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
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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.

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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).

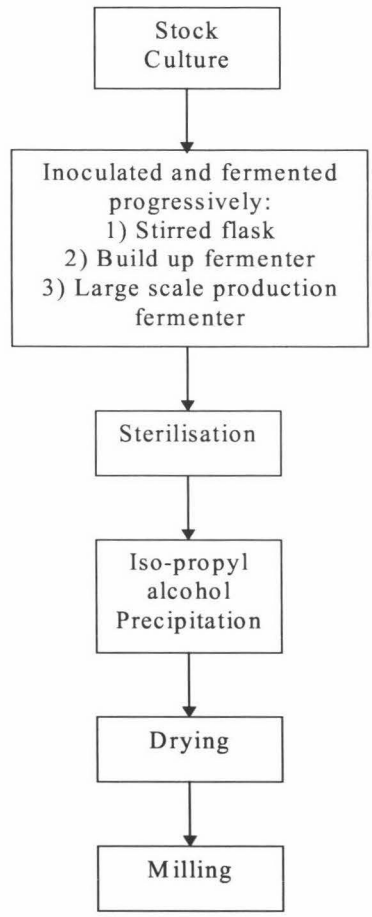

Figure 1.1 Commercial production of Xanthan from *Xanthomonas campestris* (Satia, 1982)

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)

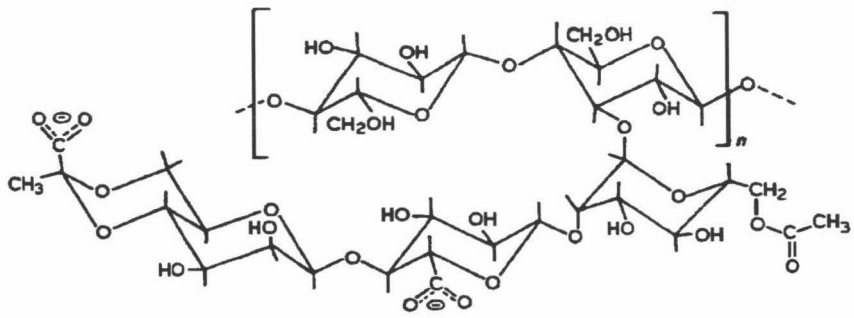

Figure 1.2 Xanthan molecular structure with repeating pentasaccharide unit

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).

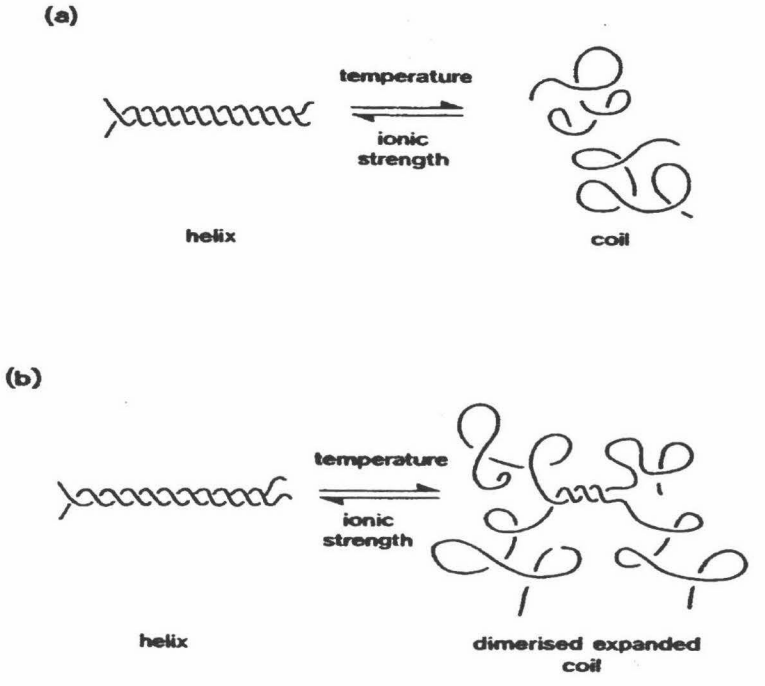

Figure 1.4 Xanthan transition from helix to a) Randomised coil b) Dimerised coil (from Morris, 1995)

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).

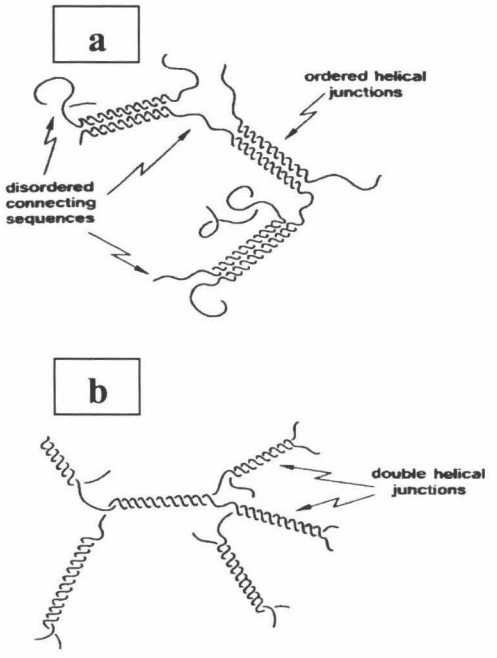

Figure 1.5 Possible aggregating tendencies of xanthan molecules a) single helix, b) double helix (from Morris, 1995)

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).

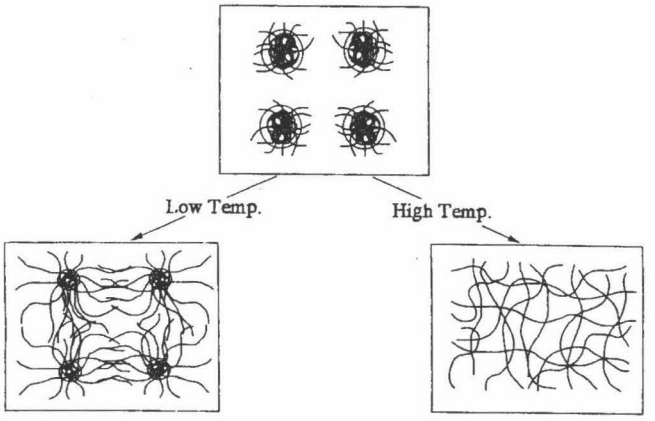

Figure 1.6 Effect of high and low annealing temperatures on the interaction of xanthan molecules (from Iseki et al. 2001).

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).

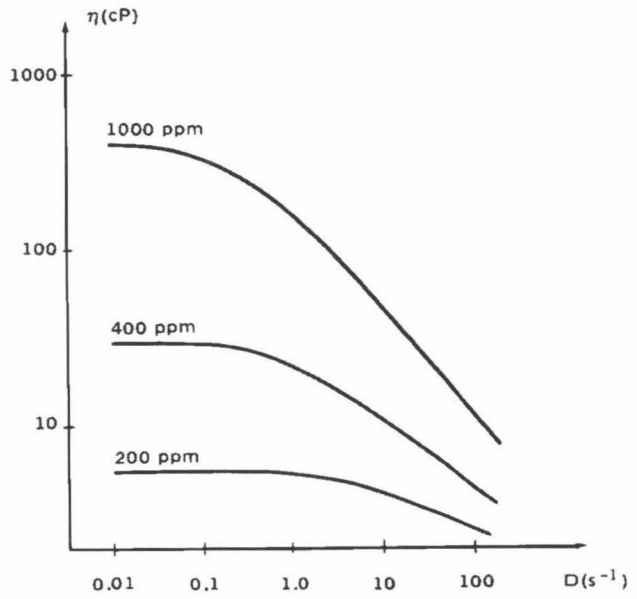

Figure 1.7 The effect of shear rate on the viscosity of various concentrations of xanthan in 0.5% sodium chloride (from Satia 1982)

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).

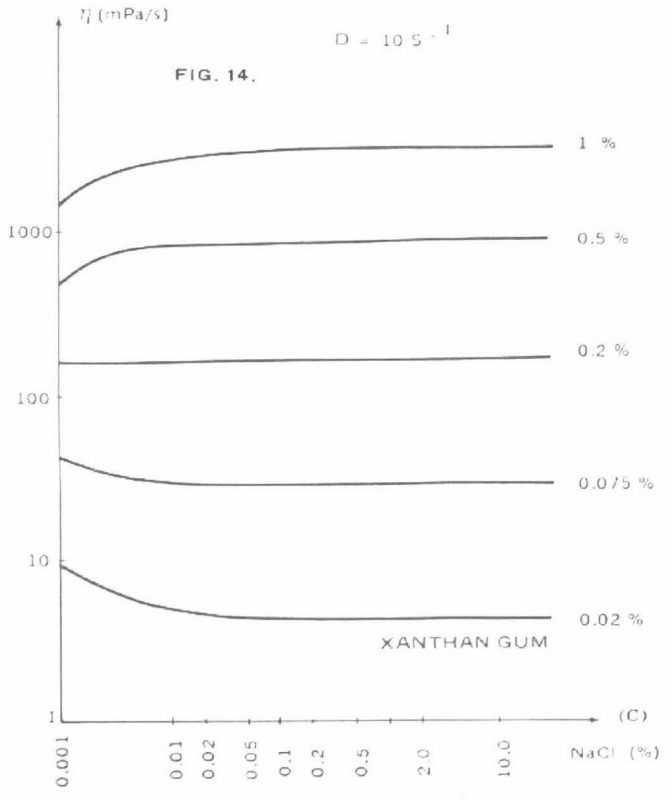

Figure 1.8 Effect of NaCl addition on viscosity for xanthan solutions at a shear rate of $10s^{-1}$ (from Satia, 1982).

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.

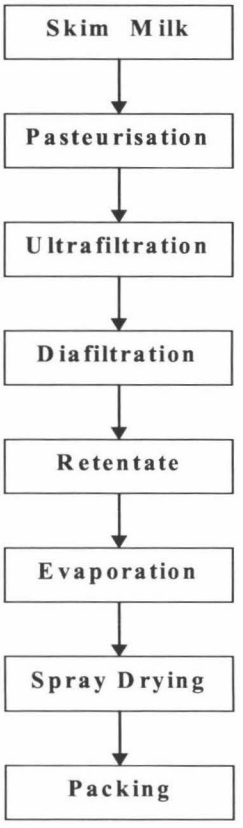

Figure 1.9 Flow diagram of MPC production

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.