-cresol from the conjugate form can occur through enzymic or chemical means (Lopez and Lindsay, 1993).

The enzyme (β-galactosidase) added to hydrolyse the lactose in milk permeate to glucose and galactose for fermentation was tested to see whether it was responsible for hydrolysing the p-cresol conjugates. β-Galactosidase was added to a sample of milk permeate, without inoculation of the microorganism, and was stored overnight at 47°C. The enzyme did not increase the levels of p-cresol present. Hence, the β-galactosidase was not responsible for the high levels of p-cresol.

The microorganism was thus the most likely agent for release of the cresol odour compounds. While the hydrolysis of the conjugates causing the release of p-cresol was the most likely explanation of the high p-cresol levels, another alternative was that the microorganism may have been producing these odour compounds through its metabolism. An experiment was conducted to investigate this possibility. The organism was inoculated into a medium of 10g/l glucose and 10g/l galactose. This imitated the composition of 50% milk permeate but contained no conjugates. The sample was used directly for GC/MS analysis. No p-cresol was detected before and after inoculation. It was therefore deduced that the p-cresol is released by the microorganism in the presence of milk permeate as a result of conjugate hydrolysis.

3.3 Conclusions

- The unpleasant odour in ferment EPS comprised p-cresol and other compounds and is produced by the microorganism hydrolysing conjugates present in the milk permeate.

- UF was effective for odour removal, decreasing the p-cresol levels from 138ppb too less than 5ppb. This made the ferment EPS more acceptable to the consumer.

- GAC reduced the odour effectively after one pass at 1.78 H.S.V; however the product was contaminated with carbon fines.

- The full effectiveness of the odour removal will not be known until product sensory tests are conducted on powders containing the purified ferment.

CHAPTER 4 INCORPORATION OF EPS INTO MILK

4.1 Introduction

Reconstitution into a thickened milk product is one potential outlet for a spray-dried milk powder containing xanthan, and this application was investigated in this section. Polysaccharides such as carrageenan are extensively used in milk products for thickening and stabilizing purposes (Langendorff et al.1997). Xanthan has been used for the same reason in milk systems but may be less effective than carrageenan because of its easier tendency to cause phase separation. Phase separation in milk systems is thought to be largely due to depletion flocculation of the casein micelles by the polysaccharide. Unlike carrageenan, xanthan appears to have little affinity for casein micelles and tends to be excluded from the space between micelles. This creates an osmotic imbalance in the solution, and water molecules move from between micelles to dilute the xanthan outside the micelle cluster. This causes the flocculation of casein micelles leading to phase separation with casein falling out of suspension (Dickinson, 1998; Hemar et al. 2001). Previous investigators have used commercially processed xanthan which may differ from the ferment xanthan produced from milk permeate (Thorne et al. 1988; Shatwell et al. 1990, Capron et al 1998). It was therefore important to determine the conditions for reconstitution and the viscosity of the reconstituted milk, as well as the possible occurrence of phase separation, which could well determine the product life.

4.2 Results and Discussion

4.2.1. Spray-drying

Milk powders containing EPS were produced by reconstitution of the milk powder, mixing of appropriate levels of EPS, followed by spray-drying. As the addition of the EPS substantially increased the viscosity, spray-drying of powders at normal operating conditions of 45-55% total solids was unsuccessful. The high viscosity clogged the atomizer and spray-drying equipment. Therefore spray-drying was conducted at around 20% total solids depending upon the EPS concentration required.

The powders produced after drying were very low in moisture ranging between 1.71 and 2.32%.

The colour and appearance of spray dried milk powders containing commercial EPS were similar to the existing control powders. In initial experiments, powders containing ferment EPS had a yellow tinge due to the yellow pigment present in the ferment. This pigment was not present in commercial EPS, which is not made from cows' milk. UF of the ferment EPS prior to spray-drying removed a considerable amount of this pigment, which was then not apparent in the spray-dried powders.

Phenol/sulphuric assays were used to determine the EPS concentration of the powders. The powders yielded results close to the amount EPS expected based on levels of constituents added. However the assay was difficult, as fat and other components interfere with the determination. Because of this, the EPS contents were calculated from the levels of ferment EPS or commercial EPS used in making the powders, rather than by assay of the powder.

4.2.2. Powder Solubility

Solubility is a crucial factor in the determination of powder quality (Masters, 1976). Incorporation of EPS in the milk powders might be expected to affect the ease of dispersion and levels of insoluble constituents present. The solubility analysis measured the amount of sediment produced upon centrifugation of the reconstituted powders.

The solubility indices of standard WMP and SMP produced by Fonterra Co-operative Group Limited (termed factory powders), and three lots of WMP and SMP, reconstituted and spray dried with or without EPS were determined. Two lots contained either commercial EPS or ferment EPS spray dried at a level of 2.3% EPS on the powder. The third lot was factory powder, reconstituted, and spray dried to mimic the spray-drying process of the EPS containing powders. This powder was termed the 'control'.

With SMP there was no significant difference in terms of solubility between any of the powders as seen in figure 4.1. Thus the spray-drying and addition of EPS had a minimal affect on the solubility of the powder in water. Figure 4.2 shows a graph of the solubility for the WMPs. A significant difference did exist between factory powder and the powders containing EPS. Lower solubility index values for the EPS containing powders were obtained. It is unlikely that the EPS containing powders actually had less insoluble material than the controls. These results may be attributed to the higher viscosity of the EPS powders in solution, which kept the insoluble particles in suspension after reconstitution. At 2.3% EPS on the powder, the amount of EPS in the powder was much higher than what would be used for a commercial application.

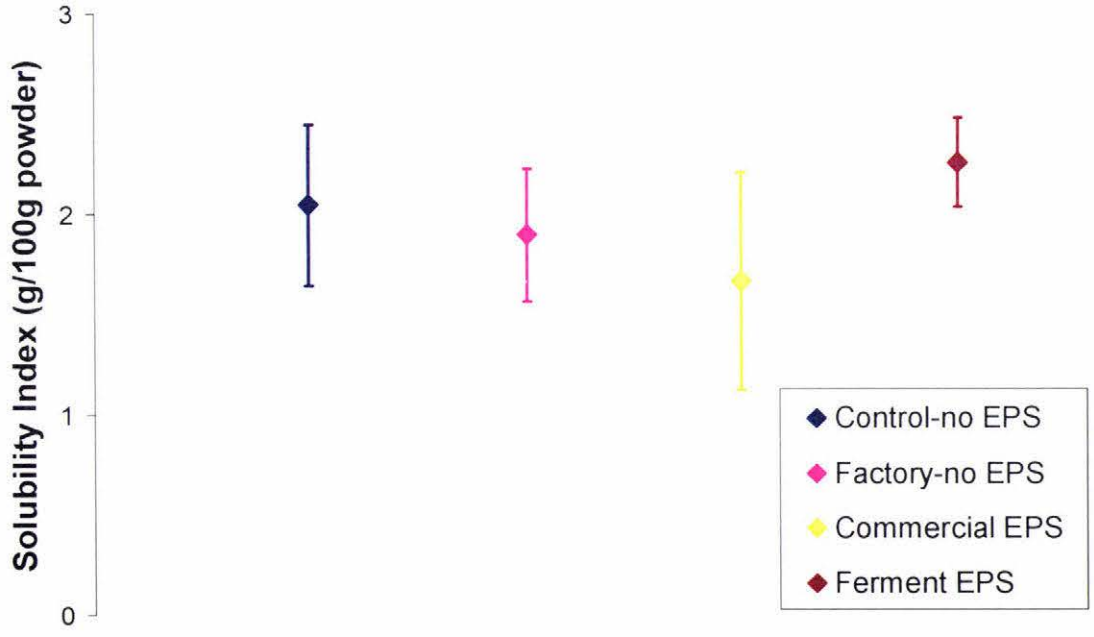


Figure 4.1 Solubility of reconstituted SMP containing 2.3% EPS on powder solids basis with 95% confidence intervals

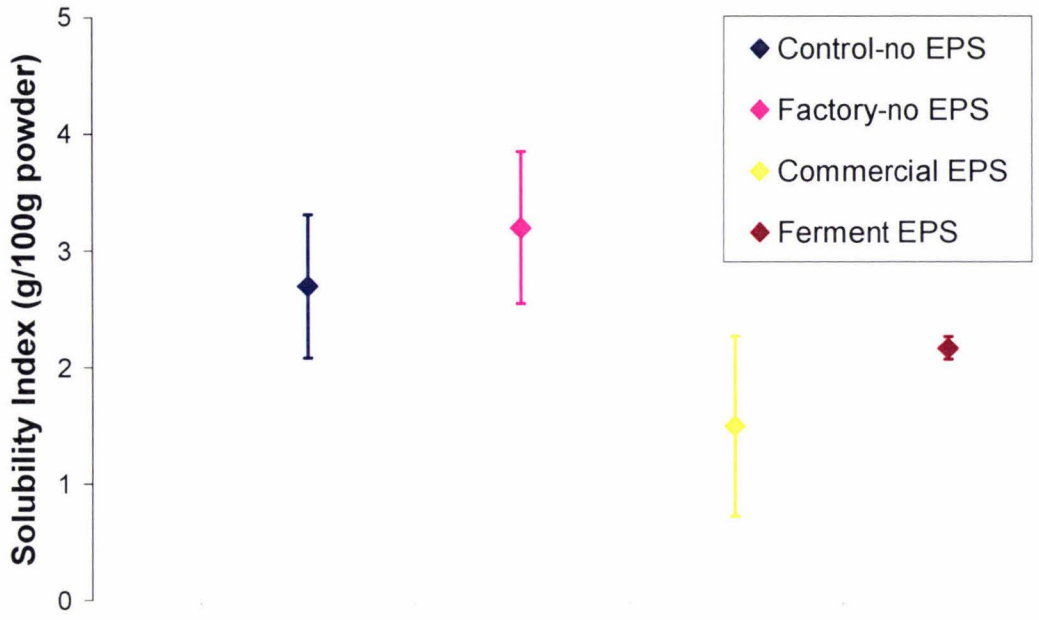


Figure 4.2 Solubility of reconstituted WMP containing 2.3% EPS on powder solids basis with 95% confidence intervals

4.2.3. Viscosity of Reconstituted Powders

The reconstituted milk powders containing EPS represented thickened milks targeted toward a consumer application. After reconstitution, consumers would probably store the product in a refrigerator for a period of time. The viscosity of reconstituted powders containing EPS were measured at both low and high shear rates, after storage at 5°C.

The viscosities of reconstituted WMP and SMP containing commercial and ferment EPS were measured using two different instruments. The Rheometrics system was used to measure the viscosity over a range of shear rates, and the Ferranti Shirley viscometer was used for the controlled shear measurements. The viscosity at a shear rate of $100s^{-1}$ for reconstituted 15% SMP containing ferment and commercial EPS is shown in figure 4.3. There was little difference between ferment and commercial EPS. The viscosity increased substantially from around 16 to 85 mPa.s for commercial EPS and to 78 mPa.s for ferment EPS as the EPS concentration increased from 0 to 0.345%. Similar observations were made with the WMP containing commercial and ferment EPS under the same conditions (figure 4.4). The viscosity of reconstituted WMP was found to increase from 15.2 to 89 mPa.s for commercial

EPS and to 98 mPa.s for ferment EPS. The slightly higher viscosities observed for the WMP solutions probably reflected the presence of the fat globules.

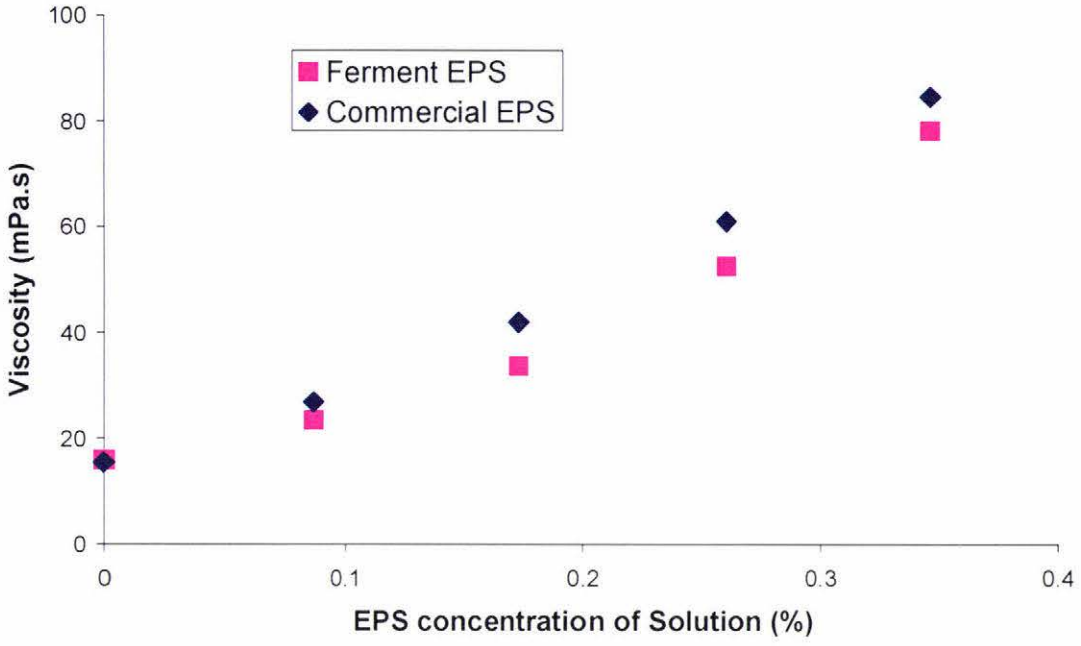


Figure 4.3 **Viscosity of reconstituted 15% SMP at $100s^{-1}$ containing ferment and commercial EPS at various concentrations.**

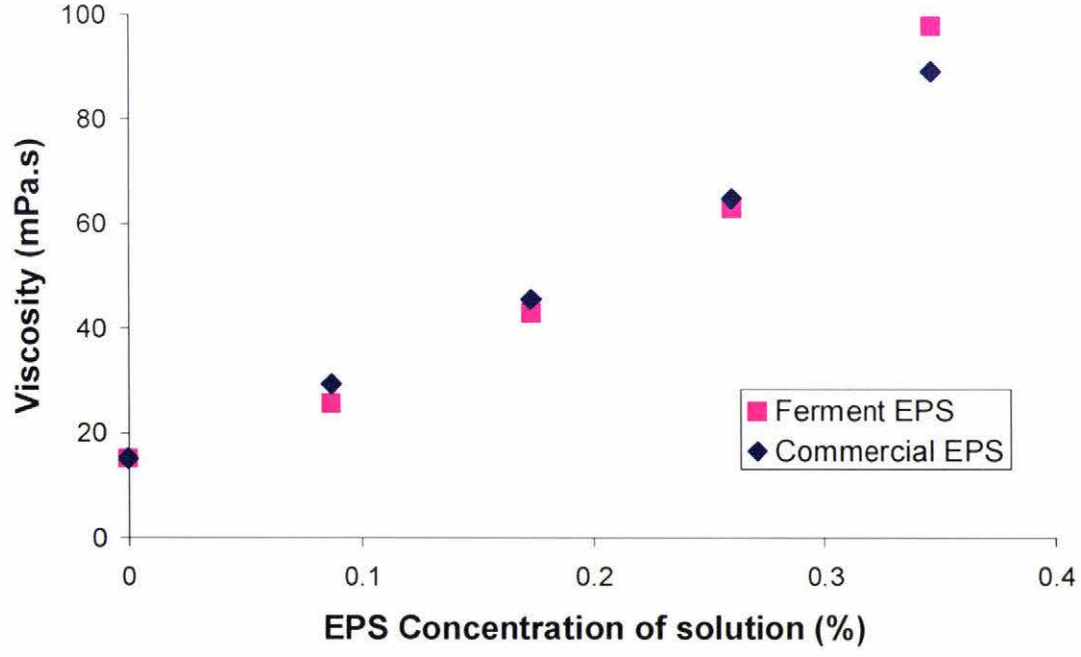


Figure 4.4 **Viscosity of reconstituted 15% WMP at $100s^{-1}$ containing ferment and commercial EPS at various concentrations.**

Greater differences between the powders containing the two types of EPS were found when the viscosities were measured at a range of shear rates. Figure 4.5 shows the viscosities over the shear rate range of 0.001 to 1000s^{-1} of 10% SMP solutions containing various concentrations of commercial and ferment EPS. The viscosities obtained at low shear rates were markedly higher than at 100s^{-1}. Of note is the difference between the ferment and commercial EPS. For the same concentration of EPS at low shear rates the ferment had a much lower viscosity than the commercial EPS. At higher shear rates such as 100s^{-1} almost identical values for the ferment and commercial EPS were obtained. This could possibly be due to a greater degree of molecular aggregation in the commercial EPS and is discussed later. Higher concentrations of EPS also showed greater pseudoplastic tendencies with a more distinguishable Newtonian domain at lower shear rates. As the EPS concentration increased, it tended to dominate the rheological properties of the solution. This was also found by Schmidt and Smith (1992) and Hemar et al. (2001).

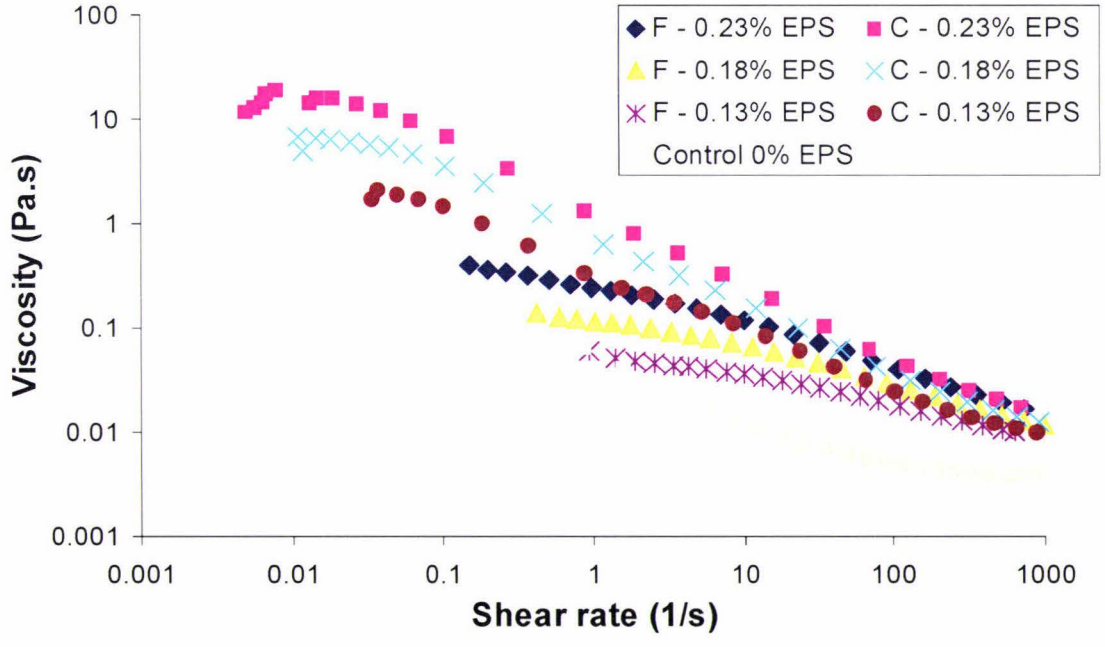


Figure 4.5 Viscosity of 10% SMP solution containing ferment EPS (F) and commercial EPS (C) at various concentrations and shear rates.

The WMP results were similar to the SMP system. Figures 4.6, 4.7 and 4.8 show the results for the reconstituted WMP concentrations of 10, 15 and 20% respectively.

The viscosity was far greater at low shear rates than at a shear rate of $100s^{-1}$, and greater pseudoplastic tendencies were observed at higher EPS concentrations. Again the WMP solutions containing commercial EPS had higher viscosities at low shear rates than the corresponding ferment EPS system.

The viscosity at low shear also increased with increasing solids concentration at the same EPS concentration. Thus the viscosity of WMP solutions containing 0.23% commercial EPS increased from ~25 Pa.s at 10% WMP to ~40 Pa.s at 20% WMP. This can be attributed to the increase in concentration of the EPS in the continuous phase as the concentration of micellar casein increased.

The difference between the low shear viscosity of the ferment and commercial EPS decreased with increasing solids concentration. The increase in volume fraction of the casein would have decreased the interparticle space. This could have encouraged more aggregation of the ferment EPS leading to a greater than expected increase in viscosity.

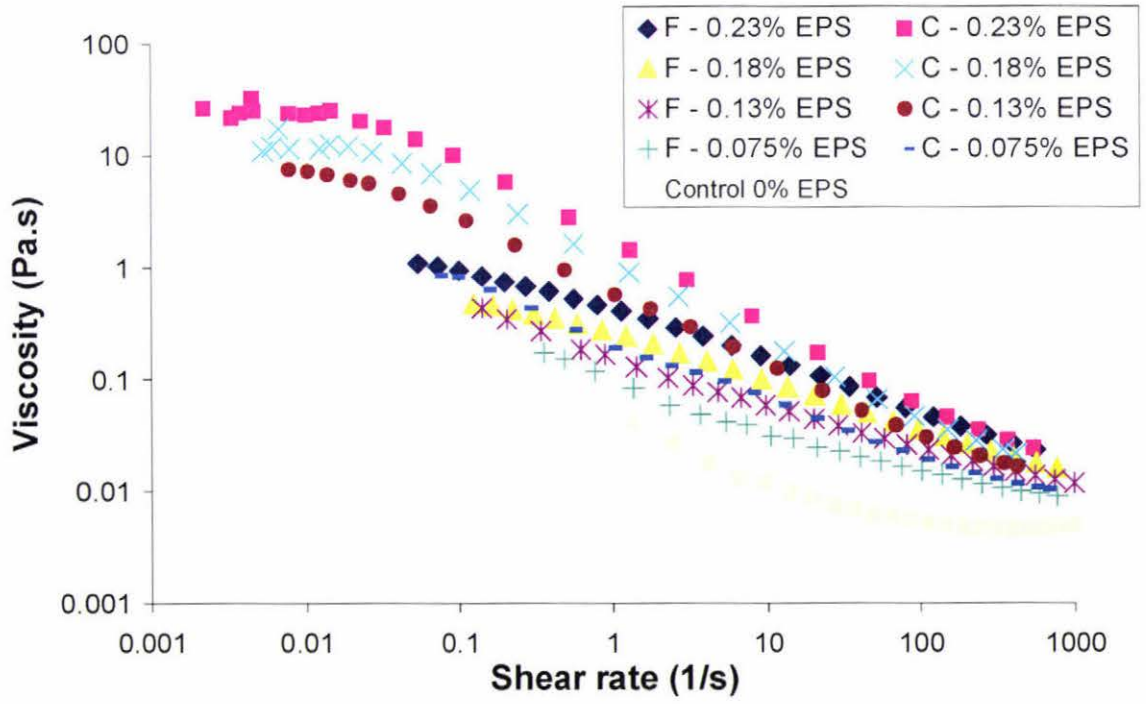


Figure 4.6 Viscosity of 10% WMP solution containing ferment EPS (F) and commercial EPS (C) at various concentrations and shear rates.

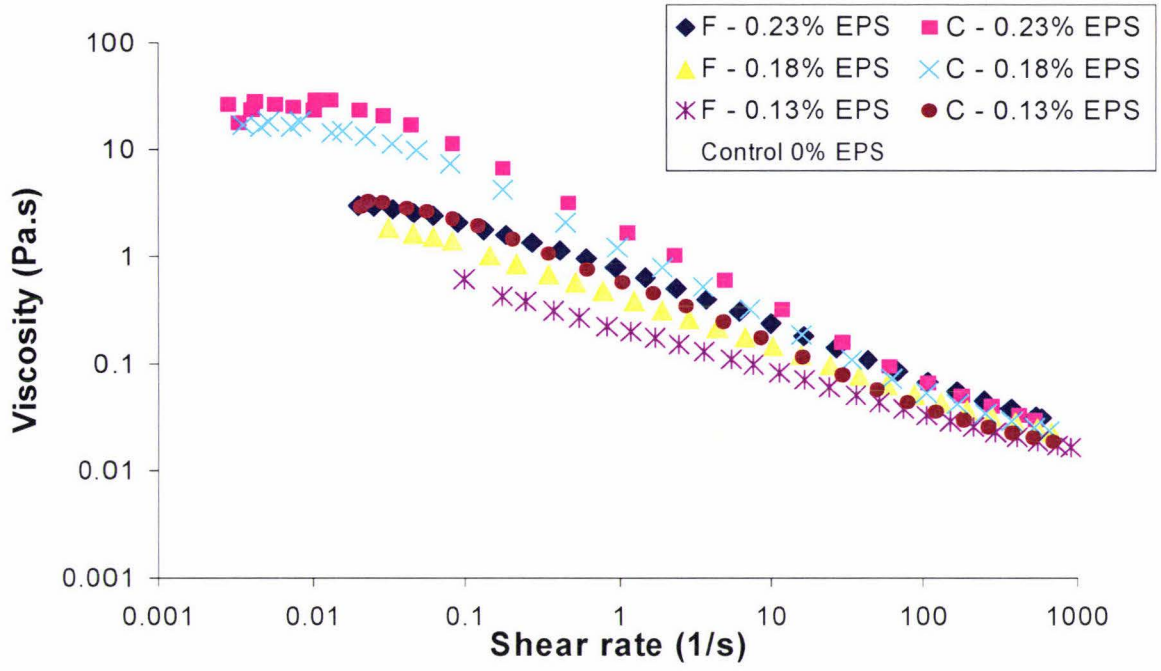


Figure 4.7 **Viscosity of 15% WMP solution containing ferment EPS (F) and commercial EPS (C) at various concentrations and shear rates.**

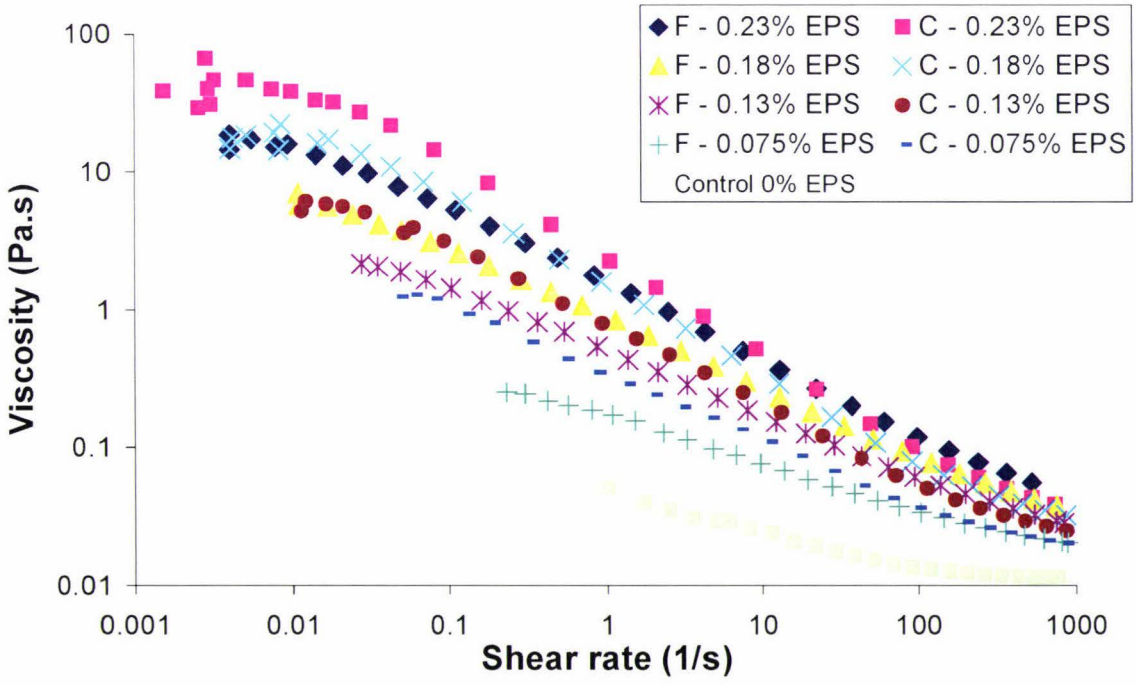


Figure 4.8 **Viscosity of 20% WMP solution containing ferment EPS (F) and commercial EPS (C) at various concentrations and shear rates.**

4.2.4. Differences Between Ferment and Commercial EPS

There are two areas where the ferment and commercial EPS were likely to differ. The first is in the molecular composition of the xanthan. The different strains of organism and media used for the production of xanthan cause variations in the proportions of pyruvate and acetate contents and the molecular weight of the molecule (Shatwell et al. 1990; Papagianni et al. 2001). Ferment EPS and commercial EPS were produced from different substrates, and were quite likely to differ in composition, molecular structure, and rheological properties.

The second and more important possible difference was in the extent of aggregation. Xanthan upon heating above the transition temperature, followed by subsequent cooling, undergoes dissociation and renaturing (Lecoutier et al. 1986; Muller et al. 1986). This process leads to a greater degree of molecular interaction and more junction points for hydrogen bonding (Iseki et al. 2001). In commercial processing, heat treatment is used to increase aggregation and hence the viscosity of the xanthan (Rinaudo, 2001).

Figures 4.6-4.8 show that in milk powders commercial EPS at low shear rates has a higher viscosity than ferment EPS in WMP. This difference decreases at higher shear rates. Because the difference is dependent upon shear rate it is most likely to be linked to the degree of aggregation. A greater amount of aggregation would cause a greater viscosity at low shear rates, disappearing as the shear rate increased and the aggregates break up.

To test this assumption the rheological properties of ferment and commercial EPS (no milk powder) were determined by viscosity measurements (figure 4.9) and dynamic frequency sweeps (figure 4.10). Figure 4.9 shows that as in the milk powders, commercial EPS has a higher viscosity than ferment EPS at low shear rates. Increasing the shear rate causes the viscosity to decrease for both types of EPS with the commercial EPS having a steeper gradient compared to the ferment EPS. At higher shear rates the viscosities are almost identical. This indicates a greater amount of aggregation in the commercial EPS. The dynamic frequency sweeps supported these results. The ferment EPS had a lower G′ value compared to the commercial

EPS especially at lower frequencies. As G′ is an indication of the elastic properties the lower value for the ferment EPS indicates that it is less elastic. This also suggests that there is a much greater degree of intermolecular bonding in the commercial EPS.

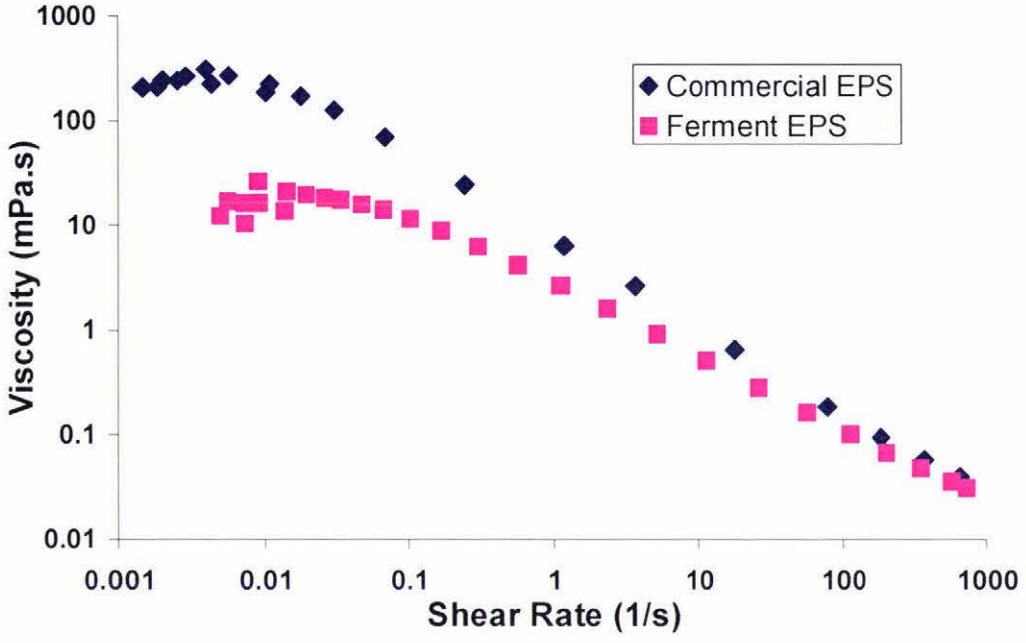


Figure 4.9 Viscosities profiles of ferment and commercial EPS at 0.502% EPS (no milk powder).

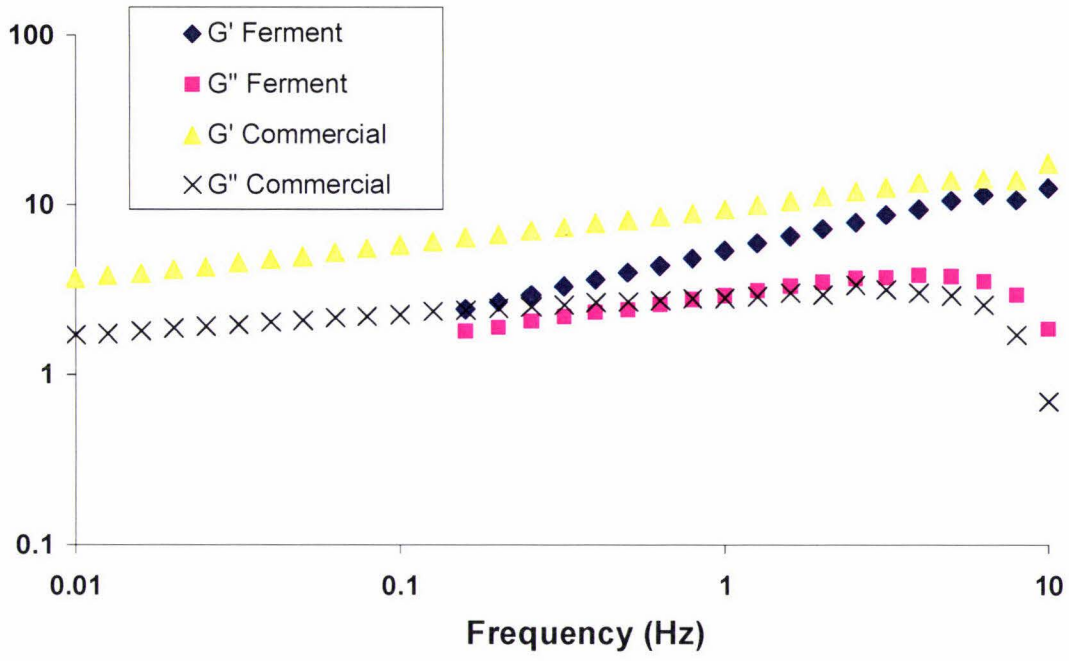


Figure 4.10 Dynamic frequency sweeps of pure ferment and commercial EPS at 0.502% EPS (no milk powder).

4.2.5. Phase Separation

4.2.5.1 Reconstituted SMP

Milk powders containing commercial and ferment EPS before and after UF were reconstituted at 10%, 15% and 20% solids in RO (or milliQ) water. The EPS levels were varied by mixing powders spray dried with and without EPS. Figure 4.11 shows a picture of 10% reconstituted SMP with the varying concentrations of EPS after 66 hours storage at 5°C. Phase separation had occurred in tubes c, d, and e. The bottom phase consisted of casein while the top layer (supernatant) was relatively clear and consisted of EPS in milk serum. The amount of supernatant is a reflection of the extent of phase separation that has occurred. The full results are shown in figure 4.12. There was a considerable difference between commercial and ferment EPS milks. In the 10% SMP milks containing commercial EPS, phase separation started at a very low EPS concentration (0.0075%), increased to a maximum, and then decreased until at EPS concentration of 0.18% and higher there was no longer any apparent separation. Where phase separation only occurs between an upper and a lower concentration, this is termed the 'critical region'. Commercial EPS in Figure 4.12 consequently has a critical region from 0.0075 and 0.18% EPS. A critical

region probably also exists for ferment EPS but the maximum limit lies above the concentrations tested.

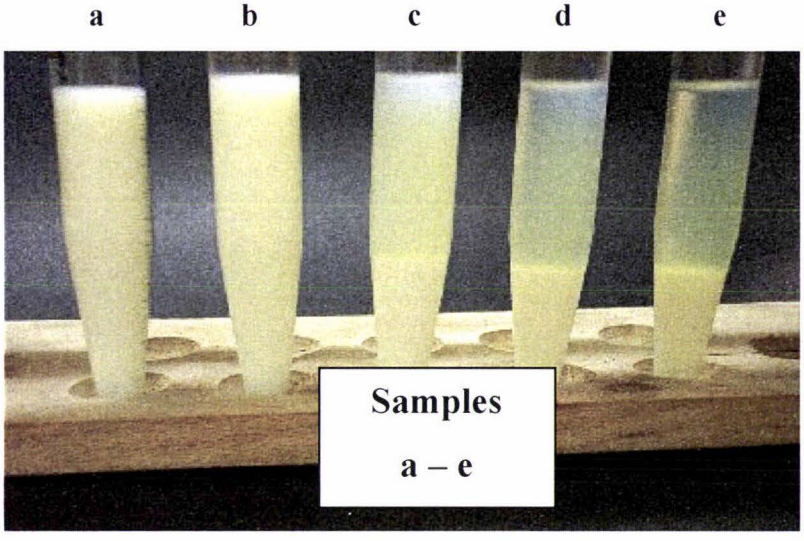


Figure 4.11 Phase separation of Ferment EPS in 10% SMP after 66 hours. EPS concentrations: a - 0%, b - 0.023%, c - 0.075%, d - 0.127%, e - 0.18%.

Increasing the total solids concentration increases the range of the critical region. This can be seen in comparing figure 4.12 and 4.13, which show the effect of total solids (10 and 15%) of SMP on phase separation. The minimum EPS concentration for phase separation decreased for all EPS sources at 15% total solids. Ferment EPS decreased to 0.0023% compared to the 0.023% for 10% SMP. The maximum limit for the critical region increased for commercial EPS exceeding 0.23% EPS. The levels of EPS tested, which do not cause phase separation, have a minimal effect on the viscosity of the solution at 15% total solids.

Figure 4.14 shows reconstituted SMP at the solids concentration of 20%. The results are virtually identical to 15% solids with phase separation occurring at almost all concentrations for the commercial and ferment EPS. There was no clear boundary between supernatant and sedimented casein at high concentrations of EPS. However the casein was visibly flocculated. Flocs of casein were held in suspension (indicated with 100% supernatant) for ferment and commercial EPS at 0.23%. At high EPS concentrations the high viscosity of the solution limited the sedimentation of the flocs, which was also found by Hemar et al (2001).

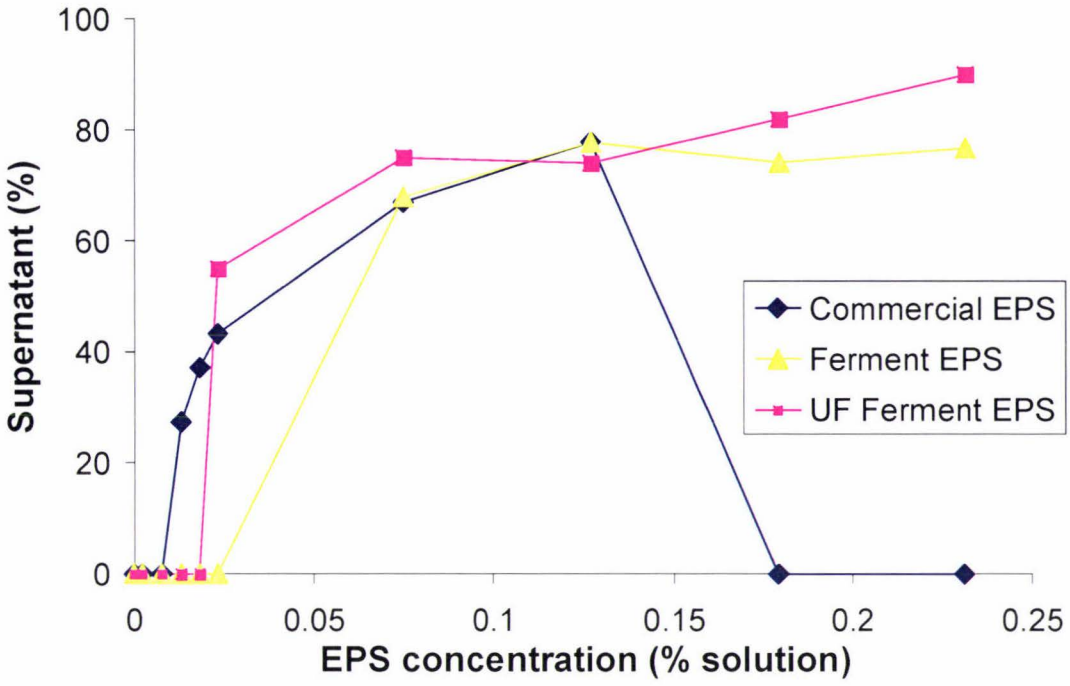


Figure 4.12 Degree of phase separation for commercial, ferment, and UF ferment EPS in 10% reconstituted SMP after 72 hours storage at 5°C.

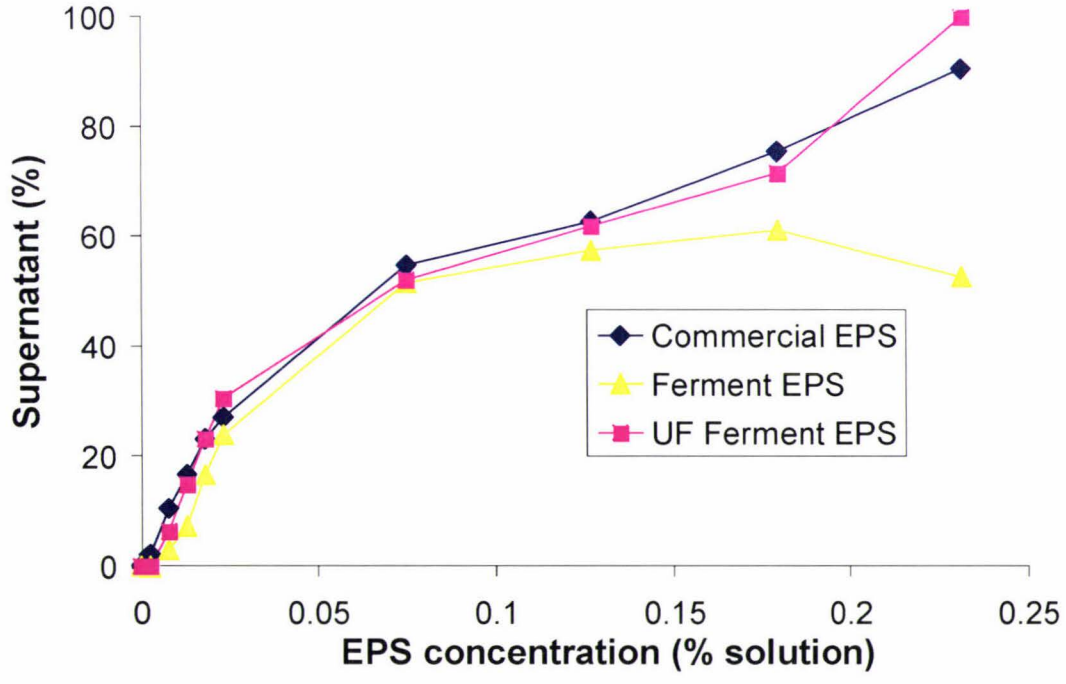


Figure 4.13 Degree of phase separation for commercial, ferment, and UF ferment EPS in 15% reconstituted SMP - 72 hours storage at 5°C.

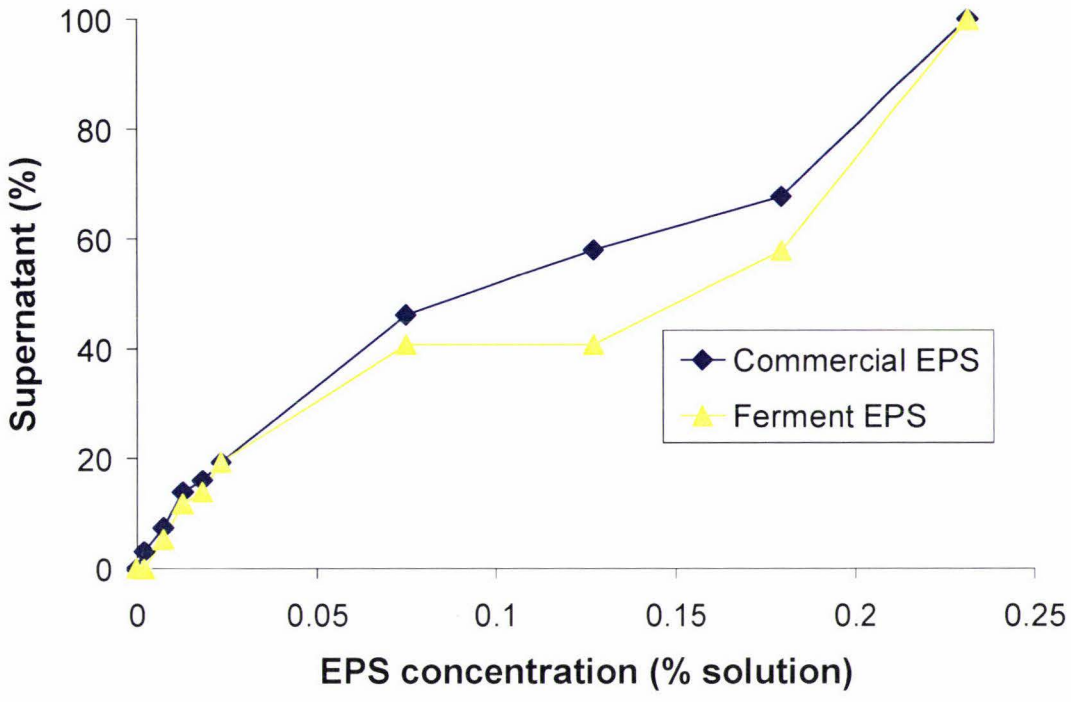


Figure 4.14 Degree of phase separation for commercial and ferment EPS in 20% reconstituted SMP after 72 hours storage at 5°C.

4.2.5.2 Reconstituted WMP

Like SMP, phase separation occurred with reconstituted WMP. The critical region was dependent upon the EPS and total solids concentration. Phase separation results for reconstituted 10% WMP are shown in figure 4.15; these results were similar to reconstituted 10% SMP. A difference did occur with the upper limit for phase separation for the commercial EPS being lower at 0.13% as opposed to 0.18% for SMP. Flocculation was also apparent with the ferment EPS at 0.23%.

Figure 4.16 shows the phase separation for reconstituted 15% WMP. As the total solids content increased the critical region is reduced at the higher levels of both ferment and UF ferment EPS. The maximum concentration of 0.23% was enough to stabilise the suspensions, due to higher viscosity at 15% total solids. Figure 4.17 shows a graph of WMP reconstituted at 20% total solids. Compared to the 10% solids levels the critical region is again reduced for both commercial, ferment and UF ferment EPS.

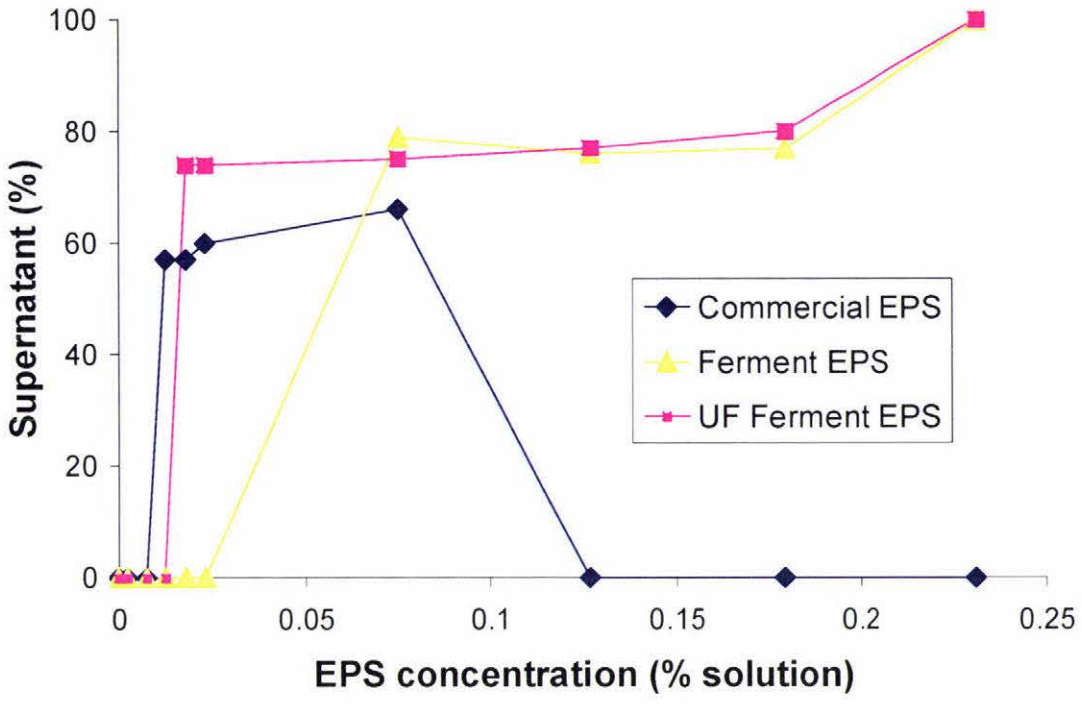


Figure 4.15 Degree of phase separation for commercial, ferment, and UF ferment EPS in 10% reconstituted WMP -72 hours storage at 5°C.

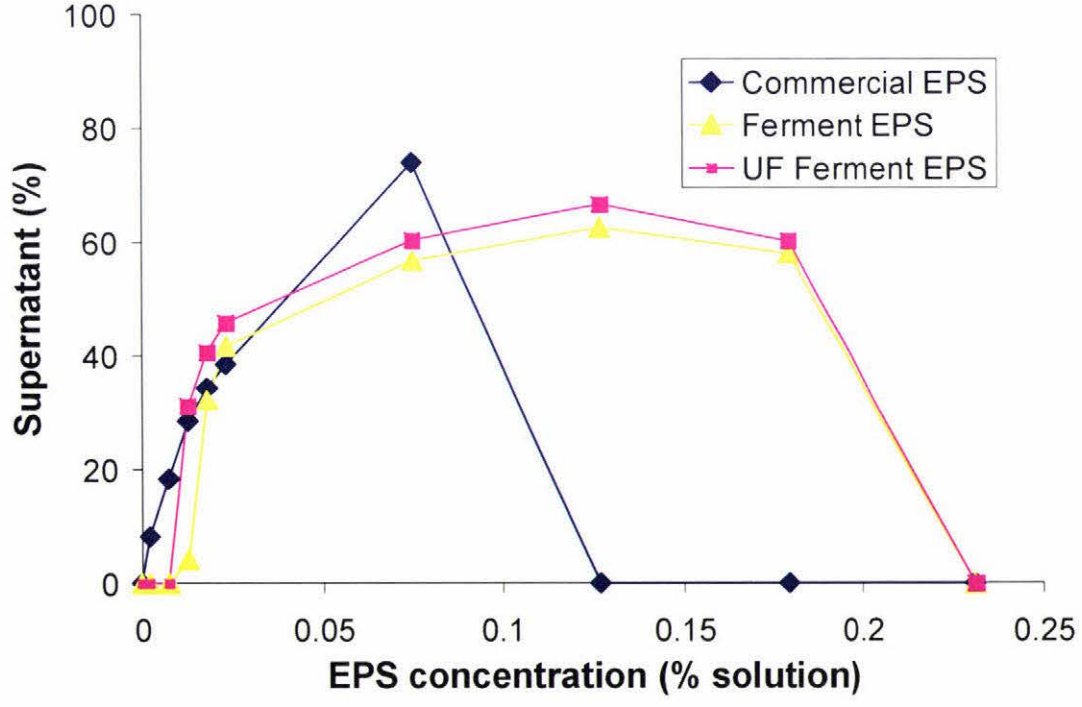


Figure 4.16 Degree of phase separation for commercial, ferment, and UF ferment EPS in 15% reconstituted WMP - 72 hours storage at 5°C.

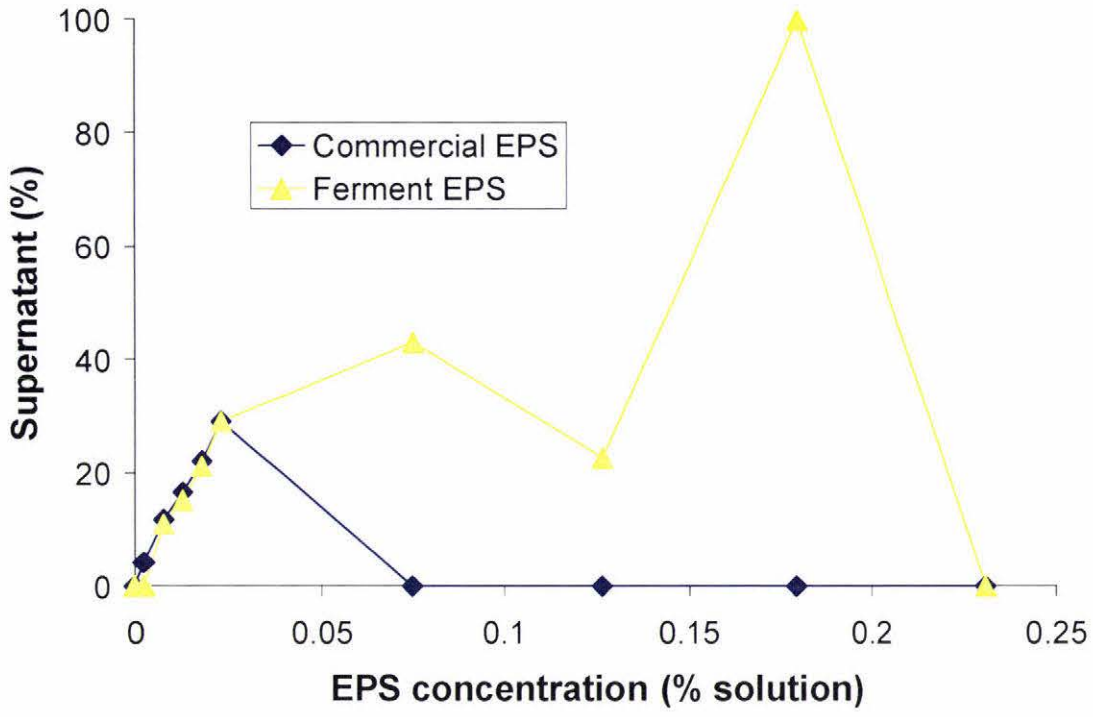


Figure 4.17 Degree of phase separation for commercial and ferment EPS in 20% reconstituted WMP after 72 hours storage at 5°C.

Phase separation was dependent upon the EPS concentration. At low concentrations of EPS, no phase separation occurs, until the concentration is sufficient to induce depletion flocculation. At the upper end of the critical region, the high viscosity of the solution limits phase separation. Flocculation did occur at these high concentrations but the casein was prevented from sedimenting. The high viscosity restricts the movement of the casein micelles, and holds the particles in suspension. The systems containing commercial EPS showed signs of phase separation at lower EPS concentrations than the ferment EPS, but were generally more stable at higher EPS concentrations. As the system was essentially in a steady state during the phase separation, it was the viscosity at very low shear rates which was relevant. The critical region difference between the commercial and ferment EPS is likely due to the higher viscosity of the commercial EPS at the low shear rates as was shown in figures 4.5 to 4.8. Table 4.1 shows the critical regions for the reconstituted powders containing commercial, ferment, and UF ferment EPS.

Table 4.1 Critical concentrations for phase separation of reconstituted milk powders containing EPS. (*) Denotes no maximum upper limit reached (For values less than 0.23%).

Powder Type / Solids Concentration	Commercial EPS (% of solution)	Ferment EPS (% of solution)	UF Ferment EPS (% of solution)
WMP / 10%	0.0075 – 0.13	0.023 – *	0.013 – *
WMP / 15%	0 – 0.13	0.0075 – 0.23	0.0075 – 0.23
WMP / 20%	0 – 0.075	0.0023 – 0.23	Not Tested
SMP / 10%	0.0075 – 0.18	0.023 – *	0.0185 – *
SMP / 15%	0 – *	0.0023 – *	0.0023 – *
SMP / 20%	0 – *	0.0023 – *	Not Tested

4.2.5.3 Rate of Phase Separation

The level of supernatant gives an approximation of the extent of phase separation. Phase separation occurs quickly at certain EPS concentrations. The first visible signs occurred after 1½ hours for ferment EPS and 5 hours for commercial EPS in 10% SMP. A majority of the phase separation occurred over the first 24 hours while the 24 - 72 hour period showed minimal change. These results were dependent upon EPS concentration as the systems containing high concentrations show slower rates of separation. Figure 4.18 illustrates this for WMP containing 15% solids and various levels of UF ferment EPS. At 0.18% EPS most phase separation occurred over the 24 - 48 hour period.

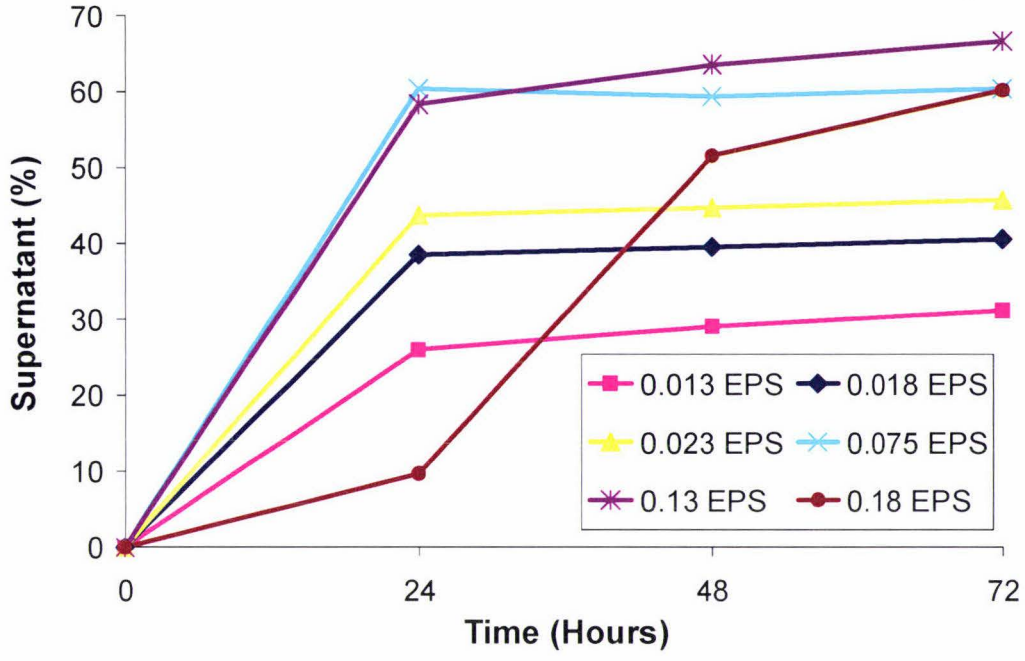


Figure 4.18 Rate of phase separation in 15% WMP solution containing UF Ferment EPS at varying concentrations.

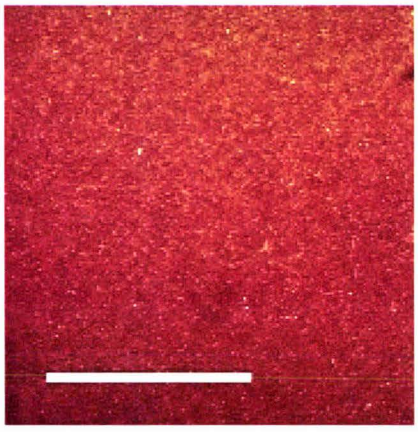
4.2.5.4 Confocal Scanning Laser Microscopy (CSLM)

CSLM was used to look at the physical effect created by the presence of EPS in the reconstituted SMP and WMP. Fast green and Nile blue dyes were used to stain the protein and lipid, respectively in WMP solutions while fast green only was used to stain the protein in the SMP solutions. The protein showed up as orange on the CSLM pictures while the lipid showed up as green. CSLM micrographs for 10% reconstituted SMP and WMP without added EPS are shown in Figures 4.19a and 4.21a, respectively. Reconstituted milk powders showed an even distribution of protein, fat and other constituents. The addition of EPS changed this distribution, causing the formation of two separate phases. Figures 4.19b-e and 4.20a-d show reconstituted SMP containing ferment and commercial EPS, respectively, at various levels of EPS. The black regions likely contain polysaccharide while the orange regions are protein. Flocculation of the protein occurs at all concentrations, although the nature of the aggregation varies. The CSLM pictures confirm that there are no attractive interactions between the polysaccharide and casein micelle, hence the formation of the separate phases. This was also observed by Hemar et al. (2001).

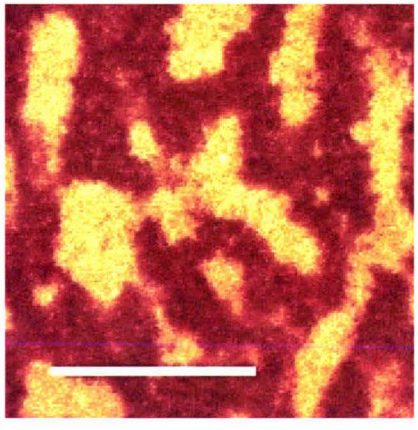
The shape adopted by the protein aggregates was driven by the osmotic pressure difference between the EPS containing external phase and the EPS depleted solution around the casein micelles. At low EPS concentrations the osmotic driving force was sufficient to cause phase separation, but too weak to cause the protein particles to pack tightly together. As the EPS concentration was increased, the driving force increased and the protein aggregates became denser. They also tended to become more spherical, so to minimise the interaction energy at the interface between the phases, and maximise the interaction between the casein micelles. At higher EPS concentrations the increasing viscosity of the system would have impeded these changes. In this work the samples were mixed immediately before CSLM, so the pictures show the state of the systems at this time. The results over a longer time might well be different. The shapes of the protein aggregates formed were in fact different to those obtained by Hemar et al. (2001). Their samples were prepared and left overnight and were not mixed immediately before CSLM.

Ferment and commercial EPS formed different shaped protein aggregates. Commercial EPS generally had smaller aggregates than ferment EPS at the same EPS concentration and the shapes were more irregular. This was probably due to commercial EPS being more viscous (at lower shear rates) and also more aggregated in solution.

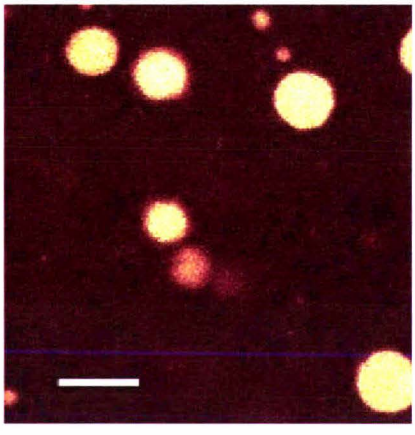
Figures 4.21a-e and 4.22a-d show the confocal microscopy pictures for WMP containing various concentrations of ferment and commercial EPS, respectively. Again, the addition of EPS caused the formation of separate phases, agglomerates of protein surrounded by the polysaccharide phase. The shapes adopted by the aggregates showed the same trend as those formed with reconstituted SMP containing EPS. Emulsified fat (green) was distributed throughout the polysaccharide and protein phases, which can be seen clearly in Figures 4.21d and 4.22c. The emulsified droplets would have had a proteinaceous exterior, including some casein, but were not flocculated like the casein micelles. The emulsion droplets were much larger than casein micelles, and evidently not subject to depletion flocculation. Figure 4.22d shows a complex arrangement of protein no longer containing an even proportion of spherical agglomerates but rather a matrix-type assembly. This is indicative of a gel-like structure and no phase separation was observed with this sample.



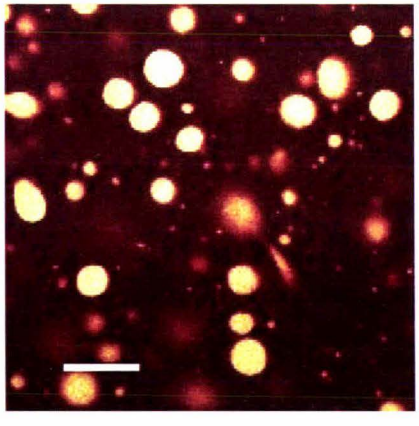
a



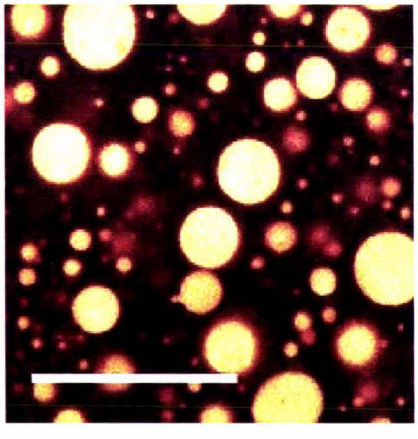
b



c



d



e

Figure 4.19 **CSLM of reconstituted SMP at 10% solids containing ferment EPS. EPS concentrations were: a) 0%, b) 0.046%, c) 0.093%, d) 0.14%, e) 0.23%. Orange = protein. Scale bar = 50μm. Figure 4.19 c and d used a 40x objective 1.0NA oil immersion lens.**

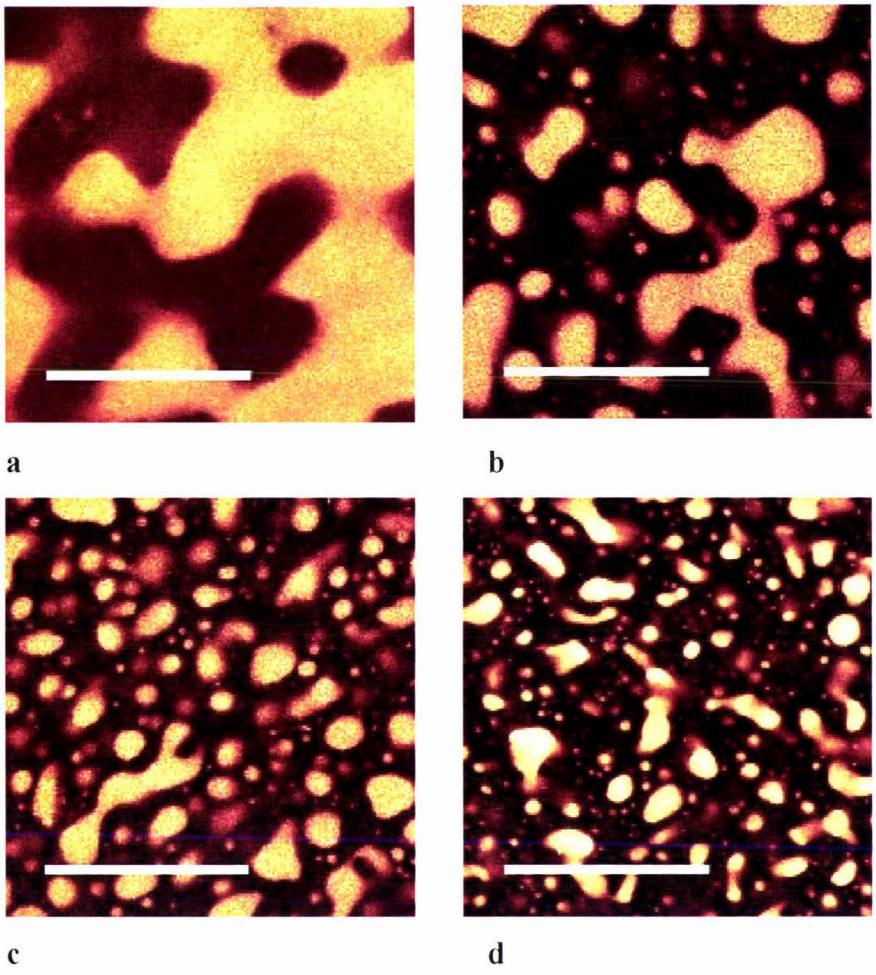
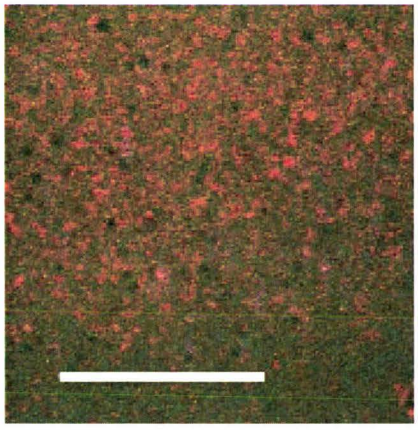
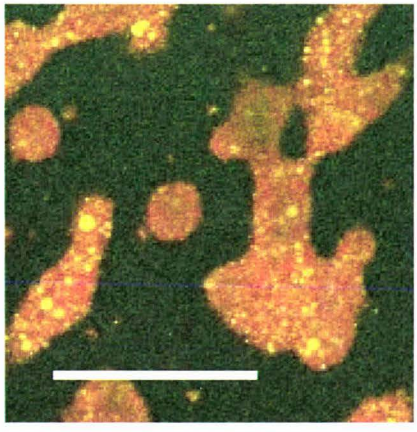


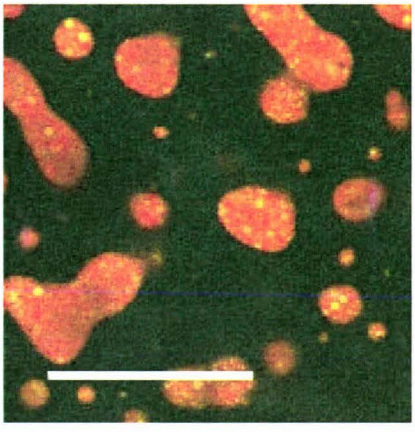
Figure 4.20 **CSLM of reconstituted SMP at 10% solids containing commercial EPS. EPS concentrations were: a) 0.046%, b) 0.093%, c) 0.14%, d) 0.23%. Orange = protein. Scale bar = 50μm.**



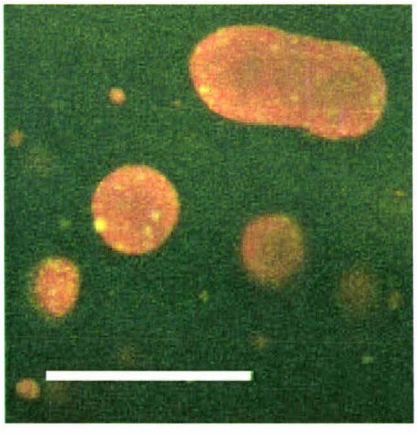
a



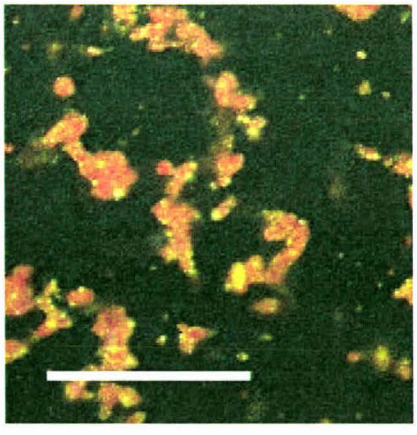
b



c

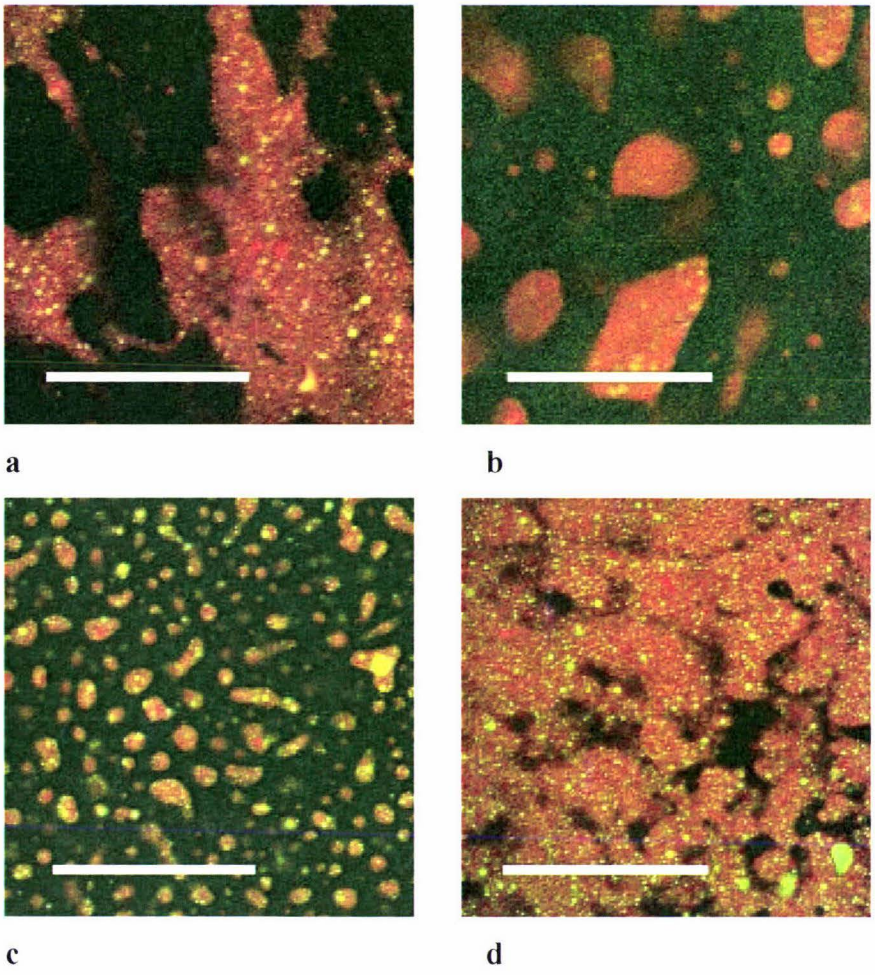


d



e

Figure 4.21 **CSLM of reconstituted WMP at 10% solids containing ferment EPS. EPS concentrations were: a) 0%, b) 0.046%, c) 0.093%, d) 0.14%, e) 0.23%. Orange = protein, green = lipid. Scale bar = 50μm.**



a b

c d

Figure 4.22 **CSLM of reconstituted WMP at 10% solids containing commercial EPS. EPS concentrations were: a) 0.046%, b) 0.093%, c) 0.14%, d) 0.23%. Orange = protein, green = fat. Scale bar = 50μm.**

4.2.6. Sensory Testing

4.2.6.1 Total solids replacement

A triangle test was used to determine whether consumers could notice the incorporation of commercial EPS replacing some milk solids. Two samples were tested; a 15% WMP solution and a 13.3% WMP solution containing 0.02% commercial EPS. 11 of the 32 consumers tested correctly identified the different sample. Using statistical tables (Larmond, 1977) this result was not statistically significant, and thus, there was no detectable difference between the two samples. This showed that commercial EPS, therefore, can substitute for WMP total solids, with 0.02% commercial EPS replacing 11.3% of the total solids of a 15% WMP solution.

4.2.6.2 Thickened milk product

As xanthan incorporation substantially increases the viscosity of milk solutions a thickened milk product is a possible application of the technology. Spray dried WMP, containing commercial EPS, was mixed with control WMP to obtain various EPS concentrations and reconstituted. The samples tested had 15% solids containing commercial EPS at 0.015, 0.04, and 0.079%. The control consisted of a reconstituted WMP (no EPS) at 20% solids. 32 people tested the samples. They were asked to compare the samples on a hedonic scale (like/dislike) for creaminess, thickness, flavour, and overall liking of the product. An intensity scale for creaminess and thickness was also included.

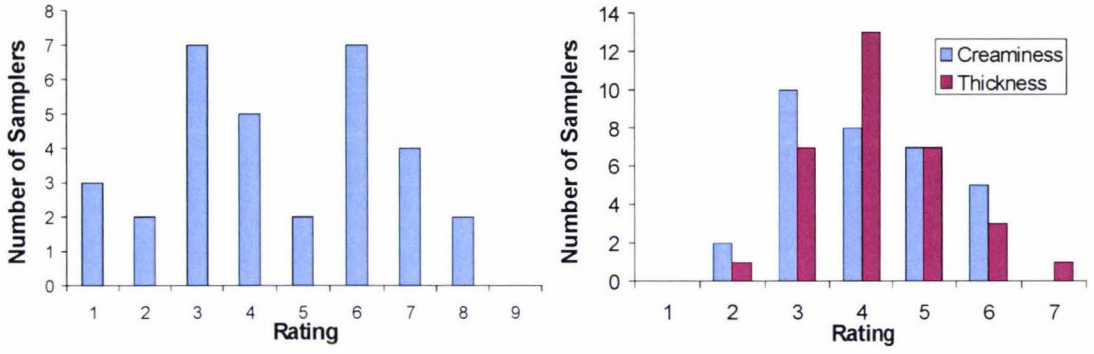
Statistical analysis showed that the flavour and the overall-liking attributes had bimodal distributions for the hedonic ratings. Figure 4.23a shows an example of this for the overall-liking rating of sample containing 15% WMP and 0.079% commercial EPS. The bimodal distribution was a reflection of people either liking or disliking the product. The people tested were not familiar with a thick milk product, and a large proportion rated the product including the control in the dislike section. A different result might be obtained with consumers accustomed to drinking thick milk. The average results were used for comparison (Appendix 1), as the distributions were very similar for both the control and commercial EPS containing samples for each

attribute. However, the average rating for a bimodal distribution does not indicate the true spread of ratings.

The statistical analysis of the hedonic (like/dislike) rating showed that there was no significant difference between the four samples for flavour, overall liking, thickness and creaminess. The addition of commercial EPS therefore had no significant effect on the tasters' liking/disliking of the product.

The intensity rating for creaminess and thickness showed a normal distribution for all four samples with the sample consisting of 15% WMP and 0.079% commercial EPS shown in figure 4.23b. For both attributes, a significant difference was found between the sample 15% WMP + 0.015% EPS and the 20% WMP control (Appendix 1). As no significant difference occurred with the hedonic rating for the two samples the results show that tasters could perceive a difference between the two samples for creaminess and thickness, however liked the samples to the same extent. No significant difference occurred for the other samples tested.

The viscosities of the samples were also measured. The viscosity of the 20% WMP control solution was very similar to the 15% WMP + 0.015% EPS at 21°C (Figure 4.24). While the viscosities were similar the two samples were found by the consumer to be different in terms of thickness and creaminess. The difference in sensory results is possibly due to the difference in solids content. Varying the solid content may change the mouth-feel of the samples. As there was minimal difference, in terms of sensory results, with the higher viscosity samples compared to the control, the higher levels of EPS may be able to compensate for the difference in solids content.



a. b.

Figure 4.23 Sensory results on sample containing 15% WMP + 0.079% commercial EPS. a) Overall liking rating, 9 = like extremely, 1 = Dislike extremely. b) Intensity rating for creaminess and thickness, 7 = high rating while 1 = low rating.

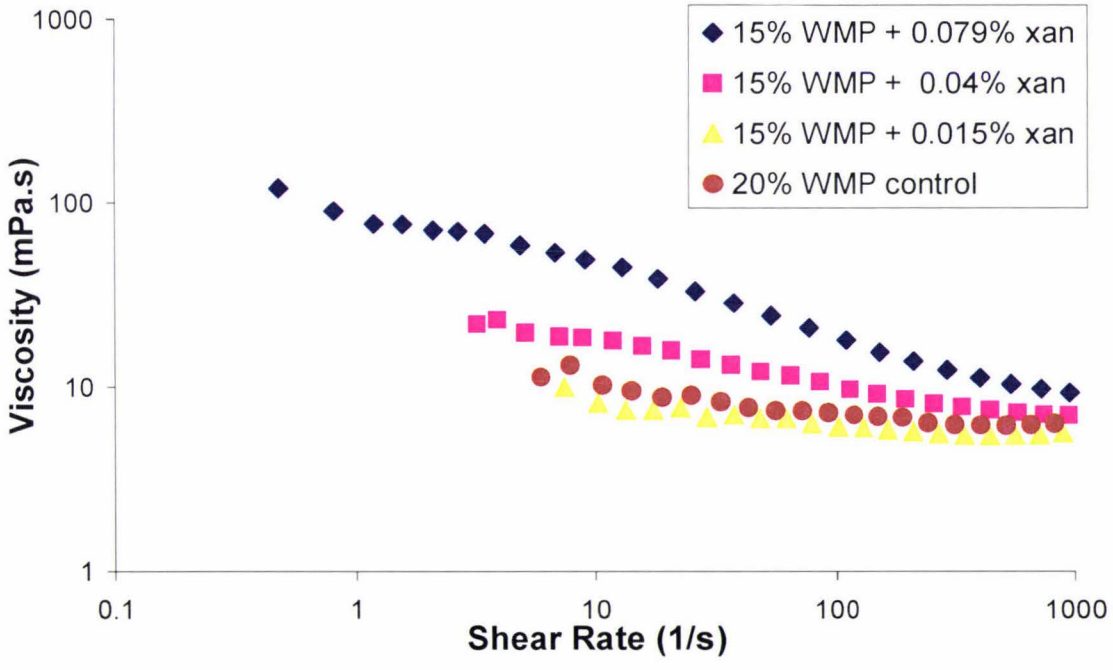


Figure 4.24 Viscosity of thickened milk samples used for sensory testing at 21°C at various shear rates.

The results of the sensory tests should not be regarded as definitive. Testing was conducted on New Zealand consumers and not the target market (Asia), and therefore

the results were not reflective of the intended consumers. New Zealand consumers are not used to this product and many indicated that that the milk was too thick.

Phase separation is a major problem with milk containing EPS. The concentrations used were in the critical region for phase separation. The samples were well mixed before sensory testing so that the panellists would not notice any separation. However a real test would be to have the product evaluated at home.

4.3 Conclusions

- Both commercial and ferment EPS were successfully added to milk solutions and spray-dried to produce milk powders containing xanthan.

- The solubility of the WMP and SMP was not affected with the addition of EPS. However the EPS addition substantially increased the viscosity and pseudoplastic tendencies of the reconstituted milk solutions.

- The commercial EPS had a higher viscosity at low shear rates and more gel-like properties compared to the ferment EPS.

- Visible phase separation occurred in reconstituted SMP and WMP at intermediate EPS concentrations. High EPS concentrations produce high viscosities that limited visual separation of the two phases. Low EPS concentrations were insufficient to cause flocculation of the casein.

- Incorporation of commercial EPS replaced milk solids with no significant influence on the sensory attributes of the product.

CHAPTER 5 INCORPORATION OF EPS INTO FRESH CHEESES

5.1 Introduction

Incorporation of the ferment EPS into MPC for cheese-making is a potential use for the polysaccharide and is covered in this section. The aim of the addition was cheese extension, which focused on the replacement of protein and the increase of cheese yield through whey retention. Polysaccharides have been shown to be effective for this purpose (Kailasapathy, 1996). As polysaccharides have different functional characteristics compared to normal cheese constituents it was important to determine the effect that the addition has on the product. Whey loss, texture and the sensory characteristics were measured, as all are important for cheese quality and could potentially be altered by the addition of EPS (Duboc and Mollet, 2001; Keogh and O'Kennedy, 1998). Ultimately the product must meet the consumer's expectations and therefore the sensory testing would be the deciding factor for the adoption of the technology.

The use of MPC for cheese-making has become a common practice and has been found to reduce the processing time and losses associated with traditional cheese making (Gilles, 1984). For these experiments, the MPC containing EPS was made into a fresh cheese similar to Panela, which has a high moisture content and a pH of 6.0. The Panela is coagulated with the use of rennet and could be consumed the day after preparation.

Cheese preparation involved the reconstitution of MPC with or without EPS. The MPC solution was mixed with emulsified milk fat, acid, salt, and water followed by the inoculation of rennet to set the cheese. Standard cheese comprised of 71.06% water, 17.37% MPC, 9.87% milk fat, 1.18% sodium chloride, 0.37% lactic acid, and 0.15% calcium lactate. Low MPC cheese comprised of the same proportion of ingredients, except for the water and MPC, which equalled 74.06% and 14.37%, respectively.

5.2 Results and Discussion

5.2.1. Solubility of MPC Powder

The solubility results for MPC powders with or without EPS are shown in Figure 5.1. A significant difference existed between all powders except between the MPC containing ferment EPS and the control. The differences may be partially attributed to the extra pre-heat treatments given to the reconstituted powders before spray-drying. The temperature was held at 50°C at times for a few hours while the spray-drying took place due to the low throughput of the spray dryer. The prolonged mild heat treatment may cause a greater degree of whey protein denaturation altering the distribution of some calcium ions and hence the aggregation of casein micelles (Muldoon and Liska, 1972; Walstra and Jenness, 1984c; Singh and Waungana, 2001). The different solubility index obtained for MPC, containing ferment EPS, compared to commercial EPS is difficult to explain as the solubility index for MPC containing ferment EPS is much lower than for MPC containing commercial EPS. A possible reason for the difference is that the commercial EPS has been more extensively processed while ferment EPS contains components and products of the fermentation which may affect the EPS/protein interaction. However, this difference was not observed for the SMP and WMP solutions containing ferment and commercial EPS even though they contained the same types of EPS.

Figure 5.1 Solubility in water of MPC, containing EPS at 2.3% EPS, on solid basis with 95% Confidence Intervals

5.2.2. Loss of Whey

For whey loss determination, fresh cheese was cut into cubes and centrifuged for 10 minutes. The whey was then poured off, weighed and the percentage whey loss calculated in relation to the initial mass of cheese. The addition of EPS to cheese decreased the amount of whey lost. The reduction for commercial EPS can be seen in Figure 5.2. An EPS concentration of 0.161% decreases the whey loss from ~18.5% to ~2.8% in cheese made with 17.37% MPC. It seems that even a small addition (0.05%) of EPS is sufficient to reduce whey loss significantly. UF ferment EPS had the same effect as commercial EPS as shown in Figure 5.3. The addition of 0.161% EPS reduced the whey loss from ~18.7% to ~4.7% for cheese made with 17.37% MPC. The increase in cheese yield is considerable.

Whey loss is determined by the contraction pressure of the casein and the ability to vacate the cheese (Castillo et al. 2000). These factors were the basis for the reduction of the whey loss upon addition of the EPS. The reduction is probably caused by the limiting of the flow of whey, through the binding and interaction of EPS with water and other constituents causing a marked increase in the viscosity of the whey portion

(Duboc and Mollet, 2001). The tendency to aggregate, particularly of the commercial EPS, also has the potential to block pores again limiting flow. The second mechanism leading to the decreased whey loss is caused by some inhibition of casein aggregation. The separate phases formed with the addition of EPS limits the casein aggregation, which was also found by Olsen (1989). This means the curd formed is not as compact leading to less whey being released from within the casein matrix.

Figures 5.2 and 5.3 show that the protein concentration is important in determining the amount of whey lost from the cheese. The lower the amount of MPC in the cheese, the greater the amount of whey lost. The MPC concentration is, therefore, important as the increasing water content may affect the final cheese product with regards to texture and other sensory attributes.

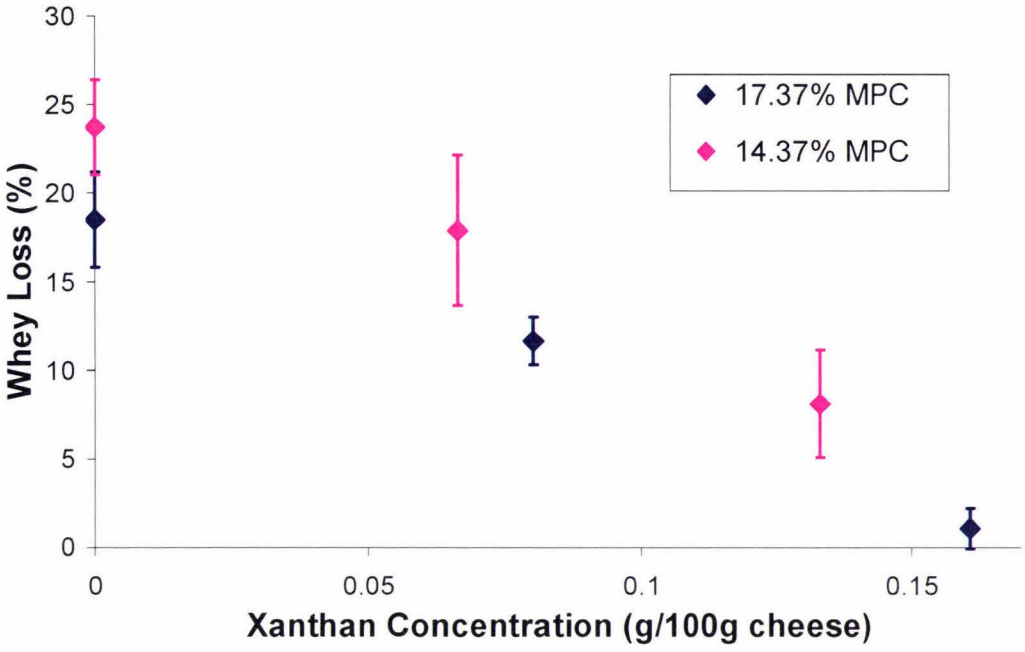


Figure 5.2 **Loss of whey from cheese made with MPC containing different levels of commercial EPS, with 95% confidence intervals**

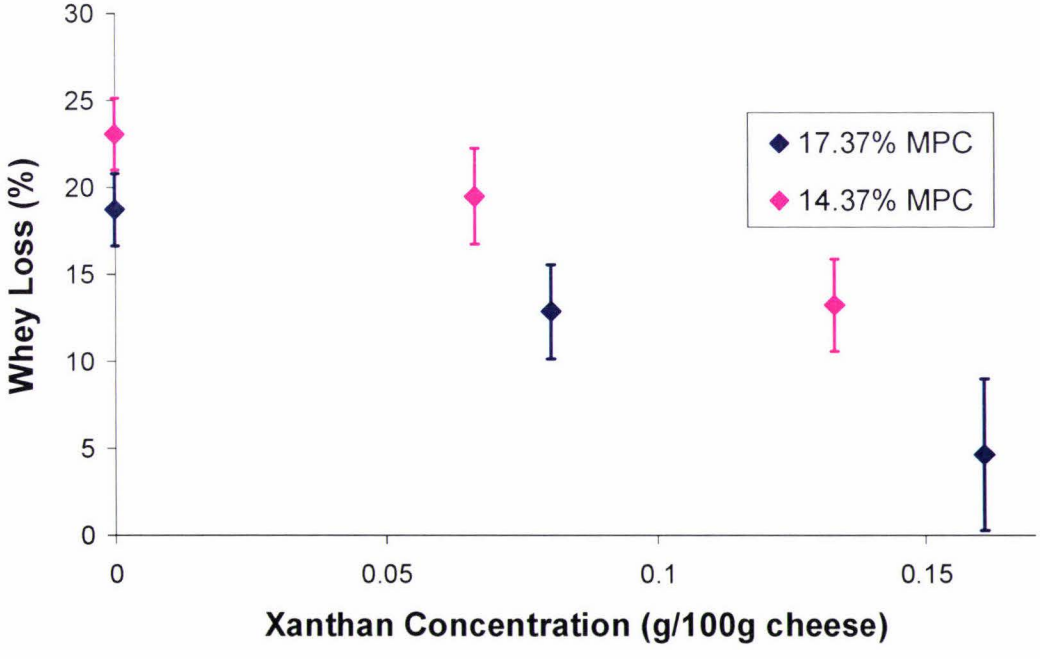


Figure 5.3 **Loss of whey from cheese made with MPC containing different levels of ferment EPS, with 95% confidence intervals**

5.2.3. Cheese Texture

As cheese texture is an important attribute for cheese quality, the effect of the EPS addition on texture was determined. A compression test with a TA-XT2 texture analyser was used for analysis on 1cm^3 samples. Figure 5.4 shows the results of the compression test for the cheese containing commercial EPS. Increasing EPS concentration caused a decrease in cheese firmness, which was also observed previously by Olsen (1989). The addition of 0.161% EPS to 17.37% MPC cheese reduced the firmness from 5.56N.s to 3.56N.s. This was a decrease of 36%. The ferment EPS showed similar results with the addition of 0.161% ferment EPS decreasing cheese firmness from 5.76N.s to 3.44N.s for 17.37% MPC cheese (Figure 5.5). This was a decrease of ~40%. While the ferment and commercial EPS results were almost identical for cheese made with 17.37% MPC they did differ slightly at the 14.37% MPC level. At this level, the cheese is considerably softer compared to the 17.37% cheese indicating that the total solids content also has a large influence on firmness.

The formation of separate phases of polysaccharide and protein was largely responsible for the decrease in firmness. Olsen (1989) observed that the curd formation was slowed by the formation of the separate phases. Phase separation therefore reduces the amount of casein aggregation taking place over the 40-minute setting period. Along with this, the formation of separate phases causes localised aggregation. This would reduce the number of bonding sites and the overall strength of the network (Olsen, 1989). The separate phases also make the cheese matrix less homogeneous. During the compression test, the heterogeneous texture of the EPS would not dissipate the force evenly throughout the system. This would cause fractures to appear earlier leading to faster breakdown of the structure at a lower applied force (Prentice, 1992).

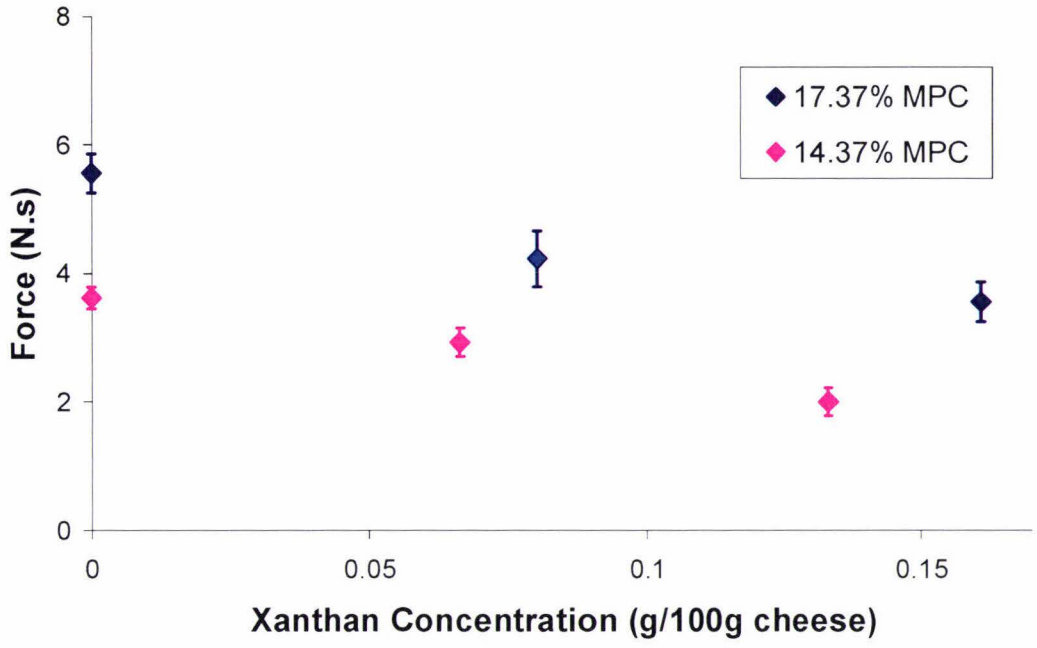


Figure 5.4 **Firmness of cheese made with MPC and commercial EPS with 95% confidence intervals**

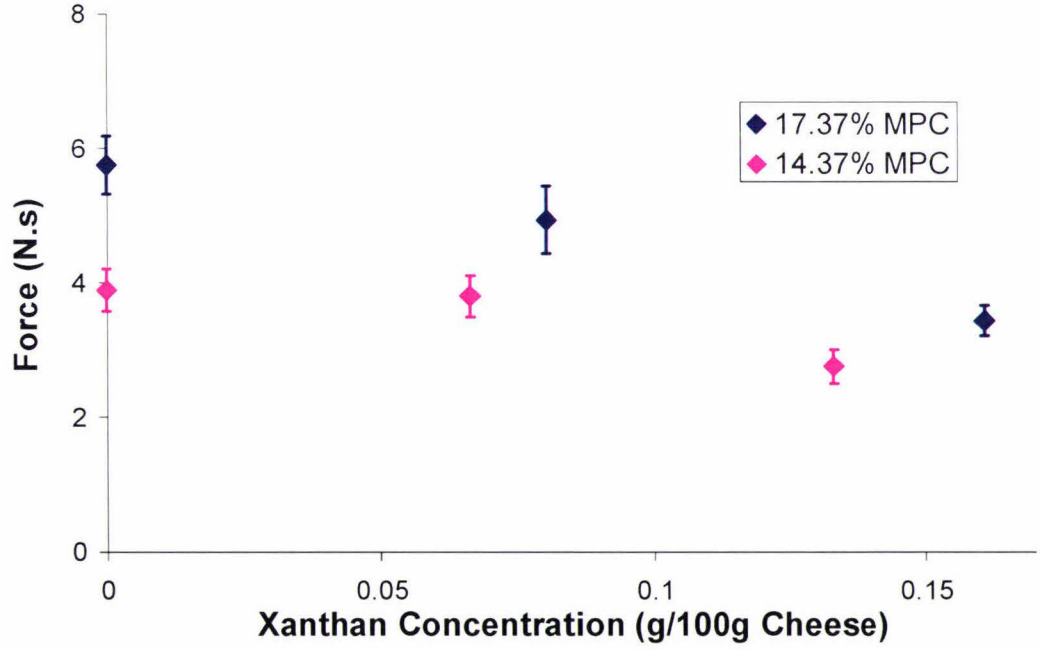


Figure 5.5 **Firmness of cheese made with MPC and ferment EPS with 95% confidence intervals**

5.2.4. Incorporation of EPS into Other Cheeses

The effect of EPS addition to other cheese varieties would probably differ from the results obtained for Panela. The large proportion of water within Panela allows xanthan both greater mobility and water interaction. Hard cheeses comprise low water contents and higher proportions of casein and fat, which would interact differently with xanthan (Dickinson, 1998). The pH of Panela (6.0) is also higher compared to the pH for a majority of cheeses. As the extent of casein micelle aggregation increases with decreasing pH, the degree of micelle aggregation would be reduced at this high pH. Both attributes could potentially affect the whey loss and texture of the product.

The amount of heat denatured whey proteins in the cheese in these tests would also have been higher than that of normal Panela made from MPC. The cheese made in the trials had undergone an extra heating and spray-drying step potentially increasing the amount of whey protein that had been denatured (Wheelock and Kirk 1973; Singh et al. 1988). Denatured whey protein (namely β-lactoglobulin) has been shown to form a complex with κ-casein. This interferes with aggregation producing a softer cheese (Schreiber and Hinrichs, 2000; Singh and Waungana, 2001). The control MPC was also spray-dried twice to accommodate this difference.

5.2.5. Confocal Microscopy of Cheese

The cheese structure was examined using confocal microscopy to determine the physical changes occurring upon the addition of the EPS. The results clearly showed the formation of separate protein and polysaccharide phases. Cheese containing no EPS had a relatively even distribution of protein (orange/yellow) as seen in Figure 5.6a. Figures 5.6b-d shows the effect of the addition of ferment EPS leading to the formation of separate phases of polysaccharide and protein. The polysaccharide phase increased in size with increasing EPS concentration, decreasing the size of the protein aggregates formed. This was also observed with the addition of commercial EPS (Figures 5.7 a-d).

The CLSM results support the previous explanation for the effects of a softer cheese and of EPS on texture and whey loss. The localised casein aggregation decreased the

overall amount and extent of bonding throughout the network. This decreased the overall pressure exerted by the casein network expelling the whey leading to a reduction of the whey expelled. The increased heterogeneous nature of the system also leads to a softer cheese. The addition of either polysaccharide at concentrations of 0.0155 % is enough to cause significant phase separation.

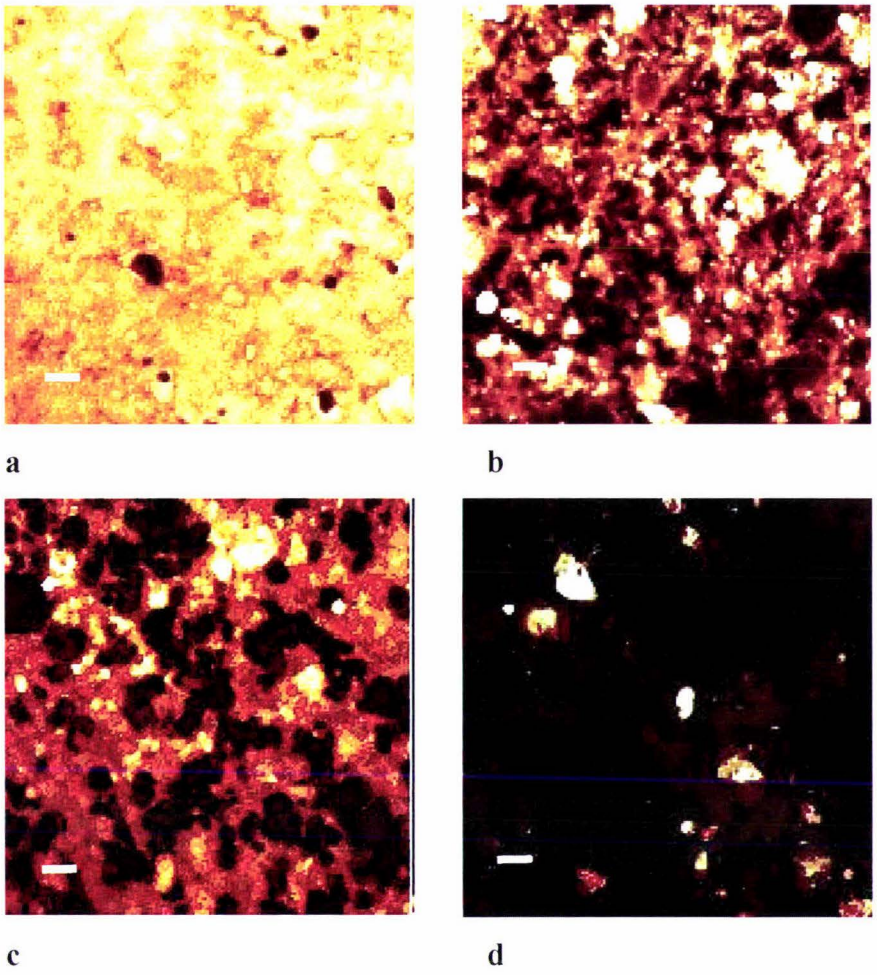


Figure 5.6 **CSLM of Panela cheese containing ferment EPS at a) 0% EPS (control), b) 0.0155% EPS, c) 0.0773% EPS, d) 0.155% EPS. Scale bar = 50μm.**

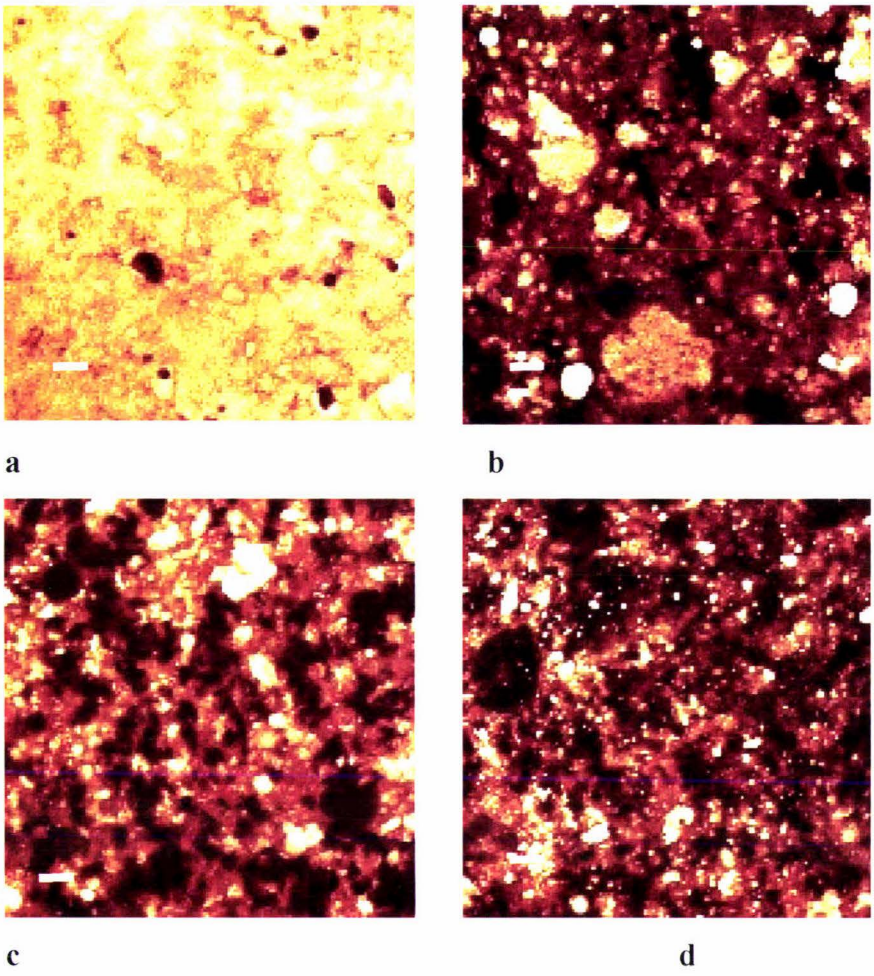
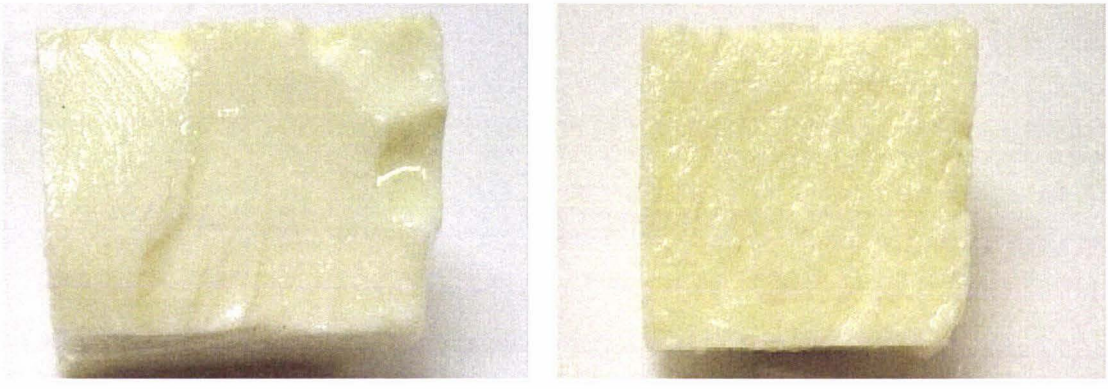


Figure 5.7 **CSLM of Panela cheese containing commercial EPS at. a) 0% EPS (Control), b) 0.0155% EPS, c) 0.0773% EPS, d) 0.155% EPS. Scale bar = 50μm.**

5.2.6. Sensory Properties of Cheese

EPS addition altered the visual appearance of the cheese. Panela, with no addition of EPS, had a smooth surface when it was cut or broken as shown in Figure 5.8a. Addition of low amounts of commercial EPS such as 0.062%, led to the formation of a granular appearance (Figure 5.8b). This granular appearance was present at almost all concentrations tested and the severity increased with increasing EPS concentration. The granular appearance is a consequence of the formation of separate phases seen with CSLM. The incorporation of high levels of EPS would be noticeable during sensory trials and may affect the mouth-feel of the product.



a. b.

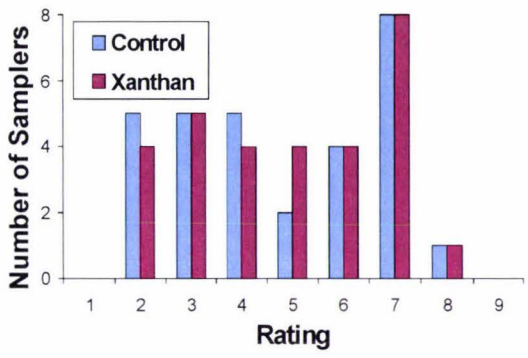
Figure 5.8 Panela cheese samples containing 17.37% MPC: a) 0% EPS (control), b) 0.062% EPS.

Consumer sensory analysis was conducted on two cheese samples. The aim was to determine if consumers could detect differences between cheese with or without EPS. The control cheese contained 17.37% MPC while the cheese containing EPS had 15.6% MPC + 0.045% commercial EPS to substitute for the reduced solids. Consumer testing rather than a trained panel was used. A 9-point hedonic scale of like/dislike was used for analysis with comments being made after tasting each sample. The attributes included the overall-liking, flavour and texture while an intensity scale rated the saltiness of the product.

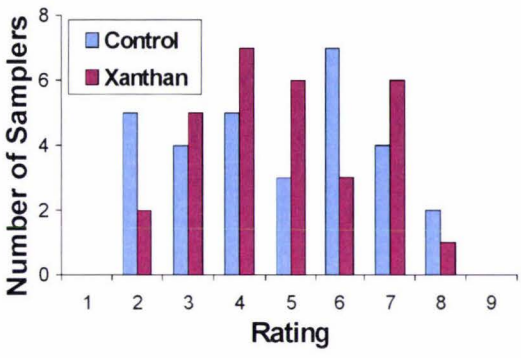
The results showed bimodal distributions and large rating spreads with the hedonic scales (Figures 5.9a-c) for the 30 people tested. One of the main problems was that the people tested were not from the countries where the products would be consumed,

so a majority had not tried these products before. A bimodal result may not have been expressed had normal consumers been trialled. Saltiness also showed a similar distribution for both the control and xanthan-containing samples (Figure 5.9d). One-way ANOVA and Tukey's pair wise comparison was used for analysis (Appendix 2). Comparison of the averages had to be done carefully due to the spread of the ratings, but was permissible as the distribution of the xanthan and control samples were similar. However, the averages did not reflect the spread well. The results showed that there was no significant difference between the two samples for all attributes tested with the averages being virtually identical.

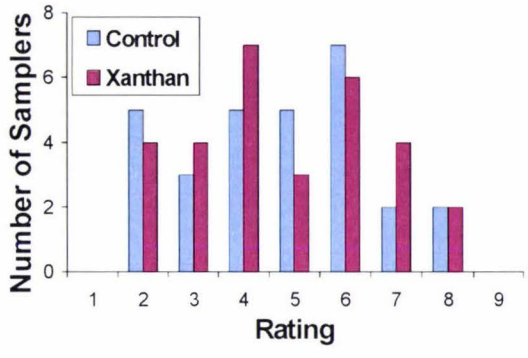
While the hedonic ratings showed no significant difference in liking of the products, the tasters' comments showed that they noticed a difference between samples. This indicates that the tasters liked the products to the same extent even though there was a difference between samples. The comments for the majority of the people tested were that both samples were bland and lacked flavour. The comments on texture indicated that the control had a rather gel like structure, described as tofu, jelly, boiled egg, leathery, and rubbery. The EPS containing sample had more people pointing towards a more granular texture with comments such as crumbly, granular and foamy. The products were clearly perceived as different with the control having a more jelly-like texture while the addition of EPS created a crumbly/granular texture at the 0.045% EPS level. Future testing should be conducted on consumers familiar with the product and a trained sensory panel should be used to determine the effects of an addition of EPS and to clarify the terminology used.



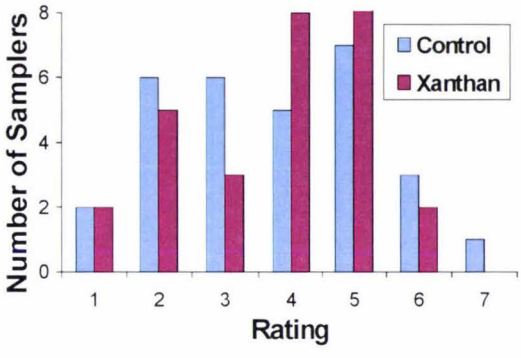
a - Overall Liking



b - Flavour



c - Texture



d - Saltiness

Figure 5.9 **Sensory ratings for cheese samples. 18a-c show hedonic ratings with 1 = dislike extremely, 9 = like extremely. Figure 18d shows an intensity rating 1 = Not Salty, 7 = Very Salty.**

5.3 Conclusions

- Commercial and ferment EPS could be successfully incorporated into fresh cheese.

- Phase separation of the EPS and casein micelle was apparent with the cheese system, which caused a granular appearance.

- Incorporation of EPS significantly reduced the loss of whey from cheese.

- Cheese firmness decreased with the addition of EPS.

- Commercial EPS addition to fresh cheese at the 0.045% level had a minimal effect on the flavour, saltiness and overall-liking however a more granular texture was apparent.

CHAPTER 6 GENERAL CONCLUSIONS AND RECOMMENDATIONS

A xanthan EPS was produced from the fermentation of *Xanthomonas campestris* on a by-product of the dairy industry, milk permeate. The main aim of the project was to incorporate EPS into milk solutions followed by spray-drying. The goal was replacement of solids or an improvement to the functional properties of the reconstituted powders. Both commercial and ferment EPS were used in the trials with incorporation into MPC, WMP and SMP. The practical application of the technology focused toward extending the yield of cheese made from MPC and a reconstituted milk drink.

6.1 Functionality of Spray Dried Powders Containing EPS.

The powders containing EPS were functionally different from the original powders. In terms of solubility there was no significant difference for SMP and WMP containing EPS compared to the control. There was a significant difference in solubility with MPC containing commercial EPS compared to the control. For all powders the amount of EPS added (2.3%) was much higher than would normally be used. Any difference in solubility for the powders would likely decrease with decreasing EPS concentration. The EPS incorporation alters many of the rheological properties of the reconstituted milk powder imparting a high viscosity and a pseudoplastic flow tendency.

Phase separation is a major problem for powders containing both xanthan and casein. When the powders containing commercial and ferment EPS were reconstituted, casein flocculated immediately and sedimented during storage. A critical region was observed for phase separation. At high EPS concentrations, the high viscosity limited flocculation while low EPS concentrations were insufficient to cause flocculation. Phase separation occurred between these high and low EPS concentrations. Production of a milk drink from the EPS containing powder would mean the drink would have to be consumed immediately and not left in the

refrigerator overnight. The incorporation may not be a problem if the powder is used as a food ingredient rather than a reconstituted dairy product. Phase separation is an undesirable property that must be overcome before the EPS incorporation can be successful.

6.2 Differences Between Ferment and Commercial EPS

Solutions of ferment and commercial EPS were found to differ in viscosity at low ($<1s^{-1}$) shear rates. This can probably be attributed to a difference in the degree of aggregation, due to the different processing treatments given to the two EPS. Commercial EPS is reported to undergo a more severe heat treatment, which could cause a greater amount of aggregation, unlike ferment EPS which was only heated to 90°C. In terms of functionality, the higher viscosity at the low shear, likely caused a difference in the critical regions for phase separation.

The ferment and commercial EPS also differed in the amount of purification. Commercial EPS had been purified through alcohol precipitation and dried removing a considerable amount of volatile and fermentation bi-products. Ferment EPS had not been purified and had an undesirable odour. The major constituents of this odour included p-cresol and m-cresol; however, other compounds were likely present that could not be extracted in sufficient quantities to measure. p-Cresol was released from the conjugate form through hydrolysis by *Xanthomonas campestris*. The odour was reduced considerably by ultra-filtration, with a dilution factor of 98 Cf reducing the concentration from 138ppb to <5ppb, making the product more acceptable to the consumers. Granulated activated carbon was very effective at removing odour compounds at 1.78 H.S.V for fresh carbon but it was difficult to remove the carbon fines. If the carbon fines can be removed from the ferment then the GAC process may be more effective and cheaper then running a UF plant for the purpose of odour removal.

6.3 Production of Products from Milk Powders Containing EPS

6.3.1. Thickened Milk Product

A thickened milk product is a possibility assuming the problem of phase separation can be overcome. An EPS addition of 0.079% EPS in a 15% WMP solution had a considerably higher viscosity than a 20% WMP solution. Sensory trials showed that the addition did not cause a significant difference with regards to the consumers' opinion of the two products. WMP at 15% with 0.015% EPS had virtually the same viscosity as a 20% WMP solution at 21°C, however a textural difference could be noticed in terms of thickness and creaminess. A higher viscosity may be required to compensate for the change in mouth-feel caused by a reduction in total solids. More in-depth sensory analysis is required on the target consumer group.

6.3.2. Total Solids Replacement

The consumer test showed that there was no detectable difference between a 13.3% reconstituted WMP solution containing 0.02% commercial EPS and a 15% reconstituted WMP solution. As the consumer will reconstitute the product to 15% total solids, lactose could be added to make up the difference in weight. Again phase separation was apparent in the EPS sample tested limiting the extent of the application.

6.3.3. Cheese

The addition of EPS to cheese leads to the retention of whey though the decreased casein aggregation and increasing viscosity of the continuous phase. At 0.161% EPS the whey loss decreased by ~75% for ferment EPS and ~85% for commercial EPS. The potential increase in yield is substantial. The addition of EPS decreased the firmness considerably by approximately 40% for 0.161% EPS. Phase separation was thought to be largely responsible for the decreased firmness caused by localised aggregation of the casein and the increasing heterogeneity of the network. Visibly the cheese appeared granular which became more apparent with increasing EPS concentration. This problem limits the application of the technology as only low EPS concentrations can be used.

6.4 Recommendations

- Conduct larger-scale EPS production and UF for incorporation into concentrated fresh milk solutions for spray-drying. The product can be analysed in terms of functionality and sensory attributes.

- Conduct sensory testing on consumer panels that are familiar with the product as bias is involved when testing on people who are not regular consumers of the product.

- Limit phase separation in the milk solutions containing EPS. This may involve using EPS concentrations outside the critical region, using a different EPS, or altering the EPS or casein structure.

- Investigate odour removal by GAC with a larger supply of ferment to determine whether the fines can be separated and establish the minimum amount of UF and Cf required to make the ferment as odour free as possible.

- Investigate the incorporation of EPS into milk powders for use as a food ingredient rather than for production of milk and cheese. Incorporation into food products where milk protein is not a major component may limit phase separation.

CHAPTER 7 REFERENCES

BeMiller, J.N. and Whistler, R.L. (1996). Carbohydrates. In *Food chemistry: 3rd ed.* O.R. Fenemma (Ed). Marcel Dekker, New York. pp 157-223.

Bendall, J.G. (2001). Aroma compounds of fresh milk from New Zealand cows fed different diets. *Journal of Agricultural and Food Chemistry,* **49**: 4825-4832.

Bennet, R. (2003). Membrane Filtration Separation Technology notes - Diploma in Dairy Technology. Institute of Food Nutrition and Human Health, Massey University, Palmerston North, New Zealand. pp 6-16.

Berth, G., Dautzenberg, H., Christensen, B., Harding, S., Rother, G., Simsrød, O. (1996). Static light scattering studies on xanthan in aqueous solutions. *Macromolecules*, **29**: 3491-3498.

Bryant, C.M. and McClements, D.J. (2000). Influence of xanthan gum on physical characteristics of heat-denatured whey protein solutions and gels. *Food Hydrocolloids*, **14**: 383-390.

Callet, F., Milas, M., Rinaudo, M. (1987). Influence of acetyl and pyruvate contents on rheological properties of xanthan in dilute solution. *International Journal of Biological Macromolecules,* **9**: 291-293.

Capron, I., Brigand, G., Muller, G. (1998). Thermal denaturation and renaturation of a fermentation broth of xanthan: rheological consequences. *International Journal of Biological Macromolecules,* **23**: 215-225.

Caric, M. (1994). Concentrated and dried dairy products. VCH Publishers, Inc. New York, USA. pp 57-95.

Caric, M. (2003). Milk Powders/types and manufacture. In *Encyclopedia of dairy sciences*. H. Roginski, J.W. Fuquay, P.F. Fox (Ed). Elsevier Science Ltd. London, U.K. pp. 1869-1880

Castillo, M., Jordan, M.J., Godoy, A., Laencina, J., Lopez, M.B. (2000). Kinetics of syneresis in fresh goat cheese. *Milchwissenschaft*, **10**: 566-569.

Cheetham, N.W.H. and Mashimba, E.N.M. (1991). Characterisation of some enzymic hydrolysis products of xanthan. *Carbohydrate Polymers,* **15**: 195-206.

Christensen, B.H. and Simrød, O. (1991). Hydrolysis of xanthan in dilute acid: Effects on chemical composition, conformation, and intrinsic viscosity. *Carbohydrate Research*, **214**: 55-69.

Christensen, B.H. and Simrød, O. (1996). Dependence of the content of unsubstituted (cellulosic) regions in prehydrolysed xanthans on the rate of hydrolysis by *Trichoderma ressei endoglucanase. International Journal of Biological Macromolecules*, **18**: 93-99.

Corbin, E.A. and Whittier, E.O. (1965).The composition of milk. In *Fundamentals of Dairy Chemistry*. B.H. Webb and A.H. Johnson (Ed). AVI Publishing Company, Inc. Westport, Connecticut. pp 1-36.

Dalgleish, D.G. (1983). Coagulation of renneted bovine casein micelles: dependence on temperature, calcium ion concentration and ionic strength. *Journal of dairy research*, **50**: 331-340.

Daviau, C., Pierre, A., Famelart, M.-H., Goudedranche, H., Jacob, D., Garnier, M., Maubois, J.-L. (2000). Characterisation of whey drainage kinetics during soft cheese manufacture in relation with the physicochemical and technological factors, pH at renneting, casein concentration and ionic strength of milk. *Lait*, **80**: 417-432.

Dickinson, E. (1998). Stability and rheological implications of electrostatic milk protein-polysaccharide interactions. *Trends in Food Science and Technology*, **9**: 347-354.

Duboc, P. and Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry. *International Dairy Journal*, **11**: 759-768.

Garnot, P., Rank, T.C., Olson, N.F. (1982). Influence of protein and fat contents of ultrafiltered milk on rheological properties of gels formed by chymosin. *Journal of Dairy Science,* **65**:2267-2273.

Gilles, J. and Lawrence, R.C. (1981). The manufacture of cheese and other fermented products from recombined milk. *New Zealand Journal of Dairy Science and Technology*, **15**: 1-12.

Gilles, J. (1984). The manufacture of white cheese using high total solids recombined milk. *New Zealand Journal of Dairy Science and Technology*, **19**: 37-42.

Gordon, D.T. and Morgan, M.E. (1972). Principle volatile compounds in feed flavoured milk. *Journal of Dairy Science*, **55**: 905-912.

Hemar, Y., Tamehana, M., Munro, P.A., Singh, H. (2001). Viscosity, microstructure and phase behaviour of aqueous mixtures of commercial milk protein products and xanthan gum. *Food Hydrocolloids*, **15**: 565-574.

Hill, A.R., Bullock, D.H., Irvine, D.M. (1982). Manufacturing parameters of Queso Blanco made from milk and recombined milk. *Canadian Institute of Food Science and Technology*, **1**: 47-53.

Holzwarth, G. (1976). Conformation of the extracellular polysaccharide of *Xanthomonas campestris. Biochemistry*, **15**: 4333-4339.

How, J.S.L. and Morr, C.V. (1982). Removal of phenolic compounds from soy protein extracts using activated carbon. *Journal of Food Science*, **47**: 933-940.

International Dairy Federation. (1989). Dried milk and dried products: Determination of insolubility index. International IDF standard 129A:1988. pp. 1-6.

Iseki, T., Takahashi, M., Hattori, H., Hatakeyama, T., Hatakeyama. H. (2001). Viscoelastic properties of xanthan gum hydrogels annealed in the sol state. *Food Hydrocolloids*, **15**: 503-506.

Jansson, P-E., Kenne, L., Lindberg, B. (1975). Structure of the extracellular polysaccharide from *Xanthmonas campestris*. *Carbohydrate Research*, **45**, 275-282.

Kailasapathy, K. (1996). Polysaccharide ingredients in dairy product applications: Increase in cheese yields. *Food Australia,* **10**: 458-461.

Kang, K.S. and Pettitt, D.J. (1993). Xanthan, gellan, welan, and rhamsan. In *Industrial Gums and their Derivatives - Third Edition*. R.L. Whistler and J.N. BeMiller (Ed). Academic Press, Inc. San Diego, California. pp341-398

Keogh, M.K. and O'Kennedy, B.T. (1998). Rheology of stirred yogurt as affected by added milk fat, protein and hydrocolloids. *Journal of Food Science*, **1**: 108-112.

Langendorff, V., Cuvelier, G., Launay, B., Parker, A. (1997). Gelation and flocculation of casein micelle/carrageenan mixtures. *Food Hydrocolloids*, **11**: 35-40.

Larmond, E. (1977). *Laboratory methods for sensory evaluation of food.* Canada Department of Agriculture Publication 1637. Kromar Printing Ltd.

Launay, B., Cuvelier, G., Martinez-Reyes, S. (1997). Viscosity of locust bean, guar and xanthan gum solutions in the Newtonian domain: a critical examination of the $\log(\eta_{sp})_o$-log $C[\eta]_o$ master curves. *Carbohydrate Polymers*, **34**: 385-395.

Lecourtier, J., Chauveteau, G., Muller, G. (1986). Salt-induced extension and dissociation of a native double stranded xanthan. *International Journal of Biological Macromolecules*, **8**: 306-310.

Lee, H. and Brant, D.A. (2002a). Rheology of concentrated isotropic and anisotropic xanthan solutions. 1. A rodlike low molecular weight sample. *Macromolecules*, **35**: 2212-2222.

Lee, H. and Brant, D.A. (2002b). Rheology of concentrated isotropic and anisotropic xanthan solutions. 2. A semiflexible wormlike intermediate molecular weight sample. *Macromolecules*, **35**: 2223-2234.

Lomholt, S.B. and Qvist, K.B. (1999). The formation of cheese curd. In *Technology of Cheesemaking*. B.A. Law (Ed). Sheffield Academic Press, Sheffield, England. pp. 66-98.

Lopez, V. and Lindsay, R.C. (1993). Metabolic conjugates as precursors for characterizing flavour compounds in ruminant milks. *Journal of Agricultural and Food Chemistry*, **41**: 446-454.

Lucey, J.A. and Fox, P.F. (1993). Importance of calcium and phosphate in cheese manufacture: A review. *Journal of Dairy Science*, **76**: 1714-1724.

Ma, L. and Barbosa-Canovas, G.V. (1997). Viscoelastic properties of xanthan gels interacting with cations. *Journal of Food Science*, **62**: 1124-1128.

Marchesseau, S., Gastaldi, E., Lagaude, A., Cuq, J.'L. (1997). Influence of pH on protein interactions and microstructure of process cheese. *Journal of Dairy Science*, **80**: 1483-1489.

Marshall, R.J. (1986). Increasing cheese yields by high heat treatment of milk. *Journal of Dairy Research*, **53**: 313-322.

Masters, K. (1976). *Spray-drying - An introduction to principles, operational practice and applications, second addition*. John Wiley & Sons Inc. New York. pp. 555-561

Melton, L.D., Mindt, L., Rees, D.A., Sanderson, G.R. (1976). Covalent structure of the extracellular polysaccharide from *Xanthomonas campestris*: evidence from partial hydrolysis studies. *Carbohydrate Research*, **46**: 245-257.

Mietton, B. (1990). Use of conventional processes for the manufacture of recombined cheese. In *Recombination of milk and milk products*. Proceedings from the International Dairy Federation and the University of Alexandria Seminar, 12-16 November 1988, Special Issue 9001. International Federation, Northfield, Illinois, USA. pp 275-278.

Milas, M., Rinaudo, M., Duplessix, R., Borsali, R., Lindner, P. (1995). Small angle neutron scattering from polyelectrolyte solutions: From disordered to ordered xanthan chain conformation. *Macromolecules*, **28**: 3119-3124.

Milas, M., Reed, W.F., Printz, S. (1996). Conformations and flexibility of native and re-natured xanthan in aqueous solutions. *International Journal of Biological Macromolecules*, **18**: 211-221.

Mohammad, K.S. and Fox, P.F. (1987). Heat-induced microstructural changes in casein micelles and after heat coagulation. *New Zealand Journal of Dairy Science and Technology*, **22**: 191-203.

Moorhouse, R., Walkinshaw, S.A., Arnott, S. (1977). Xanthan gum - molecular conformation and interactions. In *Extracellular Microbial Polysaccharides – ACS Symposium Series 45*. P.A. Sanford and A. Laskin (Ed). American Chemical Society, Washington D.C., U.S.A. pp 90- 97.

Morris, V.J. (1995). Bacterial polysaccharides. In *Food Polysaccharides and Their Application*. A.M. Stephen (Ed). Marcel Dekker, Inc. pp. 341-375.

Muldoon, P.J. and Liska, B.J. (1972). Relationship between ionized calcium and curd tension in reconstituted nonfat dry milk. *Journal of Dairy Science*, **55**: 1300-1301.

Muller, G., Anrhourrache, M., Lecourtier, J., Chauveteau, G. (1986). Salt dependence of the conformation of a single-stranded xanthan. *International Journal of Biological Macromolecules,* **8**: 167-172.

Norit. (1995). Evaluation of Activated Carbon in Liquidphase Applications: Technical Bulletin (72/07-95). Amersfoort, Netherlands. p1-16.

Nussovitch, A. (1997). *Hydrocolloid applications, gum technology in the food and other industries*. Blackie Academic & Professional. An imprint of Chapman and Hall, London, U.K. pp154-166.

O'Connell, J.E. and Fox, P.F. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: A review. *International Dairy Journal*, **11**: 103-120.

Olsen, R.L. (1989). Effects of polysaccharides on rennet coagulation of skim milk proteins. *Journal of Dairy Research*, **72**: 1695-1700.

Oviatt, H.W. and Brant, D.A. (1993). Thermal treatment of semi-dilute aqueous xanthan solutions yields weak gels with properties resembling hyaluronic acid. *International Journal of Biological Macromolecules*, **15**: 3-10.

Papagianni, M., Psomas, S.K., Batsilas, L., Paras, S.V., Kyriakidis, M., Liakopoulou-Kyriakides, M. (2001). Xanthan production by *Xanthomonas campestris* in batch cultures. *Process Biochemisry*, **37**: 73-80.

Parnell-Clunies, E.M., Irvine, D.M., Bullock, D.H. (1985). Heat treatment and homogenization of milk for Queso Blanco (Latin American white cheese) Manufacture. *Canadian Institute of Food Science and Technology*, **2**: 133-136.

Pastor, M.V., Costell, E., Izquierdo, L., Duran, L. (1994). Effects of concentration, pH and salt content on flow characteristics of xanthan gum solutions. *Food Hydrocolloids*, **8**: 265-275.

Pastorino, A.J., Hansen, C.L., McMahon, D.J. (2003). Effect of salt on structure-function relationships of cheese. *Journal of Dairy Science*, **86**: 60-69.

Patel, M.C., Lund, D.B., Olson, N.F. (1971). Factors affecting syneresis of renneted milk gels. *Journal of Dairy Science*, **55**: 913-918.

Phelan, J.A., Renaud, J., Fox, P.F. (1993) Non-European cheese varaties. In *Cheese: Chemistry, Physics, and Microbiology, Volume 2: Major Cheese Groups, Second Edition*. P.F. Fox (Ed). Chapman and Hall Inc. London, U.K. pp 421-465.

Powell, D.A. (1979). Structure, solution properties and biological interactions of some microbial extracellular polysaccharides. In *Microbial Polysaccharides and Polysaccharases*. R.C. Berkeley, G.W. Gooday, D.C. Ellwood (Ed). Academic Press, Inc. New York pp. 117-160.

Prentice, J.H. (1992). *Dairy Rheology*. VCH Publishers, Inc. New York USA pp. 89-97.

Ramet, J.P. (2000). The drainage of coagulum. In *Cheesemaking: From Science to Quality Assurance: Second Edition.* A. Eck and J.-C. Gilles (Ed). Intercept Ltd. Hampshire, U.K. pp 41-59.

Ramirez-Figueroa, E., Salgado-Cervantes, M.A., Rodriguiz, G.C., Garcia, H.S. (2002). Addition of hydrocolloids to improve the functionality of spray dried yoghurt. *Milchwissenschaft*, **50**: 87-89.

Rinaudo, M. and Milas, M. (1980). Enzymic hydrolysis of the bacterial polysaccharide xanthan by cellulase. *International Journal of Biological Macromolecules*, **1**: 45-48

Rinaudo, M. (2001). Relation between the molecular structure of some polysaccharide and original properties in sol and gel states. *Food Hydrocolloids*, **15**: 433-440.

Rodd, A.B., Dunstan, D.E., Boger, D.V. (2000). Characterisation of xanthan gum solutions using dynamic light scattering and rheology. *Carbohydrate Polymers*, **42**: 159-174.

Rose, D. (1969). A proposed model of micelle structure in bovine milk. *Dairy Science Abstract* **31**: 171-175.

Sanchez, C., Zuniga-Lopez, R., Schmitt, C., Despond, S., Hardy, J. (2000). Microstructure of acid-induced skim milk-locust bean gum-xanthan gels. *International Dairy Journal*, **10**: 199-212.

Satia. (1982). Satixane Food Grade Xanthan Gum: Technical booklet. Paris, France.

Sato, T., Norisuye, T., Fujita, H. (1984). Double-stranded helix of xanthan: Dimensional and hydrodynamic properties in 0.1M aqueous sodium chloride. *Macromolecules*, **17**: 2696-2700.

Schmidt, D.G. (1980). Colloidal aspects of casein. *Netherlands Milk Dairy Journal,* **34**: 42-64

Schmidt, K.A. and Smith, D.E. (1992). Rheological properties of gum and milk protein interactions. *Journal of Dairy Science*, **75**: 36-42.

Schreiber, R. and Hinrichs, J. (2000). Rennet coagulation of heated milk concentrates. *Lait*, **80**: 33-42.

Shaker, R.R. and Gilles, J. (1990). A Review of the manufacture of cheese from recombined milk In *Recombination of milk and milk products*. Proceedings from the International Dairy Federation and the University of Alexandria Seminar, 12-16 November 1988, Special Issue 9001. International Federation, Northfield, Illinois, USA. pp 334-341.

Shatwell, K. P., Sutherland, I.W., Ross-Murphy, S.B. (1990). Influence of acetyl and pyruvate substituents on the solution properties of xanthan polysaccharide. *International Journal of Biological Macromolecules*, **12**: 71-78.

Singh, H., Shalabi, S.I., Fox, P.F., Flynn, A., Barry, A., (1988). Rennet coagulation of heated milk: Influence of pH adjustment before or after heating. *Journal of Dairy Research*, **55**: 205-215.

Singh, H. and Waungana, A. (2001). Influence of heat treatment of milk on cheesemaking properties. *International Dairy Journal*, **11**: 543-551.

Slattery, C. W. and Evard, R. (1973). A model for the formation and structure of casein micelles from subunits of variable composition. *Biochimica et Biophysica acta*, **317**: 529-538.

Southwick, J.G., Jamieson, A.M., Blackwell, J. (1981). Quasi-elastic scattering studies of semidilute xanthan solutions. *Macromolecules*, **14**:1728-1732.

Speers, R.A. and Tung, M.A. (1986). Concentration and temperature dependence of flow behaviour of xanthan gum dispersions. *Journal of Food Science,* **1**: 96-103.

Stokke, B.T., Elgsaeter, A., Smidsrød, O. (1986). Electron microscopic study of single- and double-stranded xanthan. *International Journal of Biological Macromolecules*, **8**: 217-225.

Suemitsu, R., Fujita, S., Matsubara, H. (1965). Studies on cow's urine. Part 3. Examination of an oil obtained from cow's urine by gas chromatography. *Agricultural Biological Chemistry,* **29**: 908-911.

Sutherland, I.W. (1984). Hydrolysis of unordered xanthan in solution by fungal cellulases. *Carbohydrate Research*, **131**: 93-104.

Sutherland, I.W. (1987). Xanthan Lyases - Novel enzymes found in various bacterial species. *Journal of General Microbiology*, **133**: 3129-3134.

Thorne, L., Tansey, L., Pollock, T.J. (1988). Direct utilization of lactose in clarified cheese whey for xanthan gum synthesis by *Xanthomonas campestris*. *Journal of Industrial Microbiology*, **3**: 321-328.

Walkenstrom, P., Panighetti, N., Windhab, E., Hermansson, A.-M. (1998). Effects of fluid shear and temperature on whey protein gels, pure or mixed with xanthan. *Food Hydrocolloids*, **12**: 469-479.

Walsh, P.M., Haas, M.J., Somkuti, G.A. (1984). Genetic construction of lactose-utilising *Xanthomonas campestris*. *Applied and Environmental Microbiology*, **47**: 253-257.

Walstra, P. and Jenness, R. (1984a). *Dairy Chemistry and Physics*. John Wiley & Sons, Inc. New York. pp 109-122.

Walstra, P. and Jenness, R. (1984b). *Dairy Chemistry and Physics*. John Wiley & Sons, Inc. New York. pp 229-245.

Walstra, P. and Jenness, R. (1984c). *Dairy Chemistry and Physics*. John Wiley & Sons, Inc. New York. pp 42-57.

Wheelock, J.V. and Kirk, A. (1973). The role of β-lactoglobulin in the primary phase of rennin action on heated casein micelles and heated milk. *Journal of Dairy Research*, **41**: 367-372.

Wilson, J. (1977). Separations using activated carbons. *Process Biochemistry*, **8**: 25-28.

Winger, R.J., Taylor, M., Singh, H., Duizer, L., Glasgow, S., Ha, M. (1997). Food chemistry laboratory manual. 41391 Food chemistry I. Institute of Food Nutrition and Human Health, Massey University, Palmerston North, New Zealand. pp 23-25.

Wolfschoon-Pombo, A.F. (1997). Influence of calcium chloride addition to milk on the cheese yield. *International Dairy Journal*, **7**: 249-254.

Yang, S.T. and Silva, E.M. (1995). Novel products and new technologies for use of a familiar carbohydrate, milk lactose. *Journal of Dairy Science*, **78**: 2541-2562.

Young, S. and Torres, J.A. (1989). Xanthan: effect of molecular conformation on surface tension properties. *Food Hydrocolloids*, **3**: 365-377.

APPENDICES

Appendix 1

Statistical Analysis of Sensory Results for Thickened Milk Samples Containing EPS

Thickened Milk Samples Sensory Analysis

Sample 1 = 15% WMP solution 0.079% xanthan
Sample 2 = 15% WMP solution 0.040% xanthan
Sample 3 = 15% WMP solution 0.015% xanthan
Sample 4 = 20% WMP solution

One-way ANOVA: Overall Liking (hedonic) versus Sample

```
Analysis of Variance for Overall Liking
Source     DF        SS        MS        F        P
Sample      3     19.44      6.48     2.18    0.094
Error     124    368.44      2.97
Total     127    387.88

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ---+---------+---------+---------
1          32     4.625     1.773  (---------*---------)
2          32     4.656     1.638   (---------*---------)
3          32     5.594     1.624                  (---------*-------
4          32     5.000     1.849        (---------*---------)
                                    ---+---------+---------+---------+
Pooled StDev =     1.724                 4.20      4.80      5.40
6.00

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0104

Critical value = 3.68

Intervals for (column level mean) - (row level mean)

                1           2           3

       2     -1.153
              1.090

       3     -2.090      -2.059
              0.153       0.184

       4     -1.496      -1.465      -0.528
              0.746       0.778       1.715
```

One-way ANOVA: Flavour (hedonic) versus Sample

```
Analysis of Variance for Flavour
Source     DF        SS        MS        F        P
Sample      3     23.29      7.76     2.49    0.064
Error     123    384.18      3.12
Total     126    407.46

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+--------
1          32     4.500     2.064  (--------*--------)
2          31     4.484     1.610  (--------*--------)
3          32     5.531     1.502                  (--------*--------)
4          32     4.719     1.836      (-------*--------)
                                    -----+---------+---------+--------
Pooled StDev =     1.767                4.20      4.90      5.60
6.30

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0104

Critical value = 3.68

Intervals for (column level mean) - (row level mean)

                1          2          3

       2     -1.143
              1.175

       3     -2.181     -2.206
              0.118      0.112

       4     -1.368     -1.394     -0.337
              0.931      0.924      1.962
```

One-way ANOVA: Creaminess (intensity) versus Sample

```
Analysis of Variance for Creaminess Intensity
Source     DF        SS        MS        F        P
Sample      3     10.34      3.45     2.70    0.049
Error     124    158.63      1.28
Total     127    168.97

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ---------+---------+---------+----
1          32     4.094     1.201     (-------*-------)
2          32     4.281     0.851         (-------*-------)
3          32     3.969     1.121  (-------*-------)
4          32     4.719     1.301                  (-------*-------)
                                    ---------+---------+---------+----
Pooled StDev =     1.131              4.00      4.50      5.00

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0104
```

```
Critical value = 3.68

Intervals for (column level mean) - (row level mean)

                1           2           3

       2      -0.923
               0.548

       3      -0.611      -0.423
               0.861       1.048

       4      -1.361      -1.173      -1.486
               0.111       0.298      -0.014
```

One-way ANOVA: Creaminess (hedonic) versus Sample

```
Analysis of Variance for Creaminess Hedonic
Source     DF        SS        MS        F        P
Sample      3      3.38      1.13     0.58    0.628
Error     123    237.68      1.93
Total     126    241.06
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+--------
1          32     5.500     1.391  (-----------*-----------)
2          31     5.516     1.363   (-----------*-----------)
3          32     5.656     1.405      (-----------*-----------)
4          32     5.906     1.400            (-----------*----------
                                    -----+---------+---------+--------
Pooled StDev =     1.390                  5.20      5.60      6.00
6.40

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0104

Critical value = 3.68

Intervals for (column level mean) - (row level mean)

                1           2           3

       2      -0.928
               0.895

       3      -1.061      -1.052
               0.748       0.771

       4      -1.311      -1.302      -1.154
               0.498       0.521       0.654
```

One-way ANOVA: thickness (intensity) versus Sample

```
Analysis of Variance for thickness intensity
Source     DF        SS        MS        F        P
Sample      3     14.34      4.78     3.73    0.013
Error     124    158.66      1.28
Total     127    172.99
```

```
                                        Individual 95% CIs For Mean
                                        Based on Pooled StDev
Level       N      Mean      StDev  --------+---------+---------+-----
1          32     4.219      1.099               (-------*-------)
2          32     3.875      1.185         (------*-------)
3          32     3.531      1.077   (-------*-------)
4          32     4.406      1.160                    (-------*-------
                                     --------+---------+---------+-----
Pooled StDev =    1.131                 3.50      4.00      4.50

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0104

Critical value = 3.68

Intervals for (column level mean) - (row level mean)

                1           2           3

       2      -0.392
               1.080

       3      -0.048      -0.392
               1.423       1.080

       4      -0.923      -1.267      -1.611
               0.548       0.205      -0.139
```

One-way ANOVA: thickness (hedonic) versus Sample

```
Analysis of Variance for thickness hedonic
Source     DF        SS        MS        F        P
Sample      3      2.90      0.97     0.51    0.675
Error     124    234.28      1.89
Total     127    237.18
                                        Individual 95% CIs For Mean
                                        Based on Pooled StDev
Level       N      Mean      StDev  -------+---------+---------+------
1          32     5.406      1.411  (-----------*-----------)
2          32     5.656      1.558        (-----------*-----------)
3          32     5.719      1.250          (-----------*-----------)
4          32     5.813      1.256            (-----------*-----------
                                     -------+---------+---------+------
Pooled StDev =    1.375                 5.20      5.60      6.00

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0104

Critical value = 3.68

Intervals for (column level mean) - (row level mean)

                1           2           3

       2      -1.144
               0.644
```

3	-1.207	-0.957	
	0.582	0.832	
4	-1.300	-1.050	-0.988
	0.488	0.738	0.800

Appendix 2

Statistical Analysis of Sensory Cheese Samples Containing EPS

Sample 1 = 15.6% MPC + 0.045% commercial EPS
Sample 2 = 17.37% MPC + 0% EPS

One-way ANOVA: Overall Liking (hedonic) versus Sample

```
Analysis of Variance for Overall Liking
Source     DF        SS        MS        F        P
Sample      1      0.27      0.27     0.07    0.791
Error      58    218.07      3.76
Total      59    218.33

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ---------+---------+---------+----
1          30     4.767     1.977  (-------------*-------------)
2          30     4.900     1.900     (-------------*-------------)
                                    ---------+---------+---------+----
Pooled StDev =    1.939                   4.50      5.00      5.50

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0500

Critical value = 2.83

Intervals for (column level mean) - (row level mean)

                1

       2     -1.135
              0.869
```

One-way ANOVA: Flavour (hedonic) versus Sample

```
Analysis of Variance for Flavour
Source     DF        SS        MS        F        P
Sample      1      0.07      0.07     0.02    0.886
Error      58    185.53      3.20
Total      59    185.60

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+-----
1          30     4.767     1.906   (---------------*---------------
2          30     4.833     1.663    (---------------*--------------
                                    --------+---------+---------+-----
Pooled StDev =    1.789                 4.40      4.80      5.20

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0500
```

```
Critical value = 2.83

Intervals for (column level mean) - (row level mean)

                1

       2      -0.991
               0.858
```

One-way ANOVA: saltiness (intensity) versus Sample

```
Analysis of Variance for saltiness
Source     DF        SS        MS        F        P
Sample      1      0.10      0.10     0.05    0.831
Error      58    131.11      2.26
Total      59    131.21

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+---
1          30     3.733     1.596   (---------------*--------------)
2          30     3.817     1.405     (---------------*--------------
                                    ----------+---------+---------+---
Pooled StDev =    1.503                    3.50      3.85      4.20

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0500

Critical value = 2.83

Intervals for (column level mean) - (row level mean)

                1

       2      -0.860
               0.694
```

One-way ANOVA: texture (hedonic) versus Sample

```
Analysis of Variance for texture
Source     DF        SS        MS        F        P
Sample      1      0.09      0.09     0.03    0.872
Error      57    189.57      3.33
Total      58    189.66

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+---
1          29     4.690     1.815  (----------------*----------------
)
2          30     4.767     1.832    (----------------*--------------
                                    ----------+---------+---------+---
Pooled StDev =    1.824                    4.40      4.80      5.20

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0500

Critical value = 2.83
```

```
Intervals for (column level mean) - (row level mean)

                1

       2   -1.028
            0.874
```